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Evaluation of *Spirulina platensis* in Bicarbonate-Based Integrated Carbon Capture and Algae Production System Utilizing Different Culture Media

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A method known as Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS), is a growing study introduced as an alternative to current carbon capture and sequestration (CCS) methods. It is a closed-loop cycle involving inorganic carbon in the form of bicarbonates, which is consumed by microalgae for growth and utilizes the regenerated carbonates for another cycle of carbon capture. Existing literature requires more in-depth experimentation and analysis with regards to the viability of different microorganisms to the rising method. *Spirulina platensis* was evaluated in BICCAPS using 0.1M Na_2CO_3, employing three different culture media for growth, namely, modified Zarrouk's, NPK- based, and NPK- based with A_5 solution media. Biomass growth, productivity, and carbon dioxide utilization were investigated to determine the effectivity of BICCAPS as a carbon sequestration technique. At control conditions, NPK-based with A_5 solution medium yielded the highest productivity with a value of 10.81 mg L^{-1} day^{-1}. Likewise, using $NaHCO_3$ as a carbon source, results show that the highest productivity was achieved also under NPK- based with A_5 solution medium with a value of 6.80 mg L^{-1} day^{-1}, as well as a high carbon conversion value of 2.092 day^{-1}.

Keywords: BICCAPS, Carbon Capture, Modified Zarrouk's Medium, NPK-based Medium, Sodium Bicarbonate, *Spirulina platensis*

INTRODUCTION

Combustion or burning of fossil fuels has become a well-known and accepted method in energy production (Sayre 2010). Actuated by the demands during the industrial revolution and with the current rapid economic growth, CO_2 content in the atmosphere has drastically increased, resulting to global warming (Leung, Caramanna, & Maroto-Valer, 2014). According to the International Energy Agency (IEA), in 2018 alone, atmospheric CO_2 drastically increased by

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1.7% at a peak value of 33.1 gigatonnes or 407.4 ppm. IEA further assessed that from the 1˚C increase in global average surface temperature, 0.3˚C of which is caused solely by production of the fossil fuels. As a result, coal has been identified as the most significant lone contributor to temperature rise (IEA 2019).

Carbon Capture and Sequestration (CCS) is a method addressing global warming through CO_2 reduction by capturing, transporting, and storing CO_2 from industrial or utility plants in deep aquifer formations (CCSA 2011, Herzog and Golomb 2004, MIT 2004). However, current carbon capture methods were found disadvantageous since it generates other gaseous wastes, and requires high cost from chosen capture method and transportation (Chi et al. 2013a).

Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS) is a newly- proposed carbon capture method. This method effectively satisfies the need to capture CO_2, has lower costs from sustainable energy, provides large amounts of biomass as feedstock to valuable products. This technology applies the closed-loop process, involving the formation of bicarbonate from carbon dioxide capture using carbonate, microalgae's utilization of bicarbonate as carbon source, and regeneration of carbonate from bicarbonate's consumption (Chi et al. 2013b).

The microalgae must be able to withstand high pH values and encompass high carbon fixation rates to survive BICCAPS; additionally, this considers and prefers high biomass productivities in selecting microalgal candidates (Chi et al., 2013a). Singh and Singh (2014) compares microorganisms in terms of CO_2 fixation rate as well as biomass productivities upon loading a certain $\%CO_2$; the best results were identified with: *Synechocystis aquatilis, Botryococcus braunii, Chlorella vulgaris, Spirulina platensis,* and *Dunaliella tertiolecta*. Considering only alkaliphilic microalgae, only *Spirulina platensis and Dunaliella teriolecta* were qualified candidates. Although *Chlorella vulgaris* is a freshwater microalga, it is known to survive harsh conditions. In this regard, present literature indicates that BICCAPS has been applied to *Chlorella vulgaris* (Mokashi et al. 2016) and *Dunaliella salina* (Kim et al. 2017).

Spirulina platensis was selected for the study, considering the availability of certain microalgae in tropical countries like the Philippines, as well as the scarcity of literature present.

Previous studies have proven the ability of *S. platensis* to grow with CO_2 or HCO_3^- as a carbon source under various media. Sydney et al. (2010) conducted a study wherein air, with 5% CO_2 sparged through *S. platensis* under Zarrouk's medium, produced maximum cell productivity of 0.73 g L^{-1} day^{-1}. Likewise, Kumari, Kumar, Pathak, & Guria (2014) utilized a cost-effective NPK-based medium, generating maximum cell productivity of 0.42 g L^{-1} day^{-1}.

This study evaluates the viability of *Spirulina platensis* in BICCAPS for CO_2 mitigation and utilization. Specifically, it determines and differentiates the growth curve, biomass productivity, maximum specific growth rate, and inorganic carbon

conversion of *Spirulina platensis* under Modified Zarrouk's, NPK-based fertilizer, and NPK with added A_5 solution culture media at controlled CO_2 concentration and under BICCAPS utilizing $NaHCO_3$ as carbon source.

METHODOLOGY

Culture Media

Spirulina platensis was obtained from the Southeast Asian Fisheries Development Center (Manila, Philippines) and grown under three different culture media: NPK-based, NPK- based with A_5 solution, and Modified Zarrouk's culture media – in separate containers. Modification of the original Zarrouk's formula with regards to the removal of the $NaHCO_3$ was done to investigate the effect of $NaHCO_3$ in BICCAPS.

The Modified Zarrouk's culture medium consisted of 2.5 g-L^{-1} $NaNO_3$, 0.5 g-L^{-1} K_2HPO_4, 1 g-L^{-1} K_2SO_4, 1.0 g-L^{-1} NaCl, 0.04 g-L^{-1} $CaCl_2$•$2H_2O$, 0.01 g-L^{-1} $FeSO_4$•$7H_2O$, and 1 mL of A_5 solution. The A_5 trace elements solution is composed of 2.86 g-L^{-1} H_3BO_3, 1.81 g-L^{-1} $MnCl_2$ • $4H_2O$, 0.222 0.01 g-L^{-1} $ZnSO_4$ • $4H_2O$, 0.018 0.01 g-L^{-1} Na_2MoO_4, and 0.079 0.01 g-L^{-1} $CuSO_4$ • $5H_2O$, which was based on the formulation provided by Zarrouk (1966).

NPK-based culture medium, on the other hand, was composed of 0.076 g-L^{-1} NPK, 1 g-L^{-1} NaCl, 0.2 g-L^{-1} $MgSO_4$, 0.08 g-L^{-1} EDTA, 0.04 g-L^{-1} $CaCl_2$, 0.01 g-L^{-1} $FeSO_4$, 0.0207 g-L^{-1} urea, and 0.0469 g-L^{-1} silica, which was based on the formulation provided by Kumari, Kumar, et al. (2014), chemicals and reagents were obtained from Belman Laboratories (Philippines) and Yana Commodities (Philippines).

Experiment

Cultivation with Continuous Aeration

All experimental runs for the cultivation of the microalga involved a working volume of 8L using PET bottles at a pH of approximately 9, in 12-hour light-12-hour dark cycle with the use of a tubular LED lamp at ambient temperature. Air pumps attached with a sparger were used to continuously introduce CO_2 from air at an average volume rate of 0.8 L min^{-1}. Samples for dry weight and optical density were taken thrice a week until a stationary phase was achieved. Calibration curves were generated by relating optical density with dry weight values. Biomass productivity, maximum specific growth rate, and pH for each culture media were also evaluated.

Carbonate Dissolution and Carbon Dioxide Absorption

Under the closed system, water was reacted with CO_2 at a loading of 1 L min^{-1} to form carbonic acid solution as seen in Eq. (1). The solution then dissociates to form bicarbonates as displayed in the reaction in Eq. (2).

$$\mathrm{CO_{2(g)} + H_2O \rightarrow H_2CO_{3(aq)}} \quad (1)$$

$$H_2CO_{3(aq)} \rightleftarrows H^+ + HCO_3^- \quad (2)$$

Sodium carbonate dissolution was performed, producing 0.1M Na_2CO_3 solution; except for the control system where no carbonate was introduced. This solution was subjected to CO_2 bubbling to produce sodium bicarbonate solution as can be seen in Eq. (3).

$$H_2CO_{3(aq)} + NaCO_{3(s)} \rightarrow Na^+_{2(aq)} + 2HCO_{3(aq)} \quad (3)$$

Cultivation in BICCAPS

Resulting bicarbonate solutions were utilized in a closed system cultivation of *Spirulina platensis*. The microalgal specie was cultivated under modified Zarrouk's, NPK-based, and NPK with A_5 solution culture medium in tightly sealed PET bottles at a working volume of 5L for approximately 30 days or until the stationary phase was observed. During cultivation period, samples were obtained thrice a week to evaluate its optical density, carbonate, bicarbonate, and dissolved CO_2 content. Nomenclature list of these systems are listed in Table 1.

Table 1. Nomenclature list for the cultivation of *Spirulina platensis*

Culture Media	Carbon Source	Nomen-clature
Modified Zarrouk's	Air	ZO
	CO_2(g)	ZC
	Na_2CO_3 bubbled with CO_2(g)	ZS
NPK-Based	Air	NO
	CO_2(g)	NC
	Na_2CO_3 bubbled with CO_2(g)	NS
NPK with A5	Air	AO
	CO_2(g)	AC
	Na_2CO_3 bubbled with CO_2(g)	AS

Analytical Method

Cell Growth Measurement

Biomass was obtained by applying vacuum filtration. As seen in Eq. (4), biomass productivity is obtained from the difference between final and initial concentrations found in the exponential phase. To evaluate the maximum specific growth rate of the *S. platensis* in the culture media, Eq. (5) is used, which displays the linearized relationship of biomass concentration against time.

$$Biomass\ productivity\ = \frac{X - X_0}{t} \quad (4)$$

$$ln(X)\ = \mu_{max} t\ +\ ln(X_o) \quad (5)$$

Optical Density

The optical density was measured at a wavelength of 530 nm using a Shimadzu UV-1700 spectrophotometer (Japan) in the duplicate analysis. The Beer-Lambert's Law is applied, as seen in Eq. (6).

$$A = log_{10}\ \frac{1}{T} = log_{10}\ \left(\frac{I_0}{I}\right) A = \varepsilon \mathrm{cl} \quad (6)$$

Carbonate and Bicarbonate Concentration

Carbonate and bicarbonate concentrations were regularly evaluated using a double titration technique for a 5-mL filtered sample. The amount of the carbonate can be determined by titrating the sample with 0.1 M HCl using phenolphthalein indicator. A second titration was performed using a bromocresol green indicator with the same acid to determine the amount of bicarbonate in the solution. The following reactions, as presented in Eq. (7) and (8) were observed until the endpoint for each titration analysis was achieved.

$$\mathrm{CO_{3(aq)}^{2-}} + \mathrm{H^+} \rightarrow \mathrm{HCO_{3(aq)}^-} \quad (7)$$

$$\mathrm{HCO_{3(aq)}^-} + \mathrm{H^+} \rightarrow \mathrm{H_2CO_{3(aq)}} \quad (8)$$

Dissolved CO_2 Concentration

A 5-mL filtered sample was added with an excess volume of 0.1 M NaOH. This

reaction allows the conversion of dissolved carbon dioxide into sodium carbonate, as seen in Eq. (9). Excess 1 M $BaCl_2$ sample was added to precipitate carbonates found in the solution, forming a white-opaque solution as expressed in Eq. (10). The unreacted NaOH is back titrated with 0.1M HCl solution to a phenolphthalein endpoint, as shown in Eq. (11).

$$CO_2 + 2NaOH \rightarrow Na_2CO_3 + H_2O \quad (9)$$

$$Na_2CO_3 + BaCl_2 \rightarrow 2NaCl + BaCO_3 \quad (10)$$

$$NaHCO_3 + NaOH \rightarrow 2NaCl + H_2O \quad (11)$$

It can be determined that the amount of NaOH reacted in the solution is the total amount of CO_2 and bicarbonate. Hence, to calculate for dissolved CO_2 content ($n_{dissolved\ CO2}$) in the sample, moles of bicarbonate (n_{HCO3^-}) reacted is subtracted from moles of CO_2 (n_{CO2}) that reacted with NaOH. Eq. (12) is applied to solve for moles dissolved CO_2.

$$n_{\text{dissolved } CO_2} = \frac{n_{CO_2}\ \text{reacted}}{2} - \frac{n_{HCO_3^-}\ \text{reacted}}{2} \quad (12)$$

Inorganic Carbon Conversion

The amount of CO_2 converted into biomass per volume of culture per day is termed as inorganic carbon (C_i) conversion, as shown in Eq. (13). In BICCAPS, total amount of CO_2 utilized includes the quantity of carbonate produced after cultivation, as well as quantity of dissolved CO_2 consumed in the culture medium, as seen in Eq. (14).

$$\text{Ci conversion} = \frac{\Delta Ci}{\text{culture time}} \quad (13)$$

$$\Delta Ci = [CO_3^{2-}]_{prod} + [d.CO_2]_{consumed} \quad (14)$$

RESULTS AND DISCUSSION

Spirulina Platensis Cultivation

Cultivation of microalgae *Spirulina platensis* was regularly analyzed and monitored to maintain proper operating conditions. The average of the calibration curves generated for each culture media is found in the supplementary information, with linearity values of 0.7817, 0.8441, and 0.8935 for Modified Zarrouk's, NPK-based, and NPK with A_5 solution, respectively. The resulting linear equation from each calibration curve was used to calculate biomass concentration from absorbance value for BICCAPS.

Figure 1 displays the successful growth of *S. platensis* under modified Zarrouk's media, NPK-based, and NPK-based with A_5 solution media. This is further characterized using a maximum specific growth rate (μ_{max}) by relating the natural logarithm of dry weight with time.

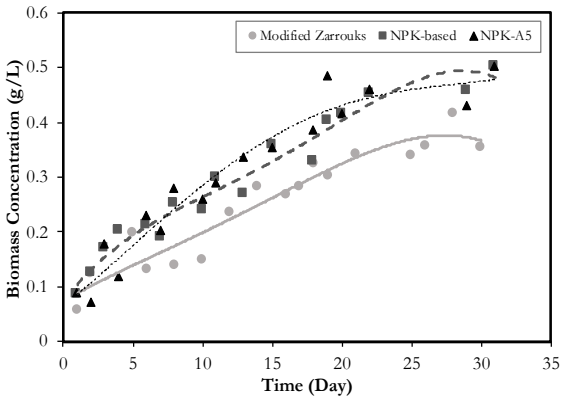


Fig. 1 Growth Curve of *Spirulina Platensis* in Modified Zarrouk's, NPK-based, and NPK-A_5

A summary of obtained biomass productivities, as well as maximum specific growth rate values, are tabulated in Table 2.

Table 2. Summary of Values Obtained Under Open System

Parameter	ZO	NO	AO
Biomass productivity	11.19	11.00	22.08
μmax	0.0469	0.0400	0.0875

From this, it can be seen that *S. Platensis* cultivated in NPK with A_5 obtained the highest biomass productivity, followed by Modified Zarrouk's and closely succeeded by NPK-based. ZO system was expected to embody the highest biomass productivity since Zarrouk's medium was formulated specifically for optimal *Spirulina platensis* growth. However, due to the removal of $NaHCO_3$, Na^+ was suggested to be insufficient for ideal biomass growth, as supported by studies of Kumari et al. (2014). Also, it has been previously established that NPK medium produces relatively lower productivities as compared to Modified Zarrouk's; however, with the addition of A_5 micronutrients, the biomass productivity of AO was seen to have doubled as compared to that of NO. Upon subjecting this to one- way ANOVA, the effect of varying culture media with biomass productivity under an open system is significant ($p<0.05$).

Carbon Dioxide Absorption of Control System and Sodium Carbonate Salt System

The behavior of CO_3^{-2}, HCO_3^-, and d. CO_2 concentrations were observed under the control system and illustrated in Figure 2a. Bicarbonate concentration increased to a maximum concentration of 5.69 mM at the 5th minute and continued to decrease after producing a new equilibrium system. Dissolved CO_2 concentration, however, increased and peaked at the 15th minute with a concentration of 9.70 mM.

For the sodium carbonate solution, CO_2 absorption was successful as the number of bicarbonates increased twice as much like that of carbonates after 150 minutes. It can be seen from figure 2b, with the initial concentration of carbonate 280.05 mM and the final concentration of bicarbonate 335.88 mM, the CO_2 absorption lead to a yield of 74.61%. Furthermore, a CO_2 absorption ratio of 1.4496 was obtained, corresponding to moles of CO_2 absorbed per mole of carbonate consumed.

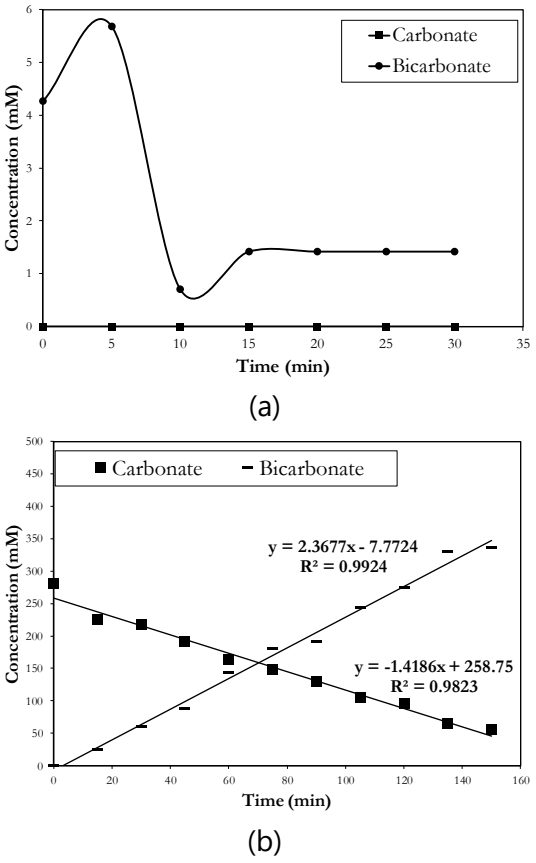


Fig. 2 Gas Absorption Response of (a) Control System, (b) Sodium Carbonate Solution

CO_2 Assimilation/Utilization Under Controlled Carbon Dioxide

Figure 3 shows successful growth utilizing the three media under a controlled environment where the stationary phase was achieved; all systems also resulted in high values of μ_{max}. Furthermore, as can be seen from Figure 4, bicarbonate concentration decreased, indicating its consumption, while carbonate concentration increased, signifying regeneration. It can be inferred that BICCAPS is taking place in the system.

Table 3. Summary of Parameters of Control System Under BICCAPS

	Biomass Productivity	μmax	Inorganic Carbon Conversion
ZC	10.42	0.0655	0.292
NC	10.51	0.0601	0.250
AC	10.81	0.0755	0.220

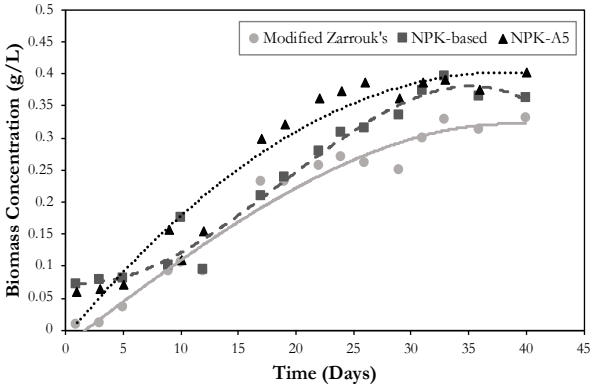
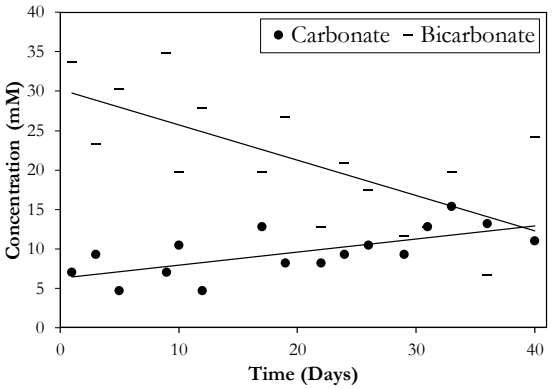
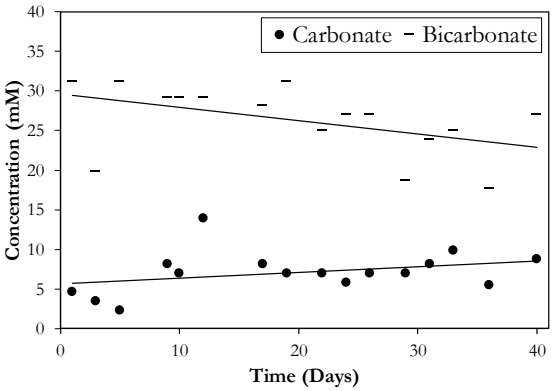


Fig. 3 Growth of *Spirulina Platensis* in BICCAPS under Modified Zarrouk's, NPK-based, and NPK-A_5 media

Dissolved carbon content, on the other hand, (Figure 5) decreases in concentration and increases thereafter. This is consistent with the provided carbonate equilibria where, at high pH values, carbon dioxide takes the form of bicarbonate. A summary of the biomass productivity, maximum specific growth rate, and inorganic carbon conversion values are tabulated in Table 3.



(a)



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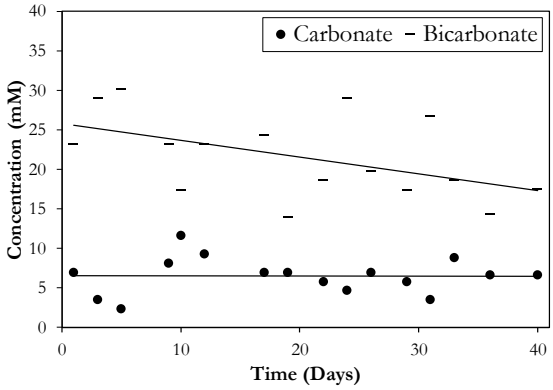


(c)

Fig. 4 Concentration Profile of Carbonate and Bicarbonate in BICCAPS for controlled set-up under (a) Modified Zarrouk's, (b) NPK-based, and (c) NPK-A_5 media

CO_2 Assimilation/Utilization Under BICCAPS

S. Platensis was successfully grown in all culture media, as seen in Figure 6 for ZS, NS, and AS systems.

Although growth was evident in all systems, only ZS and AS were able to fully utilize the bicarbonates, as seen in Figure 7a and 7c, wherein there is an observable decrease in bicarbonates and increase in carbonates. Hence, NS is not viable under the BICCAPS for *Spirulina platensis*. As for the dissolved carbon dioxide present in the systems, trends are presented in Figure 8. A summary of the different parameters for sodium carbonate is displayed in Table 4.

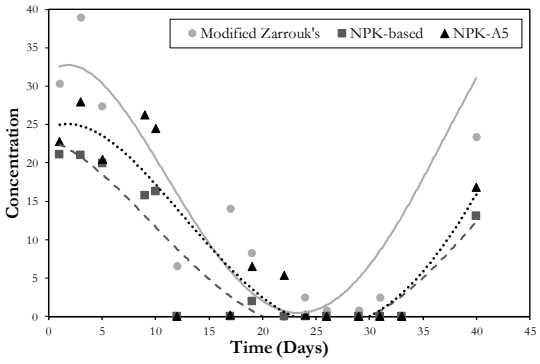


Fig. 5 Dissolved Carbon Dioxide in BICCAPS for controlled set-up under (a) Modified Zarrouk's, (b) NPK-based, and (c) NPK-A_5 media

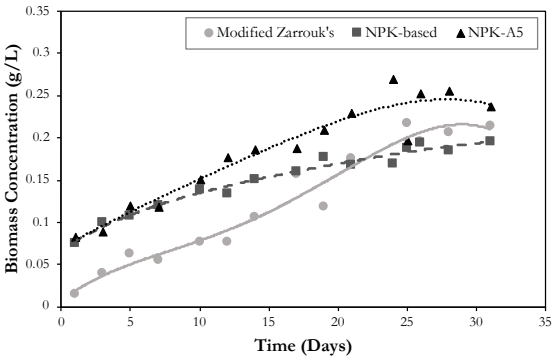
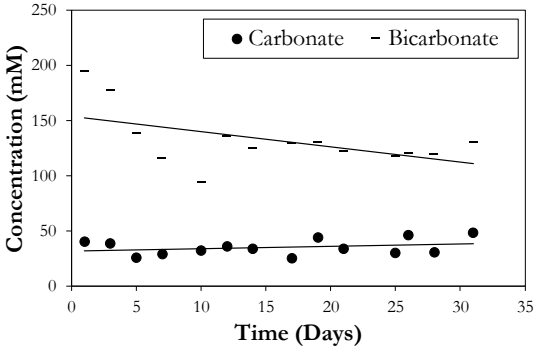
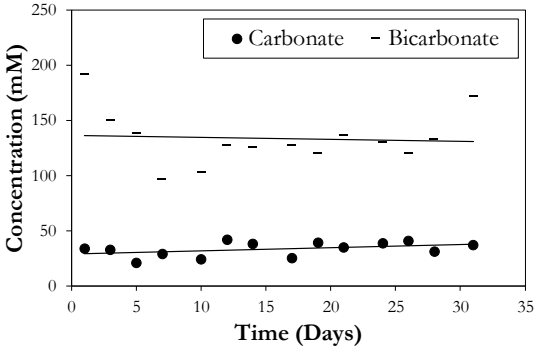


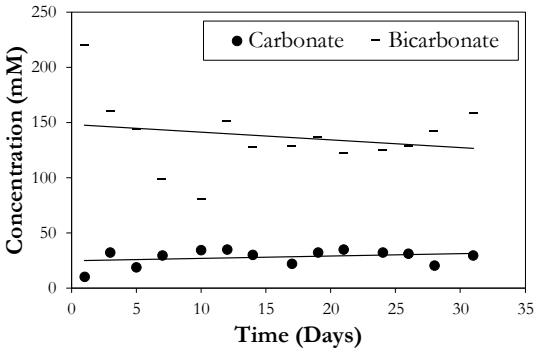
Fig. 6 Growth of *Spirulina Platensis* in BICCAPS with Na_2CO_3 under Modified Zarrouk's, NPK-based, and NPK-A_5 media



(a)



(b)



(c)

Fig. 7 Concentration profile of carbonate and bicarbonate in BICCAPS with Na_2CO_3 under (a) Modified Zarrouk's, (b) NPK-based, and (c) NPK-A_5 media

Comparison of Different Culture media Under BICCAPS

In an open system, NPK with A_5 flourished as compared to Modified Zarrouk's and NPK-based, which have similar biomass productivities and μ_{max}. Under BICCAPS, modified Zarrouk's media produced the highest productivity; therefore, proving that modified Zarrouk's

remains to be the most suitable culture medium to be used while NPK- based medium produced relatively low productivities and maximum specific growth rates.

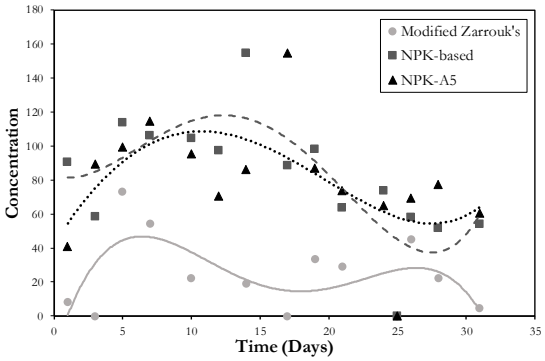


Fig. 8 Dissolved CO_2 in BICCAPS with Na_2CO_3 under (a) Modified Zarrouk's, (b) NPK-based, and (c) NPK-A_5 media

Table 4. Summary of Parameters of Sodium Carbonate System Under BICCAPS

	Biomass Productivity	μmax	Inorganic Carbon Conversion
ZS	6.56	0.0632	2.682
NS	4.03	0.0274	0.907
AS	6.80	0.0489	2.092

In general, all control systems obtained high biomass productivities but lowest carbon conversion values. Relatively small carbon conversion is due to the low bicarbonate loading while biomass productivity increases due to the absence of inhibition caused by metal ions. *Spirulina platensis* under Zarrouk's medium provided the highest carbon conversion among all three media used.

As for the carbonate systems, AS and ZS exhibited successful and similar biomass productivities and carbon conversion rates. Therefore, ZS and AS were deemed viable for *Spirulina platensis* utilization under BICCAPS. Moreover, the top system considering both parameters is ZS, making it the most suitable candidate for BICCAPS for the growth of *S. platensis*. NPK with A_5 solution, on the other hand, may be used as a sufficient cost-effective alternative to modified Zarrouk's.

Upon comparing results with those present in literature, a large difference can be observed. Table 5 summarizes the different biomass productivities obtained from various studies.

Table 5. BICCAPS literatures for *S. platensis* at different conditions

Medium/ Carbon source	Biomass Productivity (mg L^{-1} day^{-1})	Ref
Zarrouk's medium/ CO_2 – air	457.5	(Kumari et al., 2014)
NPK- 10:26:26/ CO_2 - air	422.5	
Modified Zarrouk's medium/ HCO_3	55	(Delrue et al., 2017)
Modified Zarrouk's medium/ HCO_3	0.052	(Madkour et al., 2012)
NPK-based with A_5 / CO_2-air	22.08	This study
NPK-based with A_5/ BICCAPS	6.80	
Modified Zarrouk's medium/ BICCAPS	6.56	

It can be noted from the table that studies involving CO_2 as carbon source produced superior results as compared to closed systems.

If studies involving Zarrouk's medium are compared, it can be seen that the study conducted by Kumari et al. (2014) produced best results with intending to concoct the best mixture of CO_2-air and

other minerals. It is followed by the study of Delrue et al. (2017) with the intention of large-scaling *S. platensis* production. Both studies surpass the results obtained from this study. However, it generated better results as compared to Madkour, Kamil, and Nasr's (2012) research, which aims to reduce the cost of Zarrouk's medium by replacing all nutrients with more cost-effective alternatives. It is highly possible that modifications applied in the Zarrouk's medium greatly affected the productivities.

Comparing studies under NPK fertilizers, once again, Kumari et al. (2014) produced the highest productivity, followed by this study's enhanced NPK medium and NPK-based medium. In general, the cost-effective fertilizers depict as a promising alternative to the costly Zarrouk's medium.

CONCLUSIONS

This study was able to identify that *Spirulina platensis* under BICCAPS was successful by portraying positive biomass productivities and carbon conversion values. In general, control systems resulted in the highest productivities and lowest carbon conversions. In a system of no carbonate salt, Modified Zarrouk's medium produced the highest productivity. For carbonate systems, on the other hand, NPK with A_5 solution together with Modified Zarrouk's medium displayed the highest biomass productivities as well as carbon conversions. It is therefore inferred that the growth of *S. platensis* under BICCAPS is best applied in a Modified Zarrouk's medium, closely followed by NPK with A_5 solution as a cost-effective alternative.

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