Original Research Article

Microalgae Mediated CO_2 Sequestration:A Potential Candidate for Sustainable Pollution Mitigation and Biomass Production

ABSTRACT

Introduction: Pollution control and mitigation are essential for the ecosystem's protection as well as making everyone's life safer and healthier. To eliminate pollutants, several pollution mitigation tactics and measures are used that generally include physical, chemical and biological methods. Biological approaches have been found to be more long-term, effectiveand environmentally friendly than the other two. To eliminate pollutants,technologies mostly rely on microbes such as bacteria, fungi, algae, and plants as well as their products such as enzymes and metabolic products. Algae can be cultivated utilizing basic components like CO_2, sunlightand media because of their unique photosynthetic ability and low growth needs making them a possible pollution mitigator. Chemical carbon dioxide (CO_2) absorption is a method of lessening the greenhouse impact.

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 abilityand biomass production from *S. platensis* using the Zarrouk medium and a pressured CO_2 gas diffuser instead of $NaHCO_3$ (as carbon source).

Methodology: The physical and chemical absorbent methods showed a less significant CO_2 fixing rate when compared to the control experiment. Expensive chemical absorbents are not required to fix CO_2 or produce significant amounts of biomass. It may be able to fix CO_2 without chemical absorbents, reducing pollution while producing high-nutrient biomass.

Results: The parameter differs between 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. When the concentration of CO_2-NaOH > 0.94 g/L or 1.88 g/L

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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) compared to the NaOH 317 mg/L (3.17 g) 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}).

Conclusion: Recent advances in CO_2 fixation and biomass production utilizing microalgae were compiled and analyzedwith an emphasis on how adding the CO_2 absorbent affected *Spirulina sp.* growth kinetics and biomass composition in semi-continuous CO_2 addition cultivation**.**When compared to the chemical absorbent assay, the control assay's high growth and CO_2-fixation rates provide a number of advantages**.**

Keywords: Pollution, CO_2 fixation, *Spirulinaplatensis*, 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 burning off 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. 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 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 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

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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 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 *Sirulinaplatensis*, *Scenedesmusobliquus, Botryococcusbraunii,Chlorella vulgaris and Nannochloropsisoculate*[15,16].*Spirulinasp.* 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. Material and Methods

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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 $NaHCO_3$(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; $MgSO_4{\cdot}7H_2O$, 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(b) 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 1Zarrouk medium for 1.8 L solution without $NaHCO_3$

Chemical for Macronutrients (a)	Amount (g/L)	Chemical forMicronutrients (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		

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 unitsthat areshown in fig. 2.

Fig. 2. Experimental 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 CO_2 to the Medium

1Package of Dry Baker's Yeast per bottle, 2 Cups of Sugar per bottle, 1 Teaspoon of Baking Soda per bottle. Our first step is to activate the yeast by placing it and a tablespoon of sugar in a ½ cup of warm tap water (room temperature). Mix thoroughly until the water is slightly bubbly and wait 10 minutes. The yeast will awaken and begin feeding on the available sugar. While waiting for the yeast to activate, add enough warm water to fill the soda bottles ¾ of the way. Add the rest of the sugar and baking soda into each bottle and shake 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 will continue to feed and divide. As a byproduct they create enough CO_2 that the excess gas will bleed into the line and into the media, feeding the cultivated macro-algal. How long they generate CO_2 is somewhat inconsistent but can range from 1½ to 3 weeks before the system needs 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), and recovering the pellet were used to achieve this (10 percent v v^{-1}), as shown in fig. 3.

Fig. **3.** CO_2 absorbent NaOH added to specific one flask and the other controls.

During the light period, the cell pellet was re-suspended in Zarrouk medium without a carbon source and exposed to the new carbon source (pressurized gas containing a yeast and sugar mixture or CO_2 diffuser) 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.

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 ($36mLCO_2mL_{medium}\ d^{-1}$).

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.

2.7.2. Alkalinity, pH and concentration of dissolved inorganic carbon

Medium alkalinity was determined by potentiometric titration and pH bypH 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. 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 search of weak acids.For alkalinity titration, here used a simple glassware burette of capacity of 50 ml along with the burette stands,***a*** conical flask of capacity of 250 mL**,** ***a*** measuring cylinder of 100ml, a funnel beaker and two indicator reagents with**,** H_2SO_4 Solution, methyl orange indicator**,** phenolphthalein indicator illustrate in fig. 4.

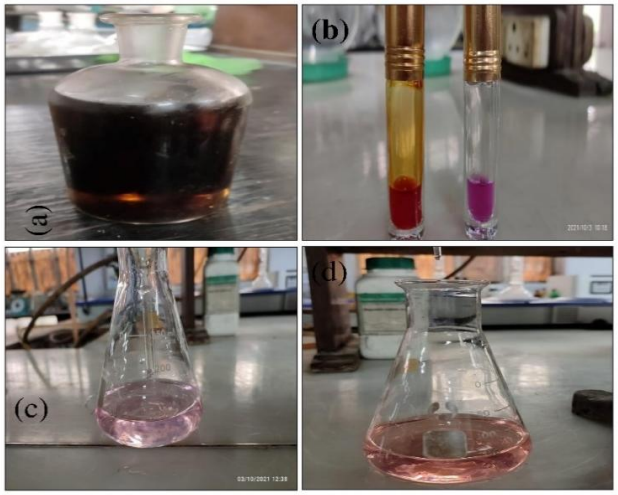

Fig. 4.(a) H_2SO_4 solution (b) Indicator (c) Solution with phenolphthalein indicator (d) Solution with methyl orange indicator

First, fill the 50 ml burette with strong acid H_2SO_4 solution. As a matter of good practice took a few drops out so as to remove air bubbles.then measured 100 ml of the sample. Then note down the initial reading add 1-2 drops of phenolphthalein indicator and titrate it with the H_2SO_4 solution noted 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

Table3 Identification and quantification of soluble ions

		Flask 01		
Ions Measurement	Cycle 01	Cycle 02	Cycle 03	Cycle 04
$\boldsymbol{CO_3^{2-}}$	14	10	6.6	3.6
$\boldsymbol{HCO_3^-}$	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 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_2fixation 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:

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

Where ΔX denotes the change in biomass concentration (g L^{-1}) during cultivation, Δt; fC denotes the carbonfraction 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 × 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}).

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. 5.

Fig.5. 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 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[26]

3. Result

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.

Table4 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 2^{nd} cycle, after the addition of chemical absorbent, the cell density reached its peak concentration, as shown in table 4.

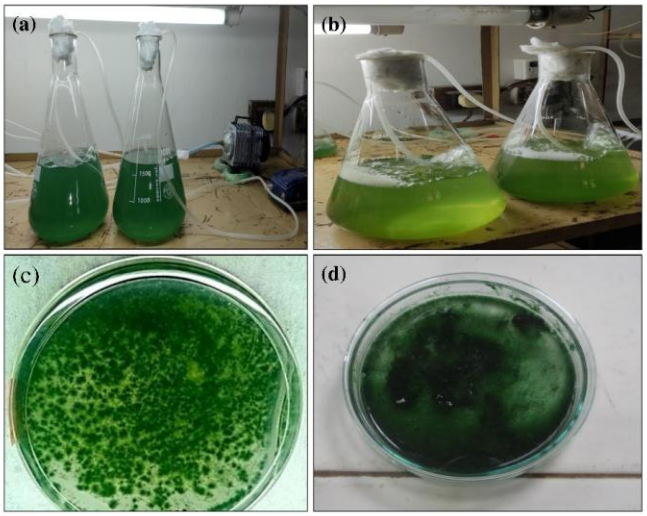

Fig. 6.(a) The culture media in 1^{st} days (b) 12^{th} days (c) 24^{th} days' final product with absorbent assay (d) with control assay

Fig.6(a), 6(b) shows the development of *Spriulina sp.* cells in the presence and absence of NaOH absorbent. Fig.7(c) and(d) show the final biomass product in the presence of NaOH and in the under-control assay.The fig. 8depicts *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(a) and (b) shows the development of *Spriulina sp.* cells in the presence and absence of NaOH absorbent. Fig. 7 (c)and (d)

shows the final biomass product in the presence of NaOH and in the under-control assay.The fig. 8 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[27]. The physiological adaptation of the cells produced by changes in nutritional circumstances, such as medium recycle, might impact the growth period.

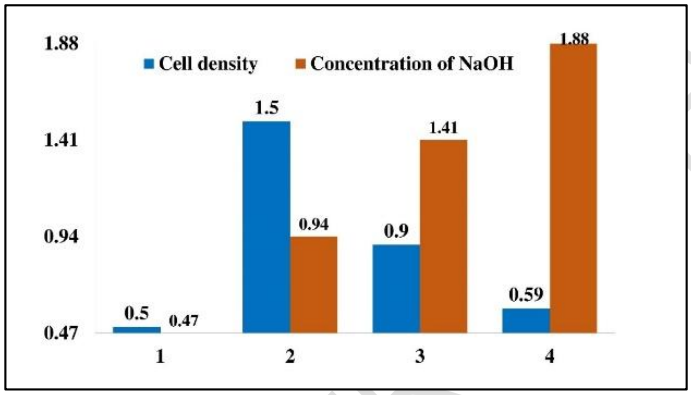

Fig.7.The effect of CO_2 absorbent (NaOH concentration) on *Spirulina sp.* cell growth

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.

Table5.*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 BetweenNaOH 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.9, the growth rate did not inhibit as NaOH absorbent.

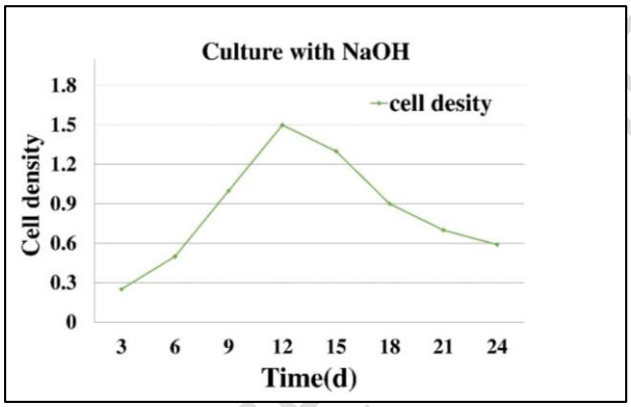

Fig. 8 Concentration of biomass culture with CO_2 absorbent (NaOH)

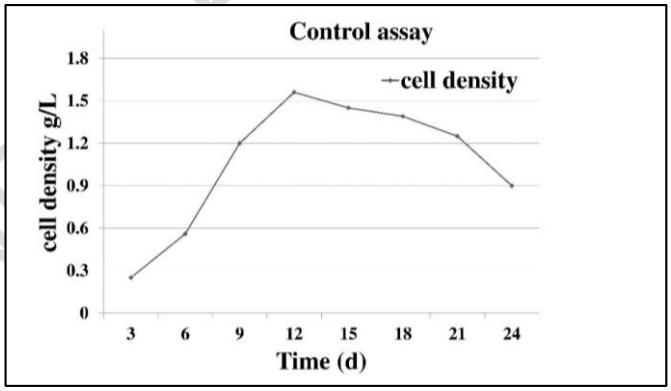

Fig. 9: Concentration of biomass in control assay

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

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

Table6 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.

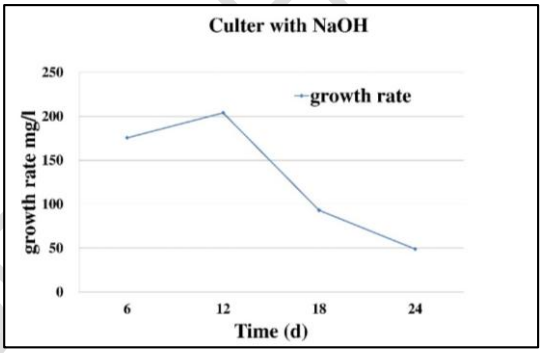

Fig. 10: Growth rate of biomass in culture with NaOH

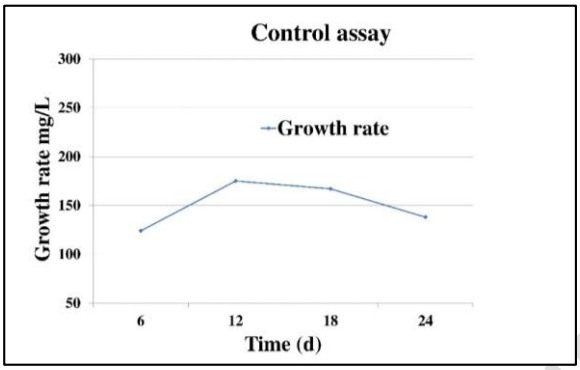

Fig. 11: Growth rate of biomass Control assay

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.

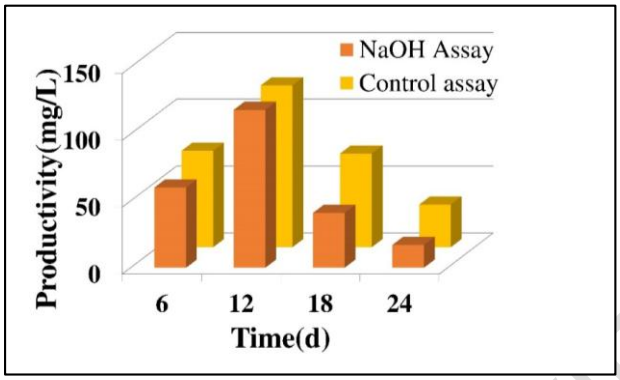

Fig. 12 Volumetric productivity of control assay and absorbent assay

3.6 The Rate of CO_2Fixation

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_2fixation rate (R_{CO_2} as g-CO_2L^{-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:

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

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 CO_2 and carbon atoms, respectively.

Table8. 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.14, when compared to the NaOH 317 mg/L (3.17 g/L) was due to the larger number of growth cycles fig.13.

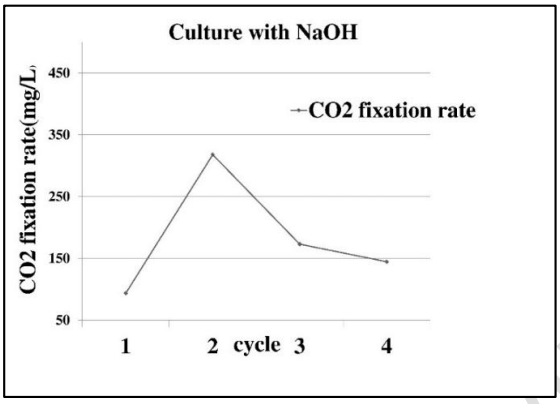

Fig.13: Rate of CO_2fixation in culture with NaOH

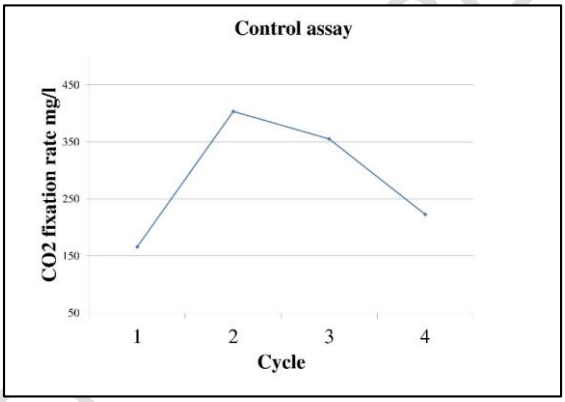

Fig. 14. Rate of CO_2fixation with Control Assay

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℃ until further analysis.

Table9. 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 [28] 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 NaOHtable 9was lower than that normally found in control assay (43.4 ± 2.9 % ww^{-1}), as shown in fig. 15.

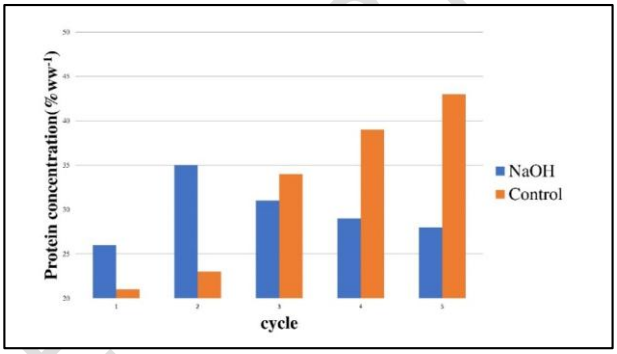

Fig.15 Comparison between Control Assay and CO_2-absorbed NaOH assay in Protein concentration

3.7.2 Carbohydrate Concentration

The carbohydrate concentration in the microalgae biomass was measured using the Dubois phenol–sulfuric technique [29]. The carbohydrate concentration outline throughout the growth cycles fig. 16in 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 NaOHtable 9 was higher than that normally found in control assay (11.5 ± 1.2 % ww^{-1}), as shown in fig. 16.

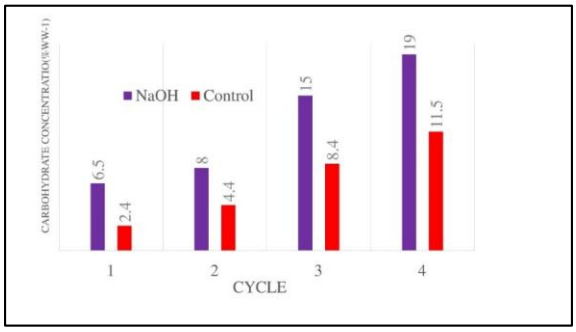

Fig.16 Comparison between Control Assay and CO_2-absorbed NaOH assay in Carbohydrate concentration.

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.8. 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. 8. 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. 11 and fig. 12. 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 8. Therefore, the mean of 35.4% for CO_2 fixation rate

increases by control assay 403 mg/L(4.03 g/L)fig.14, when compared to the NaOH 317 mg/L(3.17 g) was due to the larger number of growth cycles fig.13. 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) table9. The final protein content (28.2 ± 4.5 % ww^{-1}) in the biomass cultivated using NaOHtable 9 was lower than that normally found in the control assay (43.4 ± 2.9 % ww^{-1}), as shown in fig. 15. The protein content of the biomass grown with MEA was lower than that normally found in *Spirulinasp* (44.4, 6.9 percent w w^{-1}) and LEB 18 biomass (60.8 4.8%)[30]and the final protein content remained within the range reported by Borges[31].The carbohydrate concentration outline throughout the growth cycles fig. 16 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 NaOHtable 9was 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.

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 provideare not required to fix CO_2 or produce significant amounts of biomass.

Comment [H7]: After conclusion acknowlegement section is needed.

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