Asian Journal of Research in Biochemistry

Volume 13, Issue 3, Page 28-43, 2023; Article no.AJRB.105949
ISSN: 2582-0516

Cyanobacteria Mediated CO_2 Segregation: A Promising Alternative Method for Sustainable Bioremediation and Biomass Production

Natasha Nafisa Haque [a], Md. Ashraful Alam [b], Chapol Kumar Roy [a], Mst. Elina Akther Zenat [a] and John Liton Munshi [a*]

[a] BCSIR Laboratories Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.
[b] Institute of Glass and Ceramic Research and Testing (IGCRT), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. Author NNH designed the work plan, acquization data and wrote the original document. Author MAA co-operates to write the manuscript. Author CKR visualization the article. Author MEAZ shares the intellectual knowledge for this research. Author JLM supervised the overall work and assisted in writing and editing the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2023/v13i3257

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105949

Original Research Article

Received: 17/07/2023
Accepted: 21/09/2023
Published: 25/09/2023

**Corresponding author: E-mail: johnliton@bcsir.gov.bd;*

ABSTRACT

Aims: The purpose of this study was to examine the CO_2 absorbent (NaOH) affected *Spirulina sp.* medium culture to verify growth rate, CO_2 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 c 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 CO_2 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 CO_2 fixation and biomass production utilizing microalgae were compiled and analyzed, with an emphasis on how adding the CO_2 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 CO_2-fixation rates provide several advantages**.**

Keywords: Pollution; CO_2 fixation; Spirulina platensis; Zarrouk media; control assay.

1. INTRODUCTION

"Rising CO_2 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" and "regular droughts and desertification of regions" [1]. "Global warming occurs when carbon dioxide (CO_2) 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, (Global Warming 101, NRDC, 2021) traps the heat and causes the earth to get hotter". "These heat-trapping pollutants, especially carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), water vapour, and synthetic fluorinated gases are known as greenhouse gases and their impact is called the greenhouse effect" [2]. "As CO_2 is the primary cause of the greenhouse effect and global warming, it is mandatory to mitigate CO_2 from the earth to save the world. The capacity of microalgae cultures to capture CO_2 has been discovered (Basu S, et al, 2014) in the ongoing research for viable CO_2-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 CO_2 capture" [4]. "Microalgae-based CO_2 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 CO_2 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 CO_2 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 CO_2 fixation efficiency, and produce more high-value products, such as dietary supplements for humans, animals, and aquaculture, than terrestrial plants" [9]. "Temperature, pH, CO_2, light, and inorganic salts, all of which are necessary for maximum biomass, may be

monitored and regulated. CO_2 is fixed and converted to carbon-containing high-value compounds like carbohydrates, lipids, and proteins at the same time" [10]. "Biomass productivity reflects CO_2 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 CO_2 mitigation through microalgae has recently received significant attention among the various CO_2 mitigation strategies due to their higher CO_2 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 CO_2 mitigation. The effect of CO_2 level on microalgae growth varies depending on the strain" [13,14] "Several algal species have been found to fix CO_2 including *Sirulina platensis*, *Scenedesmus obliquus*, *Botryococcus braunii, Chlorella vulgaris and Nannochloropsis oculate"* [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]. "$NaHCO_3$ and $NaNO_3$ 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 CO_2 as a carbon source for the intense synthesis of microalgae biomass has been investigated. *Spirulina* biomass is produced utilizing CO_2 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 CO_2 bio-fixation" [25].

2. MATERIALS 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).

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 macro element: $NaNO_3$, 4.5 g; K_2HPO_4, 0.9 g; $MgSO_4{\cdot}7H_2O$, 0.36 g; K_2SO_4, 1.8 g; $FeSO_4{\cdot}7H_2O$, 0.018 g; EDTA, 1.44 g; $CaCl_2.2H_2O$ 0.072 g; and Table 1 trace metal solution, 1 mL. The 1 L of trace metal solution contains the following: H_3BO_3, 5.148 g; $MnCl_2{\cdot}4H_2O$, 3.258 g; $ZnSO_4{\cdot}7H_2O$, 0.3996 g; $CuSO_4{\cdot}5H_2O$, 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	$MnCl_2.4H_2O$	3.258g
K_2SO_4	1.8g	$ZnSO_4.4H_2O$	0.3996g
NaCl	1.8g	$Na_2MO.O4$	0.03186g
$MgSO_4.7H_2O$	0.36g	$CuSO_4.5\ H_2O$	0.1422g
$FeSO_4.7H_2O$	0.018g		
$CaCl_2.2H_2O$	0.072g		
EDTA	0.144g		
Micronutrient solution added	1.8g		

Fig. 1. (a): Zarrouk medium without $NaHCO_3$ (b) Extracting Supernatant added to the medium after autoclaving

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

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.

2.3 Supply of CO_2 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 ¾ 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 CO_2 that the excess gas bleeds into the line and into the media, feeding the cultivated macroalgae. How long they generate CO_2 is somewhat inconsistent but can range from 1½ to 3 weeks before the system is needed to fresh sugar and yeast.

2.4 CO_2 Absorbent

NaOH is a potential chemical absorbent that is widely used in CO_2-capturing processes. To investigate the influence of NaOH on cell growth and CO_2 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 CO_2, 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.

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 ml $_{CO2}$ $mL^{-1}{}_{medium}$ 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 CO_2. 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 $\mu mol_{photons}$ m^{-2} 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 CO_2 was supplied (36mLCO_2 mL_{medium} 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.

Fig. 3. CO_2 absorbent NaOH (left side) added to specific one flask and the other controls (right side)

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)).

$$CO_{2\,(aq)} \Leftrightarrow H_2CO_3 \overset{k_1}{\Leftrightarrow} HCO_3^- \overset{k_2}{\Leftrightarrow} CO_3^{2-} \quad (1)$$

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.

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

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

Where Δ X denotes the change in biomass concentration (g L^{-1}) during cultivation, Δ t; fC denotes the carbon fraction in biomass; M_{CO_2} and M_C denote the molar mass of CO_2 and carbon atoms, respectively.

2.8.3 Carbon dioxide use efficiency

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

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
pH	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
pH	5.7	6.2	6.5	8.1

Fig. 4. Recovery of biomass by Centrifuge

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 ℃, lyophilized, and stored at 20°C showed in Fig. 4.

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.

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. RESULTS

The goal of this work was to observe how adding CO_2 absorbent NaOH affected the growth kinetics, CO_2 fixation rate and biomass composition of *Spirulina sp.* under semi-continuous cultivation. Experiments were designed to verify growth rate, CO_2 fixation ability, and biomass production from *S. platensis* utilizing the Zarrouk medium, which uses a pressurized CO_2 gas diffuser instead of $NaHCO_3$. This investigated parameter differs between the control assay and the CO_2 absorbent NaOH assay. presents and discusses the effect of CO_2 absorbent on biomass production, specifically their fixation rate in comparison to the control assay.

3.1 Biomass Concentration Versus Concentration of CO_2 Absorbent NaOH

The experiments performed with X_0 = 0.2 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 CO_2 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.

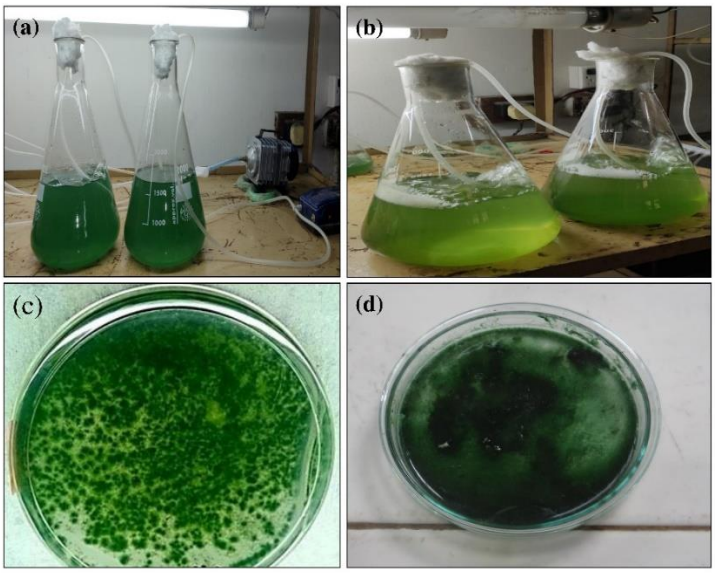

Fig. 5. (a) The culture media in 1st days (b) 12th days (c) 24th days' final product with absorbent assay (d) with control assay

Fig. 5(a), 5(b) show the development of *Spriulina 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 *Spriulina sp.* cell development in the presence of NaOH that has been absorbed by CO_2. 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 *Spriulina 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 *Spriulina sp.* cell development in the presence of NaOH that has been absorbed by CO_2.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 CO_2-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.

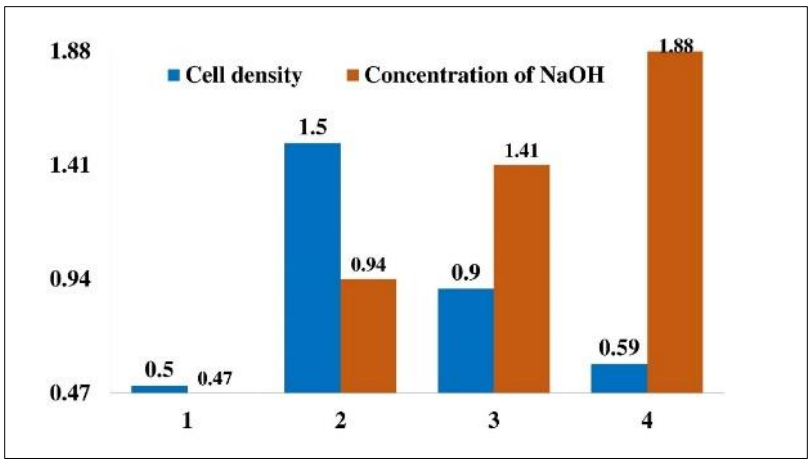

Fig. 6. The effect of CO_2 absorbent (NaOH concentration) on *Spirulina sp.* cell growth (mentioned in figures)

3.2 Control Assay

The control assay in Table 5, demonstrated similar behavior to the assays with CO_2 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 CO_2-absorbed NaOH is depicted in Fig. 8. In the presence of 1.64 – 3.28 mmol/L CO_2-NaOH, cell density reached approximately 1.5 mg/L in 12 days. Cell growth rate, on the other hand, decreased significantly when CO_2-NaOH content was greater than 4.92 to 8.56 mmol/L. These results indicate that CO_2 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 CO_2-NaOH concentrations inhibit algal cell growth. In the case of the control assay Fig. 8, the growth rate did not inhibit as NaOH absorbent.

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

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

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.

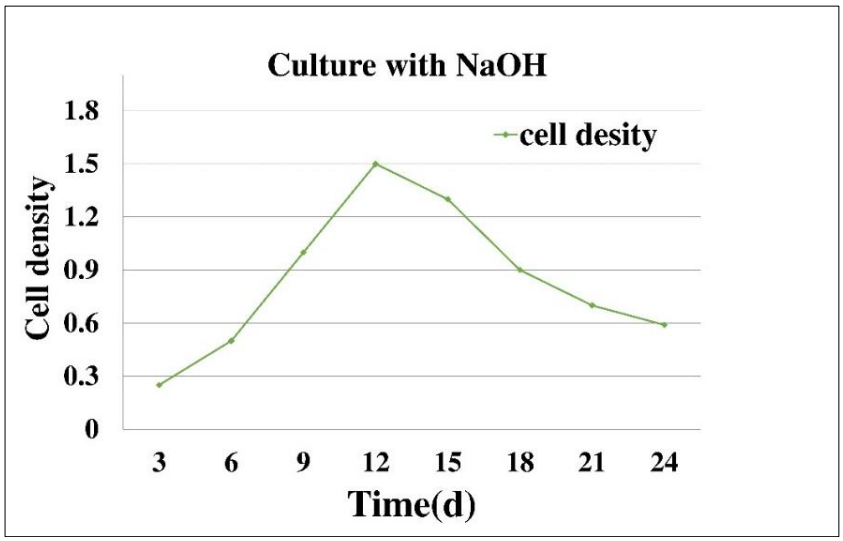

Fig. 7. Concentration of biomass culture with CO_2 absorbent (NaOH)

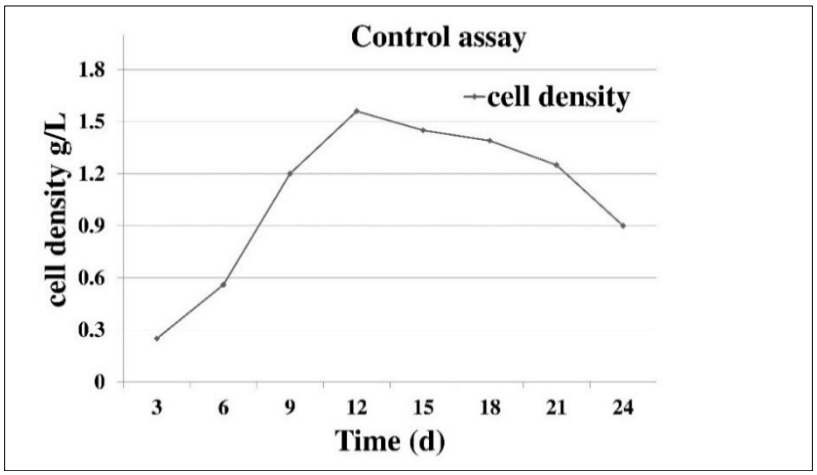

Fig. 8. Concentration of biomass in control assay

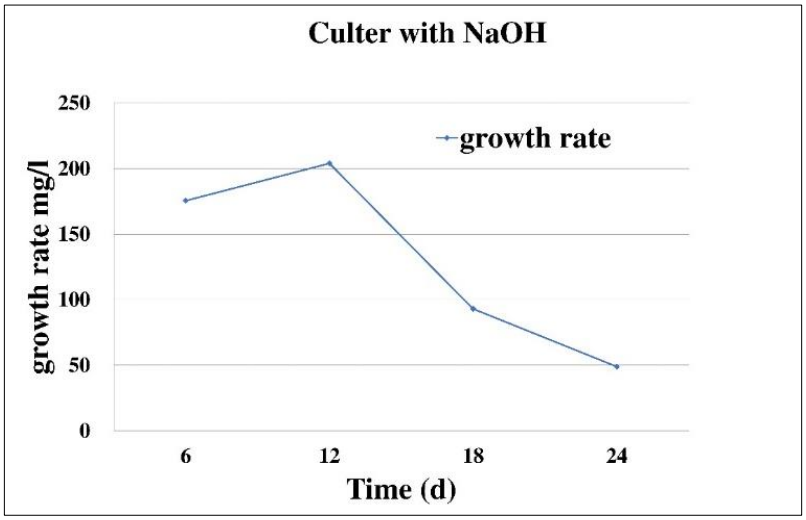

Fig. 9. Growth rate of biomass in culture with NaOH

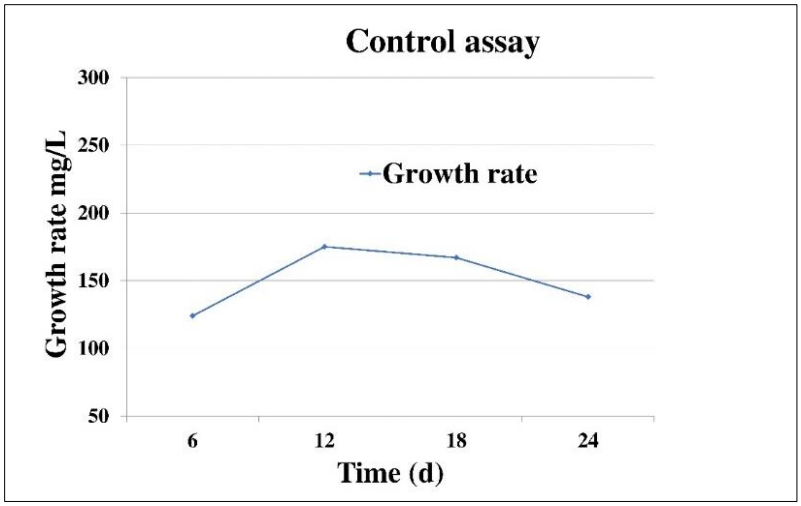

Fig. 10. Growth rate of biomass control assay

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.

3.6 The Rate of CO_2 Fixation

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

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

Where Δ X denotes the change in biomass concentration (g L^{-1}) during cultivation, Δ t denotes the time interval, f_C denotes the carbon fraction in biomass; M_{CO_2} and M_C denotes the molar mass of CO_2 and carbon atoms, respectively.

Table 8. Comparison between NaOH assay and control assay in rate of CO_2 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_{CO2} 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 CO_2 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.

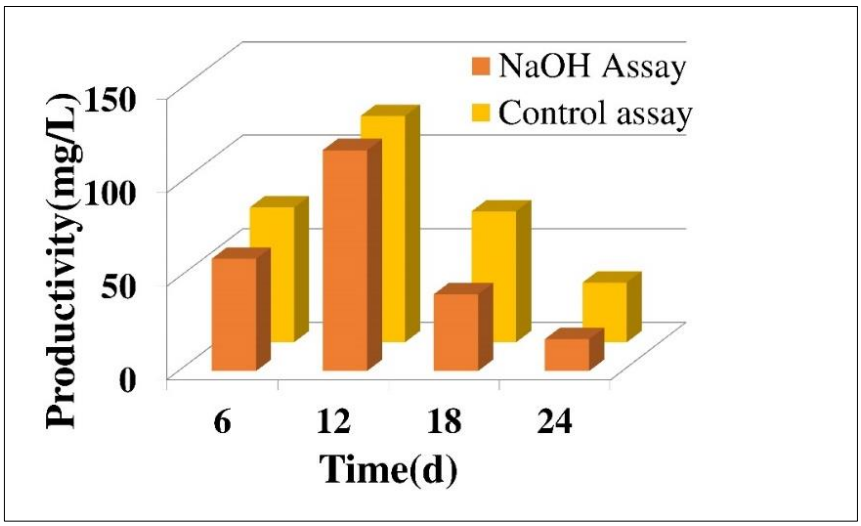

Fig. 11. Volumetric productivity of control assay and absorbent assay

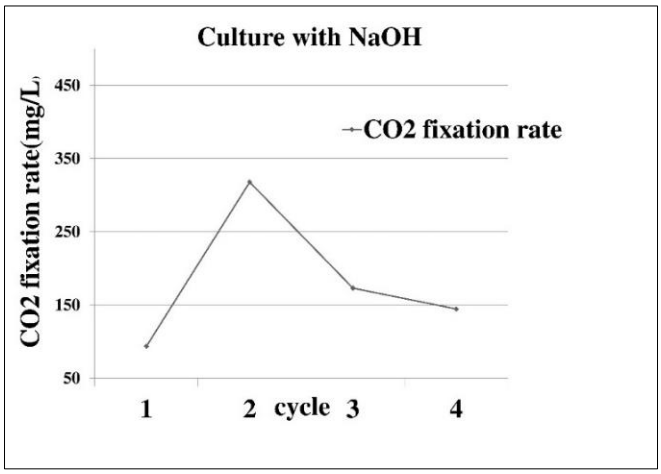

Fig. 12. Rate of CO_2 fixation in culture with NaOH

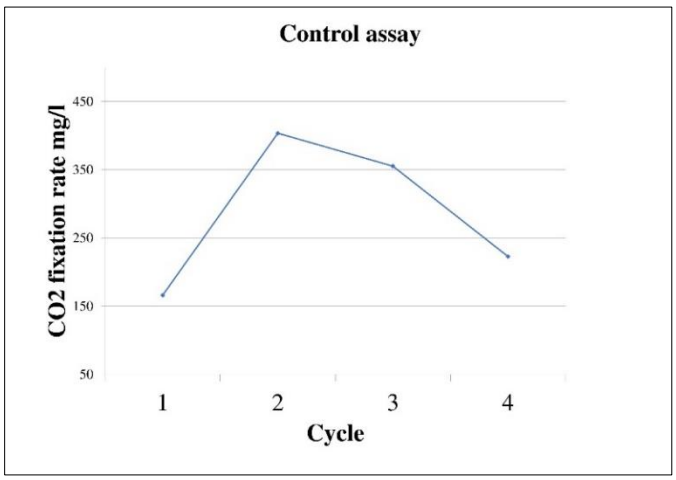

Fig. 13. Rate of CO_2 fixation with control assay

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 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 ℃ until further analysis.

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 that 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.

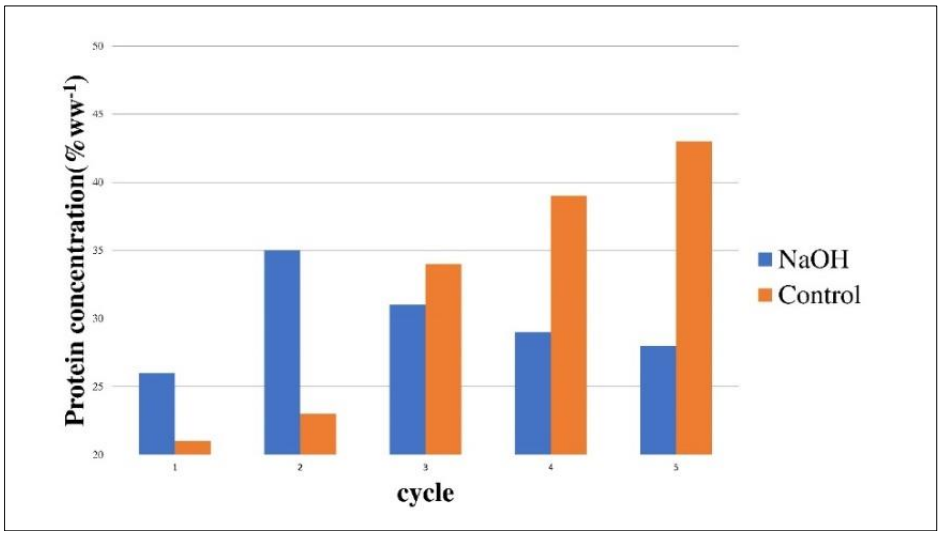

Fig. 14. Comparison between Control Assay and CO_2-absorbed NaOH assay in Protein concentration

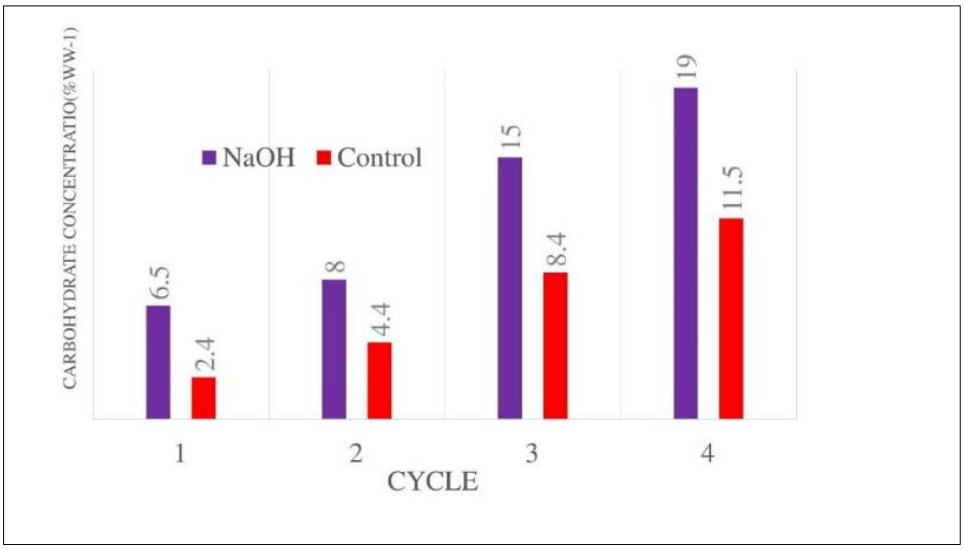

Fig. 15. Comparison between Control Assay and CO_2-absorbed NaOH assay in Carbohydrate concentration

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.

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 >0.94g/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_{CO2}) and CO_2 use efficiency (E_{CO2}) are critical parameters used to assess the potential for CO_2 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 CO_2 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 CO_2 concentration are some of the physicochemical parameters that influence CO_2 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 ± 4.5 % ww^{-1}) in the biomass cultivated using NaOH Table 9 was lower than that normally found in the control assay (43.4 ± 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 ± 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}). Increased CO_2 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 CO_2 fixing rate. Chemical absorbents, which are expensive to provide, are not required to fix CO_2 or produce substantial volumes of biomass. It may be able to fix CO_2 without the need of chemical absorbents, reducing pollution while creating high-nutrient biomass at the same time.

5. CONCLUSION

Recent advances in CO_2 fixation and biomass production utilizing microalgae were compiled and analyzed, with an emphasis on how adding the CO_2 absorbent affected the growth kinetics and biomass composition of *Spirulina sp*. in semi-continuous CO_2 addition cultivation**.** Furthermore, when compared to the chemical absorbent assay, the control assay's high growth and CO_2-fixation rates provide a number of advantages**.** The CO_2 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 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 concentration of CO_2-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 CO_2 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 CO_2 or produce significant amounts of biomass.

ACKNOWLEDGEMENTS

We also like to express our sincere gratitude to the Bangladesh Councill of Scientific and Industrial Research (BCSIR) for financial and technical support to persue the R & D project of *'Bioremediation of heavy metal in industrial waste water by using different species of microalgae and hydrophytes.' Ref. no.-39.02.0000.011.14.134.2021/900, Date 30/12/2021.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lam MK, Lee KT, and Mohamed AR. Current status and challenges on microalgae-based carbon capture. International Journal of Greenhouse Gas Control. 2012;(1);10:456-69.
2. Global Warming 101 - Definition, Facts, Causes and Effects of Global Warming | NRDC. Accessed December 3, 2021. https://www.nrdc.org/stories/global-warming-101.
3. Basu S, Roy AS, Mohanty K, and Ghoshal AK. CO_2 bifixationnd and carbonic anhydrase activity in Scenedesmus obliquus SA1 cultivated in large scale open system. Bioresource technology. 2014;(1);164:323-30.
4. Zhao B, Su Y, Tao W, Li L, and Peng Y. Post-combustion CO_2 capture by aqueous ammonia: A state-of-the-art review. International Journal of Greenhouse Gas Control. 2012;(1);9:355-71.
5. Usui N, and Ikenouchi M. The biological CO_2 fixation and utilization project by RITE(1) — Highly-effective photobioreactor system Energy Convers Manag. 1997;38: S487-S492. DOI:https://doi.org/10.1016/S0196-8904(96)00315-9
6. Ugwu CU, Aoyagi H, and Uchiyama H. Photobioreactors for mass cultivation of algae. Bioresource technology. 2008 Jul 1;99(10):4021-8.
7. Ferreira AF, Ribeiro LA, Batista AP, et al. A biorefinery from Nannochloropsis sp. microalga – Energy and CO2 emission and economic analyses. Bioresour Technol. 2013;138:235-244. DOI:https://doi.org/10.1016/j.biortech.2013.03.168
8. Moreira D, and Pires JCM. Atmospheric CO2 capture by algae: Negative carbon dioxide emission path. Bioresour Technol. 2016;215:371-379. DOI:https://doi.org/10.1016/j.biortech.2016.03.060
9. Wang B, Li Y, Wu N, and Lan CQ. CO2 bio-mitigation using microalgae. Appl Microbiol Biotechnol. 2008;79(5): 707-718. DOI:10.1007/s00253-008-1518-y
10. Danquah MK, Gladman B, Moheimani N, and Forde GM. Microalgal growth characteristics and subsequent influence on dewatering efficiency. Chem Eng J. 2009;151(1):73-78. DOI:https://doi.org/10.1016/j.cej.2009.01.047
11. Ramanan R, Kannan K, Deshkar A, Yadav R, and Chakrabarti T. Enhanced algal CO2 sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini-raceway pond. Bioresour Technol. 2010;101(8):2616-2622. DOI:https://doi.org/10.1016/j.biortech.2009.10.061
12. Yoo C, Jun S-Y, Lee J-Y, Ahn C-Y, and Oh H-M. Selection of microalgae for lipid production under high levels of carbon dioxide. Bioresour Technol. 2010;101(1,Supplement):S71-S74. DOI:https://doi.org/10.1016/j.biortech.2009.03.030
13. Duarte JH, de Morais EG, and Radmann EM, and Costa JAV. Biological CO2 mitigation from coal power plant by Chlorella fusca and Spirulina sp. Bioresour Technol. 2017;234:472-475. DOI:https://doi.org/10.1016/j.biortech.2017.03.066
14. Alam, Md Ashraful, et al. An Investigation on Synthesis of Silver Nanoparticles. Asian Journal of Research in Biochemistry 12.3 (2023): 1-10.
15. Singh S, Reviews PS-R and SE undefined. Effect of CO2 concentration on algal growth: A review. Elsevier; 2014 . Published online 2014. DOI:10.1016/j.rser.2014.05.043
16. Chen C-Y, Kao P-C, Tsai C-J, Lee D-J, and Chang J-S. Engineering strategies for simultaneous enhancement of C-phycocyanin production and CO2 fixation

with Spirulina platensis. Bioresour Technol. 2013;145:307-312.
DOI:https://doi.org/10.1016/j.biortech.2013.01.054

17. Yadav G, and Sen R. Microalgal green refinery concept for biosequestration of carbon-dioxide vis-à-vis wastewater remediation and bioenergy production: Recent technological advances in climate research. J CO2 Util. 2017;17:188-206.
DOI:10.1016/j.jcou.2016.12.006
18. Alam, Md Ashraful, et al. An Investigation on Synthesis of Silver Nanoparticles. Asian Journal of Research in Biochemistry 12.3 (2023): 1-10.
19. Belay A. Spirulina platensis (Arthrospira): production and quality assurance. Gershwin, ME, Belay, A (Eds), Spirulina Hum Nutr Heal Taylor Fr Portland. 2008;2–23.
20. Kuo C-M, Jian J-F, Sun Y-L, et al. An efficient Photobioreactors/Raceway circulating system combined with alkaline-CO2 capturing medium for microalgal cultivation. Bioresour Technol. 2018; 266:398-406.
DOI:https://doi.org/10.1016/j.biortech.2018.06.090
21. Alam, Ashraful, et al. The effect of precursor concentration on the crystallinity synchronization of synthesized copper nanoparticles. Journal of Crystal Growth. 2023;127386.
22. Morais MG, Radmann EM, Andrade MR, Teixeira GG, and Brusch LR, Costa JA. Pilot scale semicontinuous production of Spirulina biomass in southern Brazil. Aquaculture. 2009;294(1-2):60-4.
23. Alam, Md Ashraful, et al. One-pot low-temperature synthesis of high crystalline cu nanoparticles. Malaysian Journal of Science and Advanced Technology. 2023;122-127.
24. Binaghi L, Del Borghi A, and Lodi A, Converti A, Del Borghi M. Batch and fed-batch uptake of carbon dioxide by Spirulina platensis. Process Biochem. 2003;38(9): 1341-1346.
DOI:https://doi.org/10.1016/S0032-9592(03)00003-7
25. Jones, Caroline A., Natalie Bell, and Selby Nimrod, eds. Symbionts: Contemporary Artists and the Biosphere. MIT Press; 2022.
26. da Rosa, Gabriel Martins, et al. "Chemical absorption and CO2 biofixation via the cultivation of Spirulina in semicontinuous mode with nutrient recycle. Bioresource Technology. 2015;192:321-327.
27. Lowry, OliverH, et al. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265-275.
28. DuBois, Michel, et al. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;28(3):350-356.
29. Tsuzuki, Mikio, et al. Effects of CO2 concentration during growth on fatty acid composition in microalgae. Plant Physiology. 1990;93(3):851-856.
30. Classics Lowry O et al. Protein measurement with the Folin phenol reagent. J biol Chem. 1951;193(1);265-75.
31. DuBois Michel, et al. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;28(3):350-356.
32. Authors/Task Force Members, et al. ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC) Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Ep Europace. 2015;17(11):1601-1687.
33. Monegal ER, Rosset FM, Borges JL, Borges JL. Borges. Seuil; 1970.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105949