Evaluation of *Ankistrodesmus falcatus* for Bicarbonate-Based Integrated Carbon Capture System (BICCAPS)

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Abstract. This study evaluates the performance of alkaliphilic microalgae *Ankistrodesmus falcatus* in the Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS). The system utilized bicarbonate as carbon source for microalgae production. BICCAPS parameters such as pH, algal biomass productivity and CO_2 utilization (inorganic carbon conversion, C_i) were observed at different sodium bicarbonate ($NaHCO_3$) loading concentration and type of culture media. The highest productivity was observed at 10 g/L of $NaHCO_3$ loading in BRSP medium at 3.5539 mg/L/day. This value is 30% lower compared to the control experiment (continuously aerated bioreactor). The C_i values of the different system ranges from 1.17×10^{-4} to 1.51×10^{-4} moles/L/day. Both the pH of the BRSP and NPK media at 10 g/L and 30g/L loading of $NaHCO_3$ increased through time. The result shows that *A. falcatus* has a potential in BICCAPS utilization.

1 Introduction

The increasing level of carbon dioxide (CO_2) in the atmosphere had caused severe impact in global temperature. The mean CO_2 concentration in the atmosphere had passed the 400 ppm mark which is beyond the safety limit of 350 ppm [1]. The major culprit for in the increase in CO_2 in the atmosphere is the utilization of fossil fuel for energy generation and trasnportation. Several strategies is been used to reduce or prevent further release of CO_2 such as improvement in the technology, energy conservation, utilization of renewable technology and CO_2 sequestration [2].

Carbon Capture and Sequestration (CCS) system is the most viable techniques to be applied for exisiting industrial plants. In CCS system, the CO_2 gas is captured in an amine-based or carbonate-based adsorbents and stored in a vessel as compressed gas and transported to a place where it could have negligible effect on the environment [3]. Still, these technologies are energy intensive because of the thermal treatment required to regenerate the absorbents rendering it to be expensive [4]. Alternative CCS technology utilized alkaline solutions (e.g. NaOH) and microalgae in photobioreactor [5-6].

The popularity of microalgae as CCS is due to high growth rate and capture carbon capability on top of that it could be used as a potential fuels source. One of the several microalgae that has been evaluated as biofuel feedstock is *Ankistrodemus falcatus* [7-9]. Although,

study for it as potential for CCS were limited. One study reported that *A. falcatus* grew in a specialized PHM medium able to havecarbon removal rate of 20% utilizing a 2% CO_2 feed gas stream [10]. *A. falcatus* has reported to contain a lipid of more than 35%, optimal growth length of 0.20 μm and a biomass productivity of 7.9 mg/L-day in a BG-11 medium [8]. Also, it was capable to produce a lipid yield of 67.2%, but it had already undergone a morphology change. With another study, it was known that nitrogen, phosphorus and iron nutrient has a significantly effect in lipid productivity and can obtained biofuel conversion rate as high as 91% [7].

Bicarbonate-based Integrated Carbon Capture and Algae Production System (BICCAPS) is an improvement of CCS system where both chemical and biological systems together is being utlized [4]. The technology captures CO_2 in the form of bicarbonate as alkali solution and utilizes it as carbon source for microalgae. The microalgae should be able to withstand a highly alkaliphilic environment for this system to be viable thus limit the study to some cyanobacteria species.

Liu et al. conducted a study that utilized *Chlorella sp.* and *Scenedesmus obliquus sp.* in a continuous system using bicarbonate-based media and observed that the microalgae was able to grow at a rate of 0.41 g (dry weight) per gram of HCO_3^- [11]. In another study, it was determined that *Euthalothece* ZM001 species has potential for BICCAPS system due to its biomass productivity of 1.21 g/L-day while the *Spirulina* species

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has low potential due to its low biomass productivity of 0.15-0.43 g/L-day [4]. One study found out that the used of sodium bicarbonate in culture media is advantageous because of its capability to increase microalgae cells and the amount of lipids in *Tetraselmis suecica* and *Nannochloropsis salina* [12]. However, no studies were reported in utilizing bicarbonate solutions in the growth of *falcatus*.

Thus, this study attempted to evaluate the utilization of bicarbonates for the growth of *A. falcatus*. The solution pH, algal biomass productivity and CO_2 utilization as inorganic carbon conversion (C_i) were determined at different salt loading concentration and type of culture media.

2 Experimental

2.1 Materials

The following reagents and materials were used in the study: sodium bicarbonate ($NaHCO_3$, Himedia, 99.5%) and filter membrane (Pall, 0.45μm) purchased from Yana Chemodities Inc., CO_2 gas (Technical grade) obtained from Linde Philippines. Analytical grade of barium chloride (BaCl), potassium hydrogen phthalate, sodium hydroxide (NaOH) pellets, hydrochloric acid (HCl, Univar, 36%), phenolphthalein indicator and bromocresol green indicator were procured from Crimson Scientific & Laboratory Supply.

2.2 Microalgal strain, culture media and cultivation

Southeast Asian Fisheries Development Center (SEAFDEC) provided the alkaliphilic freshwater microalgae *Ankistrodesmus falcatus* maintained in NPK (14-14-14) commercial fertilizer medium and the BRSP medium (formulation from SEAFDEC) as stock culture. For culture media preparation the following formulation were followed: for BRSP media, a ratio of 1:9 of BRSP concentrated solution to distilled water and for NPK media, a 0.1g $N_{14}P_{14}K_{14}$ fertilizer per liter of distilled water.

Microalgae cultivation is done in a 12h light/12h dark cycle utilizing a light source with intensity of approximately 1 Klux. All runs were done at normal ambient temperature conditions in a 4-L PET bottle photobioreactors at a working volume of 3 L.

A. falcatus is cultivated in an open system as air pumps were used to deliver CO_2 from air. The growth curves of obtained from the experiment were used as standard for biomass production.

2.3 Algal production

Batches of 3.0 L NPK and BRSP media with 10 g/L and 30 g/L loading of $NaHCO_3$ were prepared and inoculated with 1:9 algal ratios to culture media. The microalgae were cultured in the solution for 21 days in a 12h light/12h dark cycle with a light intensity of approximately 1 Klux at normal ambient temperature

conditions at a closed system. Optical density and pH readings were measured on a regular basis.

2.4 Analysis

The microalgae growth curves in NPK and BRSP media were determined using UV-vis spectrophotometer (Shimadzu UV-170) at a wavelength of 725 nm (optical density, OD) and dry biomass weight (DBW) for 30 days cultivation. DBW were determined by obtaining the mass of the biomass from the sample by filtering it with 0.45 micron filter and dried at 100°C for 3 hrs.

Double-indicator titration method is used to obtain the bicarbonate and carbonate content of the samples with HCl solution as standard. Volumes of the standardized HCl solution were determined in the titration of 10.0 mL filtered sample (with 0.45 micron filter) until the phenolphthalein endpoint (V_{1HCl}, light pink, carbonate content) and the bromocresol green endpoint (V_{2HCl}, faint yellow, bicarbonate content). The carbonate and bicarbonate concentration were determined using (1) and (2), respectively.

$$C_{CO_3^{2-}} = C_{HCl}V_{1HCl} \times 100 \qquad (1)$$

$$C_{HCO_3^-} = C_{HCl}\left[V_{2HCl} - V_{1HCl}\right] \times 100 \qquad (2)$$

The presence of dissolve CO_2 gas in the samples was obtained using precipitation-titration method. Standardized NaOH solution (10 mL) was added to 10 mL sample which convert the dissolve CO_2 in the solution to CO_3^{2-}. The carbonate is precipitated as barium carbonate ($BaCO_3$)by adding an excess BaCl solution in the prepared sample. Excess hydroxide is titrated with standardized HCl solution to phenolphthalein endpoint (V_{3HCl}). Dissolve CO_2 gas concentration in the solution was obtained using (3).

$$C_{CO_2} = \left[C_{HCl}V_{3HCl} - C_{HCO_3^-}V_{sample}\right] \times 50 \qquad (3)$$

3 Results and Discussion

The growth curve of *A. falcatus* for both BRSP and NPK media for 30 days with air sparging is shown in Fig. 1. The results shows that the stationary phase is evident in NPK media at around day 22 but not yet visible in BRSP media during the cultivation period. Fig. 2 show the the linearized plot of a first order growth rate of *A. falcatus* for both BRSP and NPK media using (4).

$$\ln DBW = u_{max}t + \ln DBW_0 \qquad (4)$$

The slope of the line is the maximum specific growth rate (u_{max}) of the algal growth. It was determined that the u_{max} for *A. falcatus* are 0.0515 /day and 0.0349 /day for BRSP media and NPK media, respectively. These results indicates that BRSP is the more favourable growth media for *A. falcatus*. This could be due to the present of essential nutrient in BRSP for algal metabolism such as Biotin, Thiamine, and Vitamin B_{12} [13]. *A. falcatus* biomass productivity (BP) is calculated using (5), where DBW_0 and DBW_f are initial and final DBW of *A. falcatus* and t is the time of cultivation. It was determined that the BP for BRSP media is 5.09 mg/L-day while 3.59 mg/L-day for NPK media.

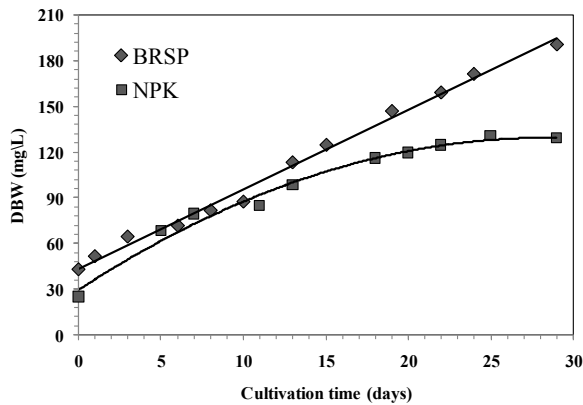$$BP = \frac{DBW_f - DBW_0}{t} \qquad (5)$$

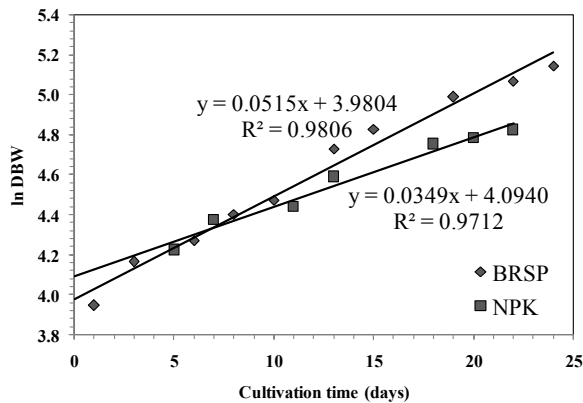**Fig 1**. Growth curve of *A. falcatus* in BRSP and NPK culture media.

Fig 2. Linearized first order growth rate of *A. falcatus* in BRSP and NPK culture media.

The algal growth and the media physico-chemical properties for bicarbonate-based media at 10 g/L loading were observed for 21 days in a closed system and the results are shown in Fig. 3 to Fig. 7.

From Fig. 3, it could be observe that even though there is no purging of air, the alga was able to grow because of the presence of bicarbonate ions as carbon source. It can be seen that both the growth in BRSP and NPK are still increasing linearly without any trace of stationary phase. Also, the growth at 10 g/L loading of $NaHCO_3$ (Fig. 3a) is much higher than 30 g/L loading (Fig. 3b). This is attributed to higher concentration of Na^+ in the media which is determental to the growth of the algae. Moreover, higher growth is observed for BRSP media with bicarbonate salt in closed system.

Biomass productivities were determined to be 3.554 mg/L-day and 0.954 mg/L-day for BRSP media at 10 g/L and 30 g/L laoding, respectively. While for NPK media, BP values are 1.801 mg/L-day and 0.936 mg/L-day for 10 g/L and 30 g/L laoding, respectively.

Fig. 4 provide the linearized plot of a first order growth rate of *A. falcatus* for bicarbonate-based media in closed system. The u_{max} observed at 10 g/L loading are 0.0604 /day and 0.0428 /day for BRSP media and NPK media, respectively. On the otherhand, u_{max} at 30 g/L

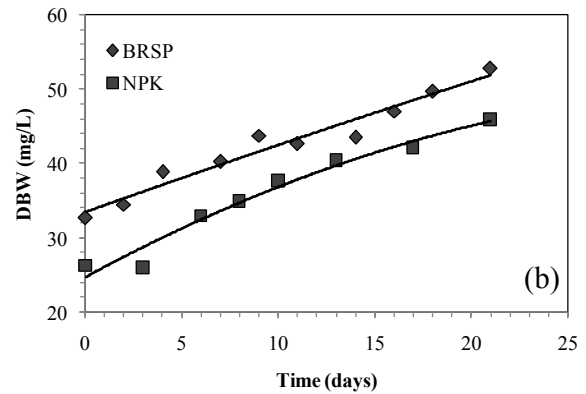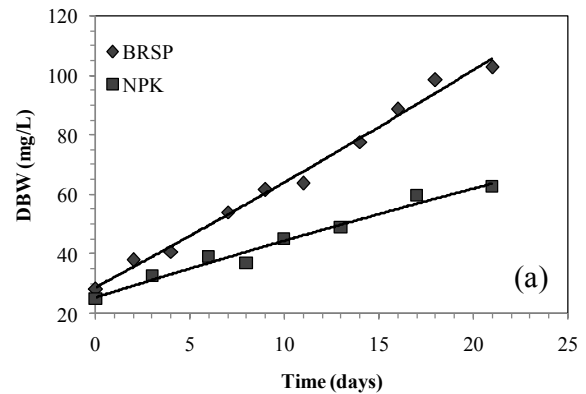loading are 0.0207 /day and 0.0286 /day for BRSP media and NPK media, respectively.

Fig 3. Growth curve of *A. falcatus* in closed BRSP-$NaHCO_3$ solution and closed NPK-$NaHCO_3$ solution media at (a) 10 g/L and (b) 30 g/L loading.
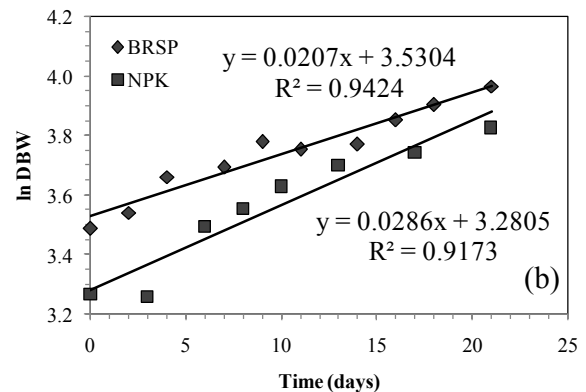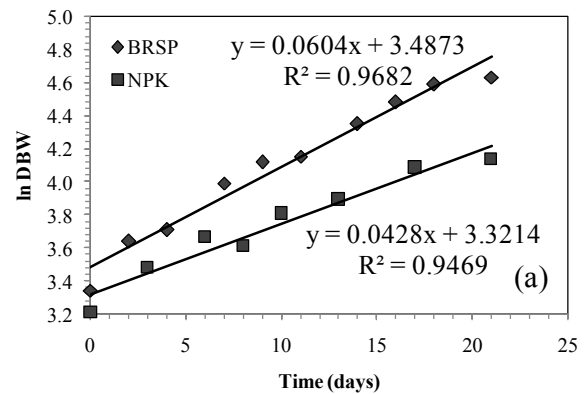

Fig 4. Linearized first order growth rate of *A. falcatus* in closed BRSP-$NaHCO_3$ solution and closed NPK-$NaHCO_3$ solution media at (a) 10 g/L and (b) 30 g/L loading..

At 10 g/L loading, for the bicarbonatete-based BRSP media, the u_{max} was higher by 17% that the airated BRSP media while for the bicarbonatete-based NPK media the u_{max} was higher by 23%. While at 30 g/L loading, the value was reduced by 60% compared to the aerated BRSP media but only 18% reduction is observed for bicarbonatete-based NPK media compared to the aerated one. The higher value for 10 g/L laoding could be due to low agitation observed in the closed system that the aerated system while the lowvalue for 30 g/L loading is caused by Na^+ inhibition.

The pH for both closed systems increases as cultivation time progressed as shown in Fig. 5. The pH increased from 8.54 to 9.07 for the BRSP media while for the NPK, the increased was from 8.64 to 9.06 at 10 g/L loading. At 30 g/L laoding, pH increased from 8.51 to 8.80 in BRSP media while in NPK media the increased was from 8.64 to 8.99.
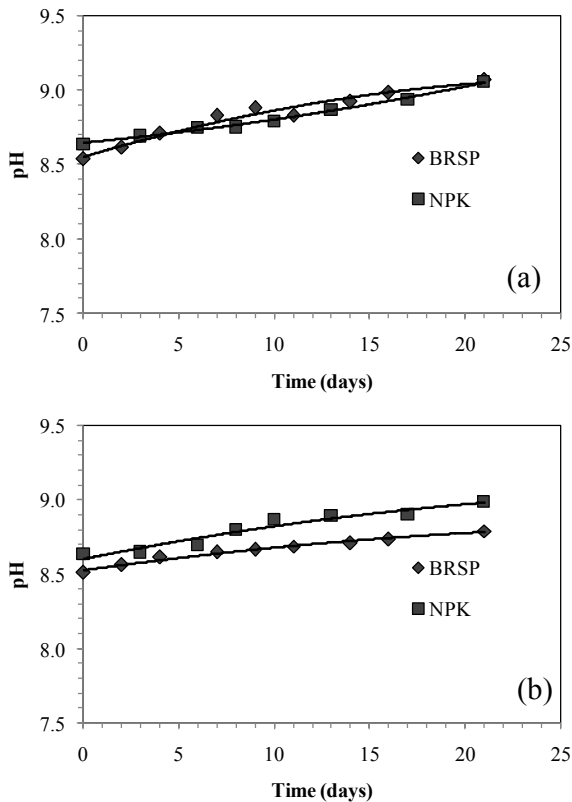

Fig 5. pH profile in closed BRSP-NaHCO₃ solution and closed NPK-NaHCO₃ solution media during growth of *A. falcatus*.

Fig. 6 and 7 show the HCO_3^- and CO_3^{2-} concentration of the closed system. As the concentration of HCO_3^- in both media goes down it can be observed the CO_3^{2-} concentration have increased. This indicates that the microalgae's growth is due to the uilization of the bicarbonate and convert it to carbonate in the system by the microorganism. Then production of carbonate then thends the pH of the system to goes up.
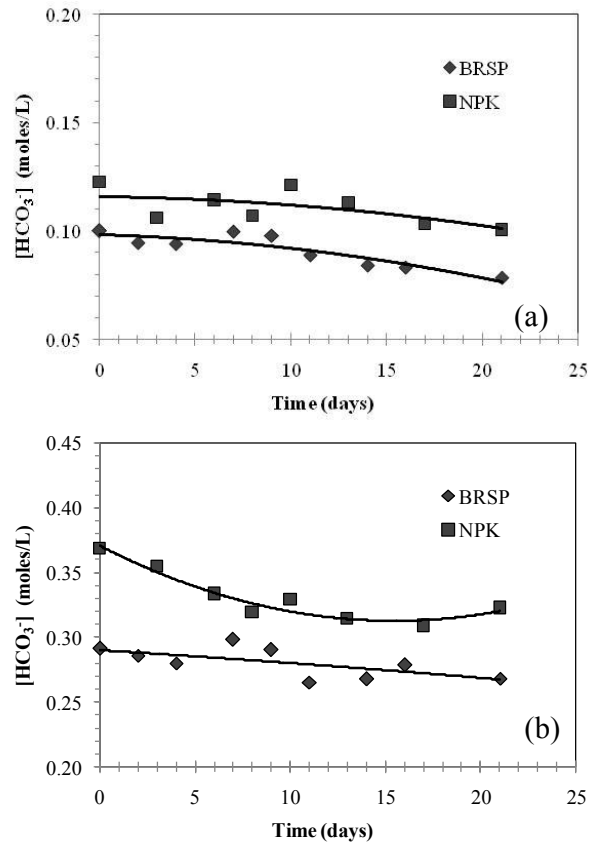

Fig 6. Bicarbonate concentration of closed BRSP-NaHCO₃ solution and closed NPK-NaHCO₃ solution media during growth of *A. falcatus*.
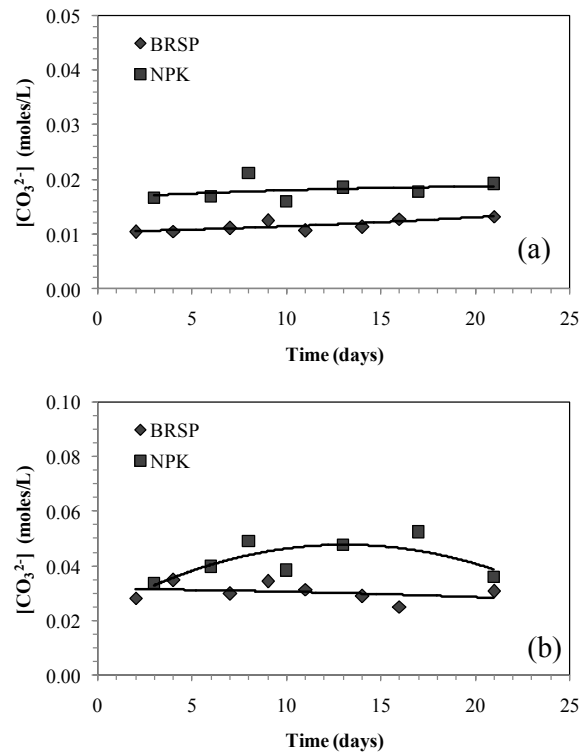

Fig 7. Carbonate concentration of closed BRSP-NaHCO₃ solution and closed NPK-NaHCO₃ solution media during growth of *A. falcatus*.

Inorganic Carbon (C_i) conversion was defined as the equivalent amount of CO_2 that is converted to biomass per culture volume per time (moles/L-day) and

was calculated using (6) where $[CO_3^{2-}]_f$ and $[CO_3^{2-}]_i$ are the initial and final concentration of CO_3^{2-} in the media, $[CO_2]_f$ and $[CO_2]_i$ are the initial and final concentration of CO_2 in the media and t is the time of cultivation. For the bicarbonate-based system, this is the amount of bicarbonate converted to biomass.

$$C_i = \frac{\left(\left[CO_3^{2-}\right]_f - \left[CO_3^{2-}\right]_i\right) + \left(\left[CO_2\right]_i - \left[CO_2\right]_f\right)}{t} \quad (6)$$

The calculated C_i for BRSP media at 10 g/L loading was 1.508 x 10^{-4} moles/L-day while 1.260 x 10^{-4} moles/L-day is determined for the NPK media. At 30 g/L laoding, Ci value calculated were 1.432 x 10^{-4} moles/L-day and 1.166 x 10^{-4} moles/L-day for BRSP and NPK media, respectively.

4 Conclusion

The utilization of bicarbonates in solution media for the growth of *Ankistrodesmus falcatus* was investigated. It was observed that growth of A. falcatus is higher in BRSP media than in NPK due to some essential nutrients necessary for algal growth. On the otherhand, bicarbonates laoding at 10 g/L show higher growth than the 30 g/L loading attrbitued to the growth inhibition of Na^+. The pH of the solution media increases as the bicarbonates is utilized and converted to carbonate ions. The result shows that *A. falcatus* can be utilized to in BICCAPS process.

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