

Cyanobacteria Mediated CO₂ Segregation: A Promising Alternative Methods for Sustainable Bioremediation and Biomass Production

ABSTRACT

Aims: The purpose of this study was to examine the CO₂ absorbent (NaOH) affected *Spirulina sp.* medium culture to verify growth rate, CO₂ fixation ability and biomass production from *S. platensis* using the Zarrouk medium.

Methodology: Cyanobacterium *Spirulina sp.* was cultured with Zarrouk's medium at 30±2°C, pH 9 which was obtained from the Applied Botany Section of BCSIR. Besides, to prevent precipitation, the medium without the carbon source was autoclaved for 30 min at 121 °C and then the carbon source and NaOH were added. Furthermore, every 6-day intervals, the biomass content, pH, and alkalinity were measured. The samples were obtained for the same analyses when the biomass concentration reached the blend concentration (0.5 g L⁻¹).

Results: The first concentration was used 0.5 g/L. 47g/L (1.64 mmol/L) of absorbent. The mean increase of 2.45% for biomass generated by the assay using control (2.09 ± 0.11 g) when compared to the NaOH (2.04 ± 0.25 g) was due to the larger number of growth cycles. Besides, a larger number of growth cycles 35.4% for CO₂ fixation rate increase compared to the control. The final protein (28.2 ± 4.5 % ww⁻¹) and carbohydrate (19 ± 2.5 % ww⁻¹) content in the biomass cultivated using NaOH was lower than normally found in the control assay (43.4 ± 2.9 % ww⁻¹) and (11.5 ± 1.2 % ww⁻¹).

Conclusion: Recent advances in CO₂ fixation and biomass production utilizing microalgae were compiled and analyzed, with an emphasis on how adding the CO₂ absorbent affected the growth kinetics and biomass composition of *Spirulina sp.* When compared to the chemical absorbent assay, the control assay's high growth and CO₂-fixation rates provide several advantages.

Keywords: Pollution, CO₂ fixation, *Spirulina platensis*, Zarrouk media, Control assay.

1. Introduction

“Rising CO₂ levels in the atmosphere have been designated as one of the century's major challenges. It increases global warming, which causes the melting of arctic ice, warming of seawater, intense heat waves that have impeded the agricultural sector and harmed human health” (Lam mk et. al., 2012) and “regular droughts and desertification of regions” [1]. “Global warming occurs when carbon dioxide (CO₂) and other pollutants accumulate in the atmosphere, absorbing sunlight and solar radiation and heating the earth's surface. Normally this radiation would escape into space but, this pollutant which can last for years to centuries in the atmosphere, traps the heat and causes the earth to get hotter”. (Global Warming 101, NRDC, 2021) “These heat-trapping pollutants, especially carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), water vapour, and synthetic fluorinated gases are known as greenhouse gases and their impact is called the greenhouse effect” [2]. “As CO₂ is the primary cause of the greenhouse effect and global warming, it is mandatory to mitigate CO₂ from the earth to save the world. The capacity of microalgae cultures to capture CO₂ has been discovered (Basu S, et al, 2014) in the ongoing research for viable CO₂-capturing technology” [3]. “According to scientific principles, chemical absorption, physicochemical adsorption, membrane, cryogenics, CLC (Chemical Looping Combustion), and terrestrial sequestration (Zaho B, et. al,2012) are the primary categories of post-combustion CO₂ capture” [4]. “Microalgae-based CO₂ fixation and energy/resource utilization, a viable and promising biological technique, has gotten a lot of attention in the recent two decades because of its techno-economic feasibility and environmental friendliness” [5]. “Several strategies are being used in the modern era to reduce and stabilize CO₂ levels. These strategies include renewable energy, chemical processes such as chemical absorption [6], and biological processes such as photosynthesis by microalgae” [7]. “Bio-energy with carbon capture and storage has the potential to significantly reduce CO₂ emissions. It is a combination of bio-energy production, carbon capture and storage, and the retention of carbon dioxide in geological reservoir” [8]. “Microalgae grow faster, have higher CO₂ fixation efficiency, and produce more high-value products, such as dietary supplements for humans, animals, and aquaculture, than terrestrial plants” [9]. “Temperature, pH, CO₂, light, and inorganic salts, all of which are necessary for maximum biomass, may be monitored and regulated. CO₂ is fixed and converted to carbon-containing high-value compounds like carbohydrates, lipids, and proteins at the same time” [10]. “Biomass productivity reflects CO₂ fixation capacity. Many microalgae species have carbon concentrating mechanisms (CCM), CCM acts as a growth promoter in microalgae and is thus preferred for carbon sequestration” [11]. “Biological CO₂ mitigation through microalgae has recently received significant attention among the various CO₂ mitigation strategies due to their higher CO₂ fixation capability and bioactive substances contained in their biomass”[12].

“Based on the research that has been conducted, it can be concluded that microalgae will undoubtedly be a cost-effective and powerful tool for CO₂ mitigation. The effect of CO₂ level on microalgae growth varies depending on the strain” [13,14] “Several algal species have been found to fix CO₂ including *Spirulina platensis*, *Scenedesmus obliquus*, *Botryococcus braunii*, *Chlorella vulgaris* and *Nannochloropsis oculata*” [15,16]. “*Spirulina sp.* is a photosynthetic filamentous cyano-bacterium that grows in water with a pH of around 10” [17,18]. “Because of its high nutritional value and the presence of bio-compounds of economic importance, it is one of the most studied microalgae” [19, 20]. “*Spirulina sp.* has several advantages over other microalgae, including the ease with which the liquid medium can be recovered due to the trichome arrangement, the high nutritional value of biomass, the ability to adapt to outdoor systems and scale up, and CO₂ bio-fixation from flue gas” [21, 22]. “NaHCO₃ and NaNO₃ have been used alone or in combination as a carbon source for *Spirulina sp.* Cultivation” [23, 24]. “Because of the low concentration of dissolved carbon attained in the medium and probable pH changes caused by gas injection, the use of gaseous CO₂ as a carbon source for the intense synthesis of microalgae biomass has been investigated. *Spirulina* biomass is produced utilizing CO₂ as a carbon source in a pressurized gas container containing a yeast and sugar mixture. On the other hand, these studies focus on the growth of this microalgae to maximize biomass production and CO₂ bio-fixation” [25].

2. Material and Methods

2.1. Microorganism

The microorganism used for cultivation was the Cyanobacterium *Spirulina sp.* which was obtained from the Laboratory of Applied Botany Section of BCSIR. The strain was maintained in 2L sterilized Erlenmeyer flasks containing 1800 mL Zarrouk’s medium at 30±2°C, pH 9.0 with continuous illumination which is shown in fig. 1(a) and 1(b).



Fig. 1. (a): Zarrouk medium without NaHCO₃ (b) Extracting Supernatant added to the medium after autoclaving.

2.2 Culture Media and Experimental Design

Zarrouk's medium has successfully served as the standard medium (SM) for *Spirulina sp.* Culture. All constituents of Zarrouk's medium were autoclaved without bicarbonate salt, which was replaced by another carbon source, and used as the standard control medium. The specific component and quantity used per litre in the Zarrouk medium were as follows: All the reagents were table 1(a) macro element: NaNO_3 , 4.5 g; K_2HPO_4 , 0.9 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.36 g; K_2SO_4 , 1.8 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.018 g; EDTA, 1.44 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.072 g; and table 1(b) trace metal solution, 1 mL. The 1 L of trace metal solution contains the following: H_3BO_3 , 5.148 g; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.258 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3996 g; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1422 g; and Na_2MoO_4 , 0.0318 g were applied.

Table 1 Zarrouk medium for 1.8 L solution without NaHCO_3

Chemical for Macronutrients (a)	Amount (g/L)	Chemical for Micronutrients (b)	Amount (g/L)
K_2HPO_4	.9g	H_3BO_4	5.148g
NaNO_3	4.5g	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	3.258g
K_2SO_4	1.8g	$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	0.3996g
NaCl	1.8g	$\text{Na}_2\text{MO} \cdot \text{O4}$	0.03186g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.36g	$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	0.1422g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.018g		
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.072g		
EDTA	0.144g		
Micronutrient solution added	1.8g		

To prevent precipitation, the medium without the carbon source was autoclaved for 30 min at 121 °C and then the carbon source was added. The composition of SM is shown in table 1. Sterilized Erlenmeyer flasks (2000 mL) containing 1800 mL of SM, were used as culture units that are shown in fig. 2.



Fig. 2. Initial aspect to the design (a) Sterilize the Erlenmeyer flask by Microwave Oven for *Spirulina* cultivation (b) Zarrouk's medium was autoclaved by an Autoclave Machine

2.3 Supply of CO₂ to the Medium

50gm of Dry Baker's Yeast, 400gm of Sugar per bottle and 5gm of Baking Soda per bottle. Our first step is to activate the yeast by placing it and 5gm of sugar in 200ml of warm tap water (room temperature). Mixed up thoroughly until the water is slightly bubbly and wait 10 minutes. The yeast is awakened and begins feeding on the available sugar. While waiting for the yeast to activate, enough warm water is added to fill the soda bottles $\frac{3}{4}$ of the way. The rest of the sugar and baking soda are added to each bottle and shaken vigorously to mix. Once the yeast is activated, add it to its new sugar-rich home, and tightly reseal each soda bottle. Over the course of hours, the yeast cells continue to feed and divide. As a byproduct they create enough CO₂ that the excess gas bleeds into the line and into the media, feeding the cultivated macroalgae. How long they generate CO₂ is somewhat inconsistent but can range from 1½ to 3 weeks before the system is needed to fresh sugar and yeast.

2.4 CO₂ Absorbent

NaOH is a potential chemical absorbent that is widely used in CO₂-capturing processes. To investigate the influence of NaOH on cell growth and CO₂ fixation, 0–8.56 mmol⁻¹ (0–1.88 g/L) NaOH was added to the microalgal cultures in intervals of every 6 days.

2.5 Maintenance of Inoculums

The carbon source for *Spirulina sp.* was CO₂, which replaced the sodium bicarbonate in the Zarrouk medium. Decanting the *Spirulina sp.* inoculums, extracting the supernatant (about 90% v v⁻¹), and recovering the pellet were used to achieve this (10 percent v v⁻¹), as shown in Fig. 3.



Fig. 3. CO₂ absorbent NaOH (left side) added to specific one flask and the other controls (right side).

During the light period, the cell pellet was re-suspended in Zarrouk medium without a carbon source and exposed to the new carbon source at a daily specific flow rate of $0.12 \text{ ml CO}_2 \text{ mL}^{-1}_{\text{medium}} \text{ d}^{-1}$ for 1 min h^{-1} during the light period [26].

2.6 Cultivation Conditions

The experiments were performed in duplicate in a 2.0L Erlenmeyer flask with a working volume of 1.8L, in a semi-continuous mode and fed with CO₂. NaOH was used as the chemical absorbent. Assays with and without (control assay) the additions of NaOH were performed. The assays were kept at 30°C in a growth room (12 hours) light/dark photoperiod. 40W daylight-type fluorescent bulbs provided illumination, resulting in a $41.6 \mu\text{mol}_{\text{photons}} \text{ m}^{-2} \text{ s}^{-1}$ illuminance. Compressed air injection with a specified flow rate of 0.05 vvm was used to stir the mixture, The experiments lasted 24 days and started at a cellular concentration of 0.20 g L^{-1} . During the light phase, 2 min h^{-1} of CO₂ was supplied ($36 \text{ mL CO}_2 \text{ mL}_{\text{medium}} \text{ d}^{-1}$).

2.7 Analytical Determinations

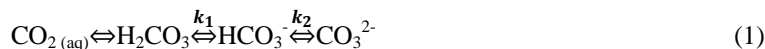
Every 6-day intervals, the biomass content, pH, and alkalinity were measured. The samples were obtained for the same analyses when the biomass concentration reached the blend concentration (0.5 g L^{-1}).

2.7.1. Biomass concentration

The biomass concentration was evaluated using a UV Spectrophotometer (Evolution 201) and a *Spirulina sp.* standard curve. The optical density of the *Spirulina sp.* inoculums was measured in a spectrophotometer (Thermo Scientific, USA) at 670 nm, and the optical density and dry weight biomass were correlated.

2.7.2. Alkalinity, pH and concentration of dissolved inorganic carbon

Medium alkalinity was determined by potentiometric titration and pH by pH meter (Hach, USA). These measurements were also used to calculate the concentration of dissolved inorganic carbon (DIC) using the equilibrium equations developed by Brune and Novak, 1981. For this calculation, it is assumed that all chemical species represented the dissolved total inorganic carbon in equilibrium (eq. (1)).



2.7.2.1. Alkalinity Titration

The alkalinity of a sample is a measure of its capacity to neutralize acid. This is due to the presence of a weak acid.

The procedures are illustrated in table 1 and table 2.

Table 2 Alkalinity Measurement of the two cultural flasks

Flask	Alkalinity measurement	Cycle 01	Cycle 02	Cycle 03	Cycle 04
01	Phenolphthalein Alkalinity	7mL	5mL	3.3 mL	1.8 mL
01	Total alkalinity	32 mL	34 mL	21 mL	19 mL
02	Phenolphthalein Alkalinity	5 mL	4 mL	2.8 mL	1.6 mL
02	Total alkalinity	38 mL	18 mL	15 mL	13 mL

Table 3 Identification and quantification of soluble ions

		Flask 01			
Ions Measurement	Cycle 01	Cycle 02	Cycle 03	Cycle 04	
CO_3^{2-}	14	10	6.6	3.6	
HCO_3^-	18	24	7.8	11.8	
p ^H	6.1	5.2	7.2	7.5	
		Flask 02			
Ions Measurement	Cycle 01	Cycle 02	Cycle 03	Cycle 04	
CO_3^{2-}	10	8	5.6	3.2	
HCO_3^-	28	10	9.4	6.6	
p ^H	5.7	6.2	6.5	8.1	

2.8 Cultivation Responses

The following characteristics were obtained for each development cycle from the cell growth outlines of *Spirulina* sp. Volumetric biomass productivity (P_X , mg L⁻¹ d⁻¹), generation time (tg, d), and CO₂ bio-fixation rate (R_{CO_2} , mg L⁻¹ d⁻¹), were the highest values for each parameter as the cycles progressed.

2.8.1 Biomass volumetric productivity

$P_X = (X_t - X_0)/(t - t_0)$ was used to calculate biomass volumetric productivity, where X_t is the biomass concentration (g L⁻¹) at time t (d) and X_0 is the biomass concentration (g L⁻¹) at time t_0 (d).

2.8.2 Carbon dioxide bio-fixation rate

The CO₂ fixation rate (R_{CO_2} as g-CO₂ L⁻¹ d⁻¹) of algal cells is computed based on the change in algal biomass when CO₂ is the only carbon source available to microalgae:

$$R_{CO_2} = \frac{\Delta X}{\Delta t} f_C \frac{M_{CO_2}}{M_C}$$

Where ΔX denotes the change in biomass concentration (g L⁻¹) during cultivation, Δt ; f_C denotes the carbon fraction in biomass; M_{CO_2} and M_C denote the molar mass of CO₂ and carbon atoms, respectively.

2.8.3 Carbon dioxide use efficiency

The CO₂ use efficiency (E_{CO_2}) was calculated according to $E_{CO_2} = R_{CO_2} \cdot V_{work} / m \times 100$, where R_{CO_2} was the daily CO₂ bio-fixation rate (mgL⁻¹d⁻¹), V_{work} was the useful working volume of the photobioreactor (L), and m was the daily CO₂ feed rate (mgd⁻¹).

2.9 Biomass Recovery and Characterization

Each experiment's total biomass was recovered by centrifugation (Hitachi Himac CR-GIII, Tokyo, Japan) at 15,200g and 20°C for 15 minutes, re-suspended in distilled water, and centrifuged again under the same conditions. This procedure was repeated once more to improve nutrient removal. The biomass was then concentrated to 50 mL in a sterile recipient, frozen at 80°C, lyophilized, and stored at 20°C showed in [fig. 4](#).



Fig.4. Recovery of biomass by Centrifuge

2.9.1 Protein concentration

The total proteins concentration of the *Spirulina* biomass was evaluated by colorimetric technique from heat and alkaline pretreatment of the microalgal biomass at each growth cycle[27] and at the end of the assays (Lowry,1951)

2.9.2 Carbohydrate concentration

Using a standard glucose curve, the carbohydrate concentration in the microalgae biomass was measured using the Dubois phenol–sulfuric technique [28]

3. Result

The goal of this work was to observe how adding CO₂ absorbent NaOH affected the growth kinetics, CO₂ fixation rate and biomass composition of *Spirulina sp.* under semi-continuous cultivation. Experiments were designed to verify growth rate, CO₂ fixation ability, and biomass production from *S. platensis* utilizing the Zarrouk medium, which uses a pressurized CO₂ gas diffuser instead of NaHCO₃. This investigated parameter differs between the control assay and the CO₂ absorbent NaOH assay. presents and discusses the effect of CO₂ absorbent on biomass production, specifically their fixation rate in comparison to the control assay.

3.1 Biomass Concentration Versus Concentration of CO₂ Absorbent NaOH

The experiments performed with $X_0 = 0.2 \text{ gL}^{-1}$ were not able to tolerate a NaOH concentration. The first dose of NaOH 1.64 mmolL^{-1} is added to the culture medium when the initial concentration reaches 0.5 g/L . As a result, the results revealed that *Spirulina sp.* has tolerance only for the initial concentration of 0.5 g/L for *Spirulina sp.* cultivation with CO₂ absorbent showed in table 4.

Table 4. Growth parameters of *Spirulina sp.* with NaOH absorbent assay

Cycle (interval of 6 days)	Cell density (g/L)	Concentration of NaOH absorbent(mmolL ⁻¹)
01	0.5	1.64
02	1.5	3.26
03	0.9	4.92
04	0.59	8.56

On the sixth day, when the concentration was 0.5 g/L, the first dose was administered. The absorbent was added at a rate of 0.47g/L (1.64 mmolL⁻¹) to the test. On the 12th day or in 2nd cycle, after the addition of chemical absorbent, the cell density reached its peak concentration.

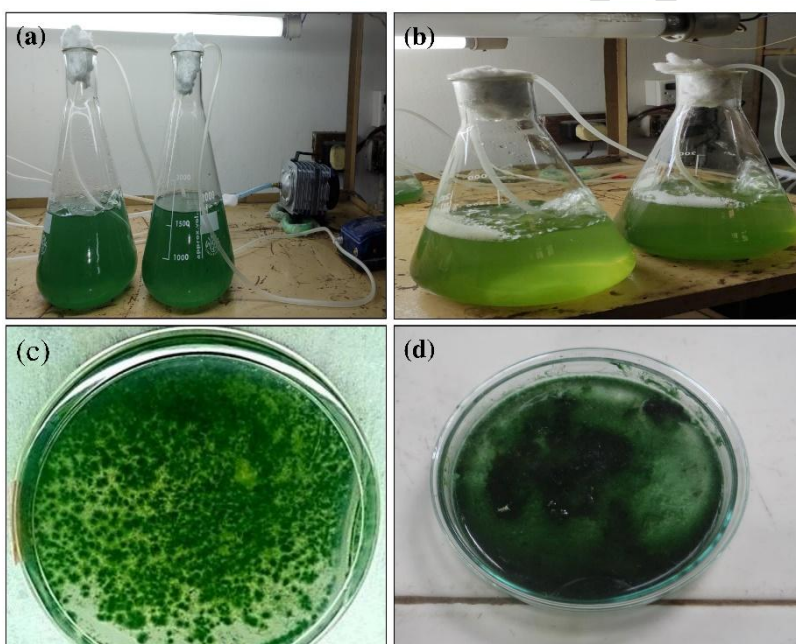


Fig. 5. (a) The culture media in 1st days (b) 12th days (c) 24th days' final product with absorbent assay (d) with control assay

Fig. 5(a), 5(b) show the development of *Spirulina sp.* cells in the presence and absence of NaOH absorbent. **Fig. 5(c) and (d)** show the final biomass product in the presence of NaOH and in the under-control assay. **Fig. 6** depicts *Spirulina sp.* cell development in the presence of NaOH that has been absorbed by CO₂. The cell density transitioned to a low productivity condition as the concentration of NaOH was increased, as shown in fig. 8. Biomass productivity would be at its peak if the concentration was maintained at 3.26 mmol L⁻¹ (0.94 g/L). **Fig. 6**

shows the development of *Spirulina sp.* cells in the presence and absence of NaOH absorbent. Fig. 7 shows the final biomass product in the presence of NaOH and in the under-control assay. The depicts *Spirulina sp.* cell development in the presence of NaOH that has been absorbed by CO₂. The cell density transitioned to a low productivity condition as the concentration of NaOH was increased, as shown in fig. 8. Biomass productivity would be at its peak if the concentration was maintained at 3.26 mmol L⁻¹ (0.94 g/L). When concentration of CO₂-NaOH >0.94g/L, however, cell growth rate was significantly reduced [29]. The physiological adaptation of the cells produced by changes in nutritional circumstances, such as medium recycle, might impact the growth period.

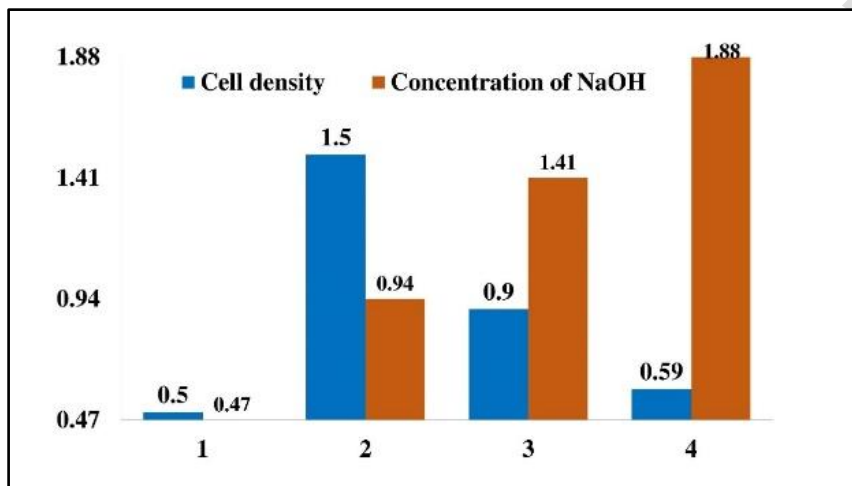


Fig.6. The effect of CO₂ absorbent (NaOH concentration) on *Spirulina sp.* cell growth (mentioned in figures)

3.2 Control Assay

The control assay in table 5, demonstrated similar behavior to the assays with CO₂ absorbents added. All cultures tended to enter the stationary phase of growth between days 10 and 12 of the experiment. However, an excessive buildup of organic matter on the inner side surface of the Erlenmeyer flask was seen in both of the study's circumstances, with or without the inclusion of the chemical absorbent. The average biomass concentrations were obtained over the 13 days of the assay.

Table 5. *Spirulina sp.* growth parameters with Control Assay

Time (days)	Biomass Concentration (g/L)
1	0.2
6	.56
12	1.54
18	1.39
24	0.95

3.3 Comparison Between NaOH Absorbent Assay and Control Assay in Biomass Concentration

Spirulina sp. cell growth in the presence of CO₂-absorbed NaOH is depicted in fig. 8. In the presence of 1.64 – 3.28 mmol/L CO₂-NaOH, cell density reached approximately 1.5 mg/L in 12 days. Cell growth rate, on the other hand, decreased significantly when CO₂-NaOH content was greater than 4.92 to 8.56 mmol/L. These results indicate that CO₂ in the absorbed state with NaOH can be fixed into the algal biomass in the range of 1.64- 3.28 mmol/L but that higher CO₂-NaOH concentrations inhibit algal cell growth. In the case of the control assay fig. 8, the growth rate did not inhibit as NaOH absorbent.

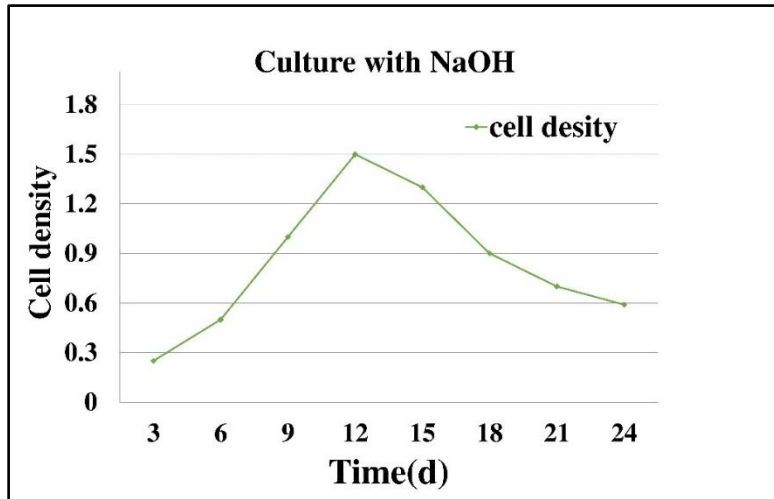


Fig. 7 Concentration of biomass culture with CO₂ absorbent (NaOH)

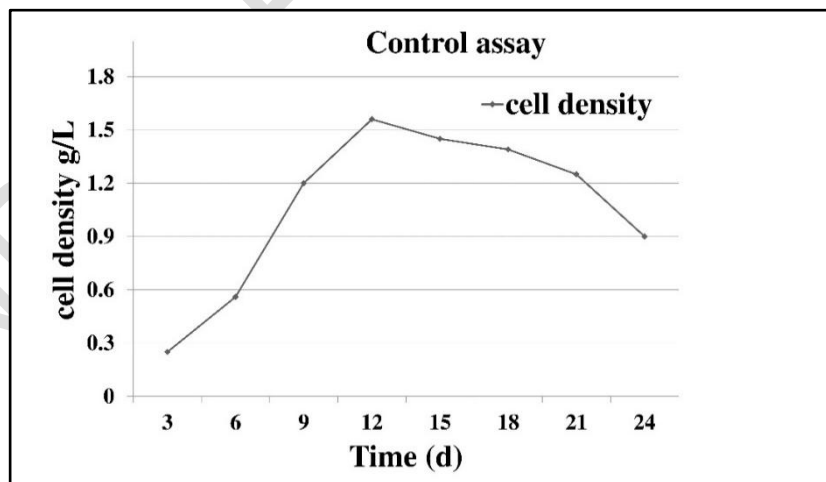


Fig. 8: Concentration of biomass in control assay

3.4 Growth Rate of Biomass

The rate of cell growth has been calculated by the concentration of biomass increase or decrease along with the time intervals. Here the following equation is

$$\text{Grow Rate, } R = \frac{V_{\text{present}} - V_{\text{previous}}}{V_{\text{previous}}} \times 100$$

Table 6 Comparison between NaOH assay and control assay in the growth rate of biomass

Cycle	Growth Rate(mg/L)	
	NaOH Absorbent Assay	Control Assay
01	174	124
02	204	209
03	93	167
04	48.9	138

The growth rates of the two assays were only slightly different, as seen in [fig. 10](#) and [fig. 11](#). Therefore, the mean increase of 2.45% for biomass generated by the assay using control assay (2.09 ± 0.11 g) when compared to the NaOH (2.04 ± 0.25 g) was due to the larger number of growth cycles.

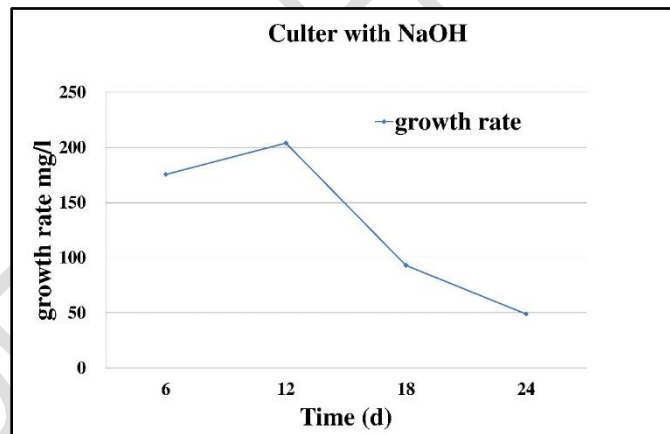


Fig. 9: Growth rate of biomass in culture with NaOH

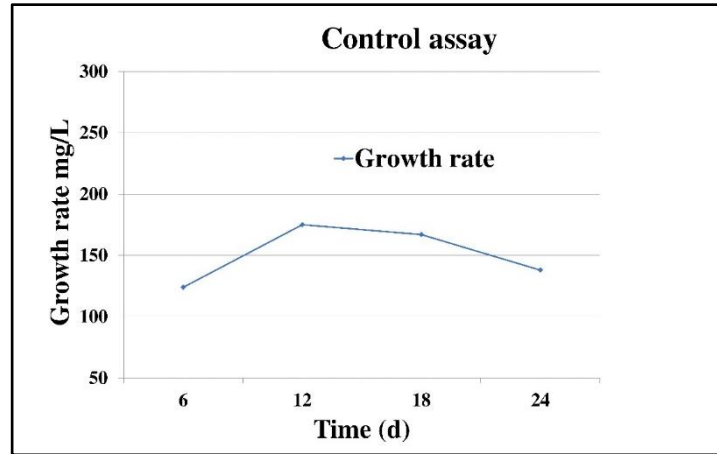


Fig. 10: Growth rate of biomass Control assay

3.5 Biomass Volumetric Productivity

Biomass volumetric productivity was calculated using the following equation

$$P_X = (X_t - X_0)(t - t_0)$$

Where X_t is the biomass concentration (g L^{-1}) at time t (d) and X_0 is the biomass concentration (g L^{-1}) at time t_0 (d).

Table 7. Volumetric productivity of NaOH assay and Control assay

Time(days)	Biomass volumetric Productivity	
	NaOH – Assay(mg/L)	Control Assay(mg/L)
6	60±0.7	72±0.9
12	118±0.3	121±0.2
18	41.1±0.9	70±0.7
24	16.95±0.8	32.60±0.4

The volumetric productivity of biomass *Spirulina sp.* declined in both circumstances during the growth cycles the means the time intervals table 7, with mean values in the control assay was considerably greater ($p < 0.001$) than the assay with NaOH absorbent assay. The maximum productivity of control assay is 121 ± 0.2 mg/L, which is greater than NaOH assay (118 ± 0.3) mg/L.

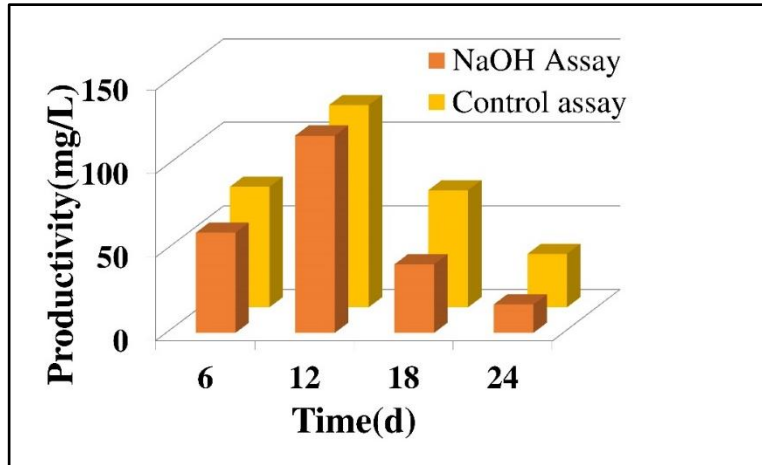


Fig. 11: Volumetric productivity of control assay and absorbent assay

3.6 The Rate of CO₂ Fixation

The maximums of CO₂ bio-fixation rate (R_{CO_2}) are significant factors to consider when assessing the potential for CO₂ removal. The CO₂ fixation rate (R_{CO_2} as g-CO₂ L⁻¹ d⁻¹) of algal cells is computed based on the change in algal biomass when CO₂ is the only carbon source available to microalgae:

$$R_{CO_2} = \frac{\Delta X}{\Delta t} f_c \frac{M_{CO_2}}{M_C}$$

Where ΔX denotes the change in biomass concentration (g L⁻¹) during cultivation, Δt denotes the time interval, f_c denotes the carbon fraction in biomass; M_{CO_2} and M_C denotes the molar mass of CO₂ and carbon atoms, respectively.

Table 8. Comparison between NaOH assay and control assay in rate of CO₂ fixation

Cycle	CO ₂ fixation Rate(mg/L)	
	NaOH Assay	Control Assay
01	93.8	165.9
02	317.9	403.6
03	172	355.3
04	144	222.84

Throughout the experiment, the R_{CO_2} obtained in the NaOH-added assay was not higher than that obtained in the control assay table: 8. Therefore, the mean of 35.4% for CO₂ fixation rate increases by control assay 403 mg/L (4.03

g/L) **fig. 12**, when compared to the NaOH 317 mg/L (3.17 g/L) was due to the larger number of growth cycles **fig. 13**.

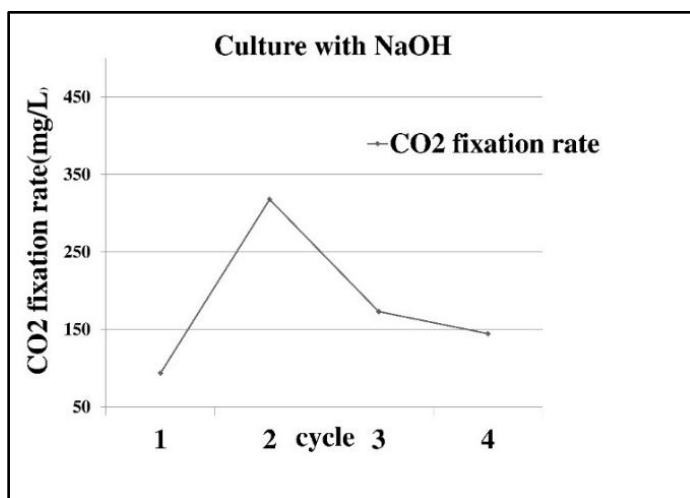


Fig. 12. Rate of CO₂ fixation in culture with NaOH

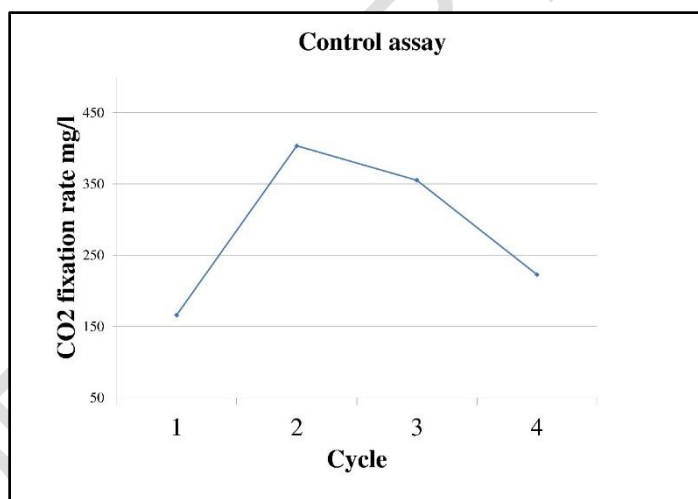


Fig. 13. Rate of CO₂ fixation with Control Assay

3.7 Biomass Recovery and Characterization

Each experiment's total biomass was recovered by centrifugation (Hitachi Himac CR-GIII, Japan) at 15,200g and 20°C for 15 minutes, resuspended in distilled water, and centrifuged again under the same conditions. This procedure was repeated once more to improve nutrient removal. The biomass was then concentrated to 50 mL in a sterile recipient, frozen at 80°C, lyophilized, and stored at 20°C until further analysis.

Table 9. Concentration of Protein and Carbohydrate

Condition	Protein(%ww ⁻¹)	Carbohydrate(%ww ⁻¹)
With NaOH	28.2 ± 4.5 ^a	19 ± 2.4 ^b
Control assay	43.4 ± 2.9 ^a	11.5 ± 1.2 ^b

3.7.1 Protein concentration

The total proteins concentration of the *Spirulina sp.* biomass was evaluated by colorimetric technique [30] from heat and alkaline pretreatment of the microalgal biomass at each growth cycle and at the end of the assays. The protein concentration in the *Spirulina sp.* biomass produced with NaOH was higher than the control assay on 1st and 2nd growth cycles, and equal to protein content until the final cycles without addition of NaOH (5th cycle) table 9. The final protein content (28.2 ± 4.5 % ww⁻¹) in the biomass cultivated using NaOH table 9 was lower than that normally found in control assay (43.4 ± 2.9 % ww⁻¹), as shown in fig. 14.

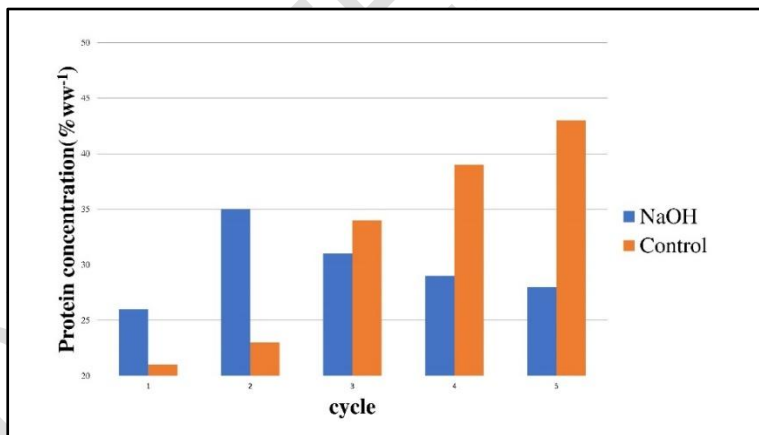


Fig. 14 Comparison between Control Assay and CO₂-absorbed NaOH assay in Protein concentration

3.7.2 Carbohydrate Concentration

The carbohydrate concentration in the microalgae biomass was measured using the Dubois phenol-sulfuric technique [31]. The carbohydrate concentration outline throughout the growth cycles fig. 15 in the assays using NaOH evidenced that *Spirulina sp.* biomass produced with NaOH was higher than that of that control assay. The

final carbohydrate content (19 ± 2.5 % ww^{-1}) in the biomass cultivated using NaOH table 9 was higher than that normally found in control assay (11.5 ± 1.2 % ww^{-1}), as shown in fig. 15.

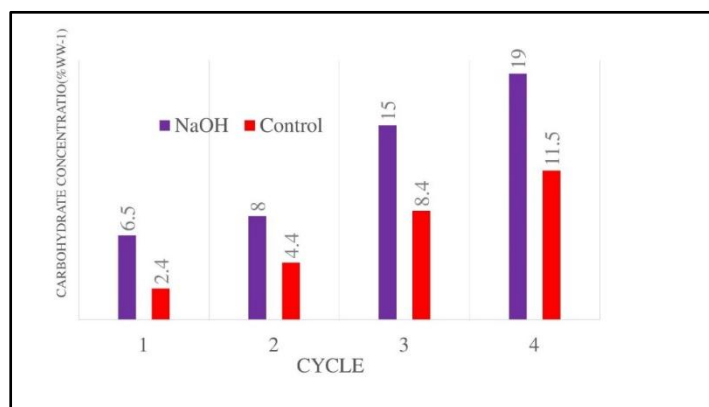


Fig. 15 Comparison between Control Assay and CO_2 -absorbed NaOH assay in Carbohydrate concentration.

4. Discussion

The purpose of this study was to observe how the CO_2 absorbent NaOH influenced *Spirulina* medium culture without the use of NaHCO_3 . We employed a pressurized gas containing a yeast and sugar mixture. This parameter differs between the CO_2 absorbent NaOH assay and the control assay, which utilizes no chemical absorbent. As chemical absorption NaOH has been used, here it affects the growth of *Spirulina* cells. The physiological adaptation of the cells produced by changes in nutritional circumstances, such as medium recycle, might impact the growth period. The results demonstrated that *Spirulina sp.* can only tolerate an initial concentration of 0.5 g/L for CO_2 absorbent *Spirulina sp.* cultivation. The first dose was given on the sixth day, when the concentration was 0.5 g/L. 47g/L (1.64 mmol/L) of absorbent was added to the test. The cell density rose to its peak concentration after the addition of chemical absorbent on the 12th day as shown in fig. 7. The concentration of CO_2 absorbent NaOH, affect the cell growth at a specific concentration. When the concentration of CO_2 -NaOH $>0.94\text{g/L}$ or 1.88 g/L content, however, cell growth rate was significantly reduced, as shown as fig. 7. Due to the shielding property of this organic material, this deposition may have impeded microalgae growth. The growth rates of the two assays were only slightly different, as seen in fig. 10 and fig. 11. Therefore, the mean increase of 2.45% for biomass generated by the assay using control assay (2.09 ± 0.11 g) when compared to the NaOH (2.04 ± 0.25 g) was due to the larger number of growth cycles. The maximum productivity of the control assay is 121 ± 0.2 mg/L, which is greater than the NaOH assay (118 ± 0.3 mg/L). The rates of CO_2 bio-fixation (R_{CO_2}) and CO_2 use efficiency (E_{CO_2}) are critical parameters

used to assess the potential for CO₂ removal. Throughout the experiment, the R_{CO₂} obtained in the control assay was greater than that obtained in the NaOH addition table 7. Therefore, the mean of 35.4% for CO₂ fixation rate increases by control assay 403 mg/L (4.03 g/L) fig. 13, when compared to the NaOH 317 mg/L (3.17 g) was due to the larger number of growth cycles fig. 12. Temperature, medium composition, pH, light intensity, and CO₂ concentration are some of the physicochemical parameters that influence CO₂ fixation. The protein concentration in the *Spirulina sp.* biomass produced with NaOH was higher than the control assay on 1st and 2nd growth cycle, and equal to protein content until the final cycles without the addition of NaOH (5th cycle) table 9. The final protein content ($28.2 \pm 4.5 \%$ ww⁻¹) in the biomass cultivated using NaOH table 9 was lower than that normally found in the control assay ($43.4 \pm 2.9 \%$ ww⁻¹), as shown in fig. 14. The protein content of the biomass grown with MEA was lower than that normally found in *Spirulina sp.* (44.4, 6.9 percent w w⁻¹) and LEB 18 biomass (60.8 4.8%) [32] and the final protein content remained within the range reported by Borges [33]. The carbohydrate concentration outline throughout the growth cycles fig. 15 in the assays using NaOH evidenced that *Spirulina sp.* biomass produced with NaOH was higher than that of that control assay. The final carbohydrate content ($19 \pm 2.5 \%$ ww⁻¹) in the biomass cultivated using NaOH table 9 was higher than that normally found in control assay ($11.5 \pm 1.2 \%$ ww⁻¹). Increased CO₂ levels (above ambient levels) are required for successful microalgal growth and metabolism, and they are currently one of the most expensive aspects of microalgal production. As compared to the control experiment, the chemical absorbent did not show a significant CO₂ fixing rate. Chemical absorbents, which are expensive to provide, are not required to fix CO₂ or produce substantial volumes of biomass. It may be able to fix CO₂ without the need of chemical absorbents, reducing pollution while creating high-nutrient biomass at the same time.

Conclusion

Recent advances in CO₂ fixation and biomass production utilizing microalgae were compiled and analyzed, with an emphasis on how adding the CO₂ absorbent affected the growth kinetics and biomass composition of *Spirulina sp.* in semi-continuous CO₂ addition cultivation. Furthermore, when compared to the chemical absorbent assay, the control assay's high growth and CO₂-fixation rates provide a number of advantages. The CO₂ absorbent NaOH assay and the control assay which utilizes no chemical absorbent. *Spirulina sp.* can only tolerate an initial concentration of 0.5 g/L for CO₂-absorbent *Spirulina sp.* cultivation. The first dose was given on the sixth day when the concentration was 0.5 g/L. 47g/L (1.64 mmol/L) of absorbent was added to the test. The concentration of CO₂-NaOH > 0.94 g/L or

1.88 g/L content, the cell growth rate was significantly reduced. The mean increase of 2.45% for biomass generated by the assay using control assay (2.09 ± 0.11 g) when compared to the NaOH (2.04 ± 0.25 g) was due to the larger number of growth cycles. The maximum productivity of the control assay is 121 ± 0.2 mg/L, which is greater than the NaOH assay (118 ± 0.3) mg/L. The mean of 35.4% for CO₂ fixation rate increases by control assay 403 mg/L (4.03 g/L) when compared to the NaOH 317 mg/L (3.17 g) was due to the larger number of growth cycles. The final protein content (28.2 ± 4.5 % ww⁻¹) in the biomass cultivated using NaOH was lower than that normally found in the control assay (43.4 ± 2.9 % ww⁻¹). The final carbohydrate content (19 ± 2.5 % ww⁻¹) in the biomass cultivated using NaOH was higher than that normally found in the control assay (11.5 ± 1.2 % ww⁻¹). Chemical absorbents which are expensive to provide are not required to fix CO₂ or produce significant amounts of biomass.

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Competing Interests

There is no known financial interest to disclose by the authors.

Authors Contributions

Natasha Nafisa Haque designed the work plan, acquisition data and wrote the original document. Md. Ashraf Alam co-operates to write the manuscript. Chapol Kumar Roy visualization the article. Mst. Elina Akhter Zenat shares the intellectual knowledge for this research. John Liton Munshi supervised the overall work and assisted in writing and editing the manuscript.

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