

CO₂ sequestration using *Characium* sp. in an airlift photobioreactor

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Abstract— Microalgae are known to have better growth rate and have higher performance at fixing CO₂ compared to other photosynthetic organisms. In addition, microalgae require less space for cultivation, hence, the strategic use of photobioreactor. Cultivation of microalgae in photobioreactor is a practical approach to mitigate CO₂ emission. In this study, *Characium* sp. was cultured in an airlift photobioreactor to determine the effect of CO₂ concentrations on its growth. Assessment was also conducted on its biomass, chlorophyll *a* and lipid contents. *Characium* sp. presented the highest CO₂ fixation rate in 5% CO₂ with 1.222 g L⁻¹ d⁻¹. During 21-day interval cultures, the specific growth rate of *Characium* sp. at CO₂ concentrations of 5%, 10%, and 15% were 0.263 d⁻¹, 0.191 d⁻¹, and 0.181 d⁻¹ respectively. Meanwhile, its biomass productivity at 5%, 10%, and 15% CO₂ concentrations was 0.048 g L⁻¹ d⁻¹, 0.038 g L⁻¹ d⁻¹, and 0.028 g L⁻¹ d⁻¹ respectively. The highest chlorophyll *a* recorded was 2.82 µg/mL in 15% CO₂ enriched medium followed by 2.412 µg/mL in 10% enriched medium, and 2.284 µg/mL in 5% CO₂. *Characium* sp. recorded the highest lipid content of 18.63 µg/mL in 15% CO₂ enriched medium followed by 6.44 µg/mL in 10% CO₂ enriched medium and 4.90 µg/mL in 5% CO₂ enriched medium.

Keywords— microalgae, *Characium* sp., carbon dioxide, airlift, CO₂ fixation

I. Introduction

Global warming is a global concern. To date, it is linked to abnormal phenomena such as heat waves, heavy precipitation and storms [1]. CO₂ is the principal greenhouse gas that contributes to global warming. The concentration of CO₂ has inclined radically throughout the decades since the onset of Industrial era through anthropogenic activities i.e. fossil fuel combustion, change of land use, extensive agricultural activities and global population. It is still showing an upward trend in the early 21st century due to heavy reliance on fossil fuel combustion. For the past decades, many attempts have been made to mitigate atmospheric CO₂ through various physical methods e.g. chemical based reaction and direct injection. However, the operating cost is a focal hindrance for these methods [2].

Biological method for mitigating CO₂ is very promising through the usage of both plants and photosynthetic organisms. Furthermore, it is environmentally friendly and economical. Microalgae are 10-50 times better at fixing CO₂ and have a faster growth rate than terrestrial plants [3]. Photoautotrophic microalgae consume inorganic carbon and are dependent on light as source of energy [4, 5]. Furthermore, microalgae can convert CO₂ into useful biomass. For 1 kg biomass, microalgae consume approximately 1.83 kg of CO₂ [6]. The biomass can be processed into valuable products e.g. biofuel, animal feed, biohydrogen, etc. [7]. In addition, microalgae can also be used in bioremediation and as biofertilizer [8,9]. Therefore, coupling of microalgal growth and CO₂ fixation is possible to offset the costs of cultivating the organism and carbon release [10].

Microalgae are normally cultivated via open system through the usage of open pond. However, this approach requires large areas and possesses multiple setbacks, including contamination, low productivity and poor control over cultivation conditions [11]. Alternatively, closed system using photobioreactors (PBRs) is shown to be effective and promising. Using vertical tubular PBRs can save up cultivation area by culturing microalgae vertically and extend the carbon dioxide residence time in the medium, and increase the efficiency of carbon dioxide utilization [12].

In this study, effects of CO₂ concentrations in airstreams on *Characium* sp. growth were investigated. The biomass, and chlorophyll *a*, and lipid contents were also studied.

II. Materials and methods

A. Microalgae culture and condition

A stock culture of *Characium* sp. was obtained from Faculty of Engineering and Built Environment, National University of Malaysia (UKM), Malaysia and cultivated at 27°C in modified BG-11 medium (per liter) containing 1.5 g NaNO₃, 0.04 g K₂HPO₄, 0.075 g MgSO₄·7H₂O, 0.036 g CaCl₂·2H₂O, 0.006 g citric acid, 0.006 g Ferric ammonium citrate, 0.001 g EDTA, 0.02 g NaCO₃, and 1 mL of trace metal solution. The trace metal (per 100 mL) contains 2.86 g H₃BO₃, 1.81 g MnCl₂·4H₂O, 0.222 g ZnSO₄·7H₂O, 0.39 g NaMoO₄·2H₂O, 0.079 g CuSO₄·5H₂O, and 49.4 mg Co(NO₃)₂·6H₂O.

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B. Experimental system with photobioreactor

Culture cell studies were conducted in a 60 L enclosed cylindrical acrylic photobioreactor with diameter of 13.8 cm and height of 100 cm at room temperature under illumination of cool white, fluorescent light. Four fluorescent lamps of 21W were uniformly aligned around the photobioreactor. Light intensity was approximately $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ on each side. Fig. 1 presented a schematic diagram of the complete experimental setup. The illumination cycle was set at 12:12 h light/dark period. The initial pH of each culture was 7. Compressed air was mixed with CO_2 to give concentrations of CO_2 of 5%, 10%, and 15%. Cultures in the photobioreactor were aerated continuously at 0.1 vvm. Each experiment was performed for 21 days for microalga tested.

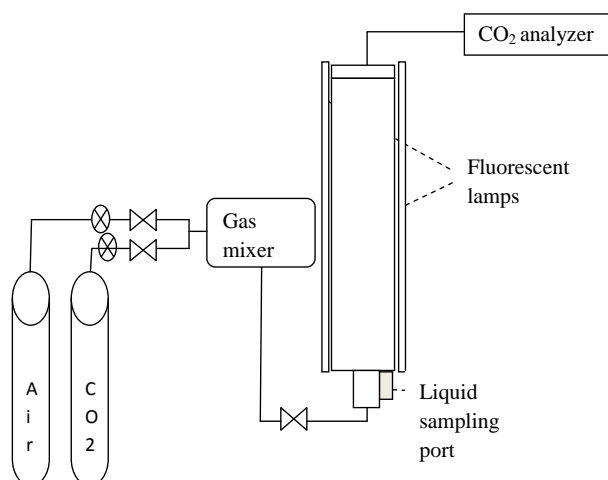


Figure 1: Schematic diagram of the photobioreactor for the experiments on CO_2 fixation for batch cultures. The airstream containing different CO_2 concentrations was controlled using individual digital mass flow controllers (Alicat M-series, USA) and the gas output was determined via CO_2 analyzer (Quantek Instruments, USA).

C. Preparation of the inoculums

A stock culture of *Characium* sp. was incubated in an Erlenmeyer flask containing 1.8 L modified BG-11 medium at 27°C and light intensity of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$. After six days, the cell density of culture was measured prior to usage. Culture was considered ready for inoculation when the OD reading is 1.

D. Batch cultivation

The photobioreactor was filled with 18 L modified BG-11. The medium was inoculated with 2 L *Characium* sp. seed culture. A mixture of air and CO_2 was obtained by mixing both air and CO_2 gas at 0.1 vvm. Concentration of CO_2 was

adjusted to 5%, 10%, and 15% CO_2 in airstreams for each air/ CO_2 mixture. Cultures were incubated for 21 days.

E. Biomass analysis

Samples were collected at an interval of 2 days for 21 days and subjected to analysis of dry weight, chlorophyll *a* content and protein content.

E. A. Dry weight

Culture was filtered using Whatman No. 1 and dried in the oven overnight at 80°C until a constant weight could be achieved. This reading was then deducted from the preliminary filter paper weight to obtain cell weight.

E. B. Chlorophyll a content

Culture was harvested via centrifugation at 5000 rpm for 10 min. The supernatant was discarded. The pellet was resuspended with 80% acetone solution and sonicated for 30 min. The sample was left overnight at room temperature. Next, the sample was centrifuged at 5000 rpm for 10 min. The filtrate was transferred into a quartz cuvette and measured at 646 nm and 663 nm using a spectrophotometer (Hach DR2800, USA) and measured according to [13]

E. C. Lipid estimation

Culture was harvested via centrifugation at 5000 rpm for 10 min. The supernatant was discarded. The pellet was precipitated in methanol/chloroform (2:1, v/v) and sonicated for 30 min. NaCl was added to give a ratio of methanol, chloroform, and NaCl of 2:2:1. The mixture was left overnight [14]. Next, the chloroform phase was collected and evaporated in the oven at 70°C to remove organic solvent. Sulfuric acid was added to the sample. The sample was incubated for 10 min in boiling water bath. Phosphovanilin reagent was added to the sample. After 30 min, sample was measured at 520 nm. Lipid standard curve was established using cholesterol ranging from $20 \mu\text{g} - 100 \mu\text{g}$.

E. D. Kinetic parameters

Biomass (X) values were used to calculate the maximum specific growth rate (μ_{max} , d^{-1}) according to $\mu = \ln(X_t - X_0) / (t - t_0)$ where X_t and X_0 are dry weight (g/L) at t and t_0 respectively [15]. The doubling time (t_d , d) was calculated as $t_d = \ln 2 / \mu_{\text{max}}$. The maximum biomass concentration was illustrated as X_{max} (g/L). Productivity (P , $\text{g L}^{-1} \text{d}^{-1}$) was derived from the equation $P = (X_t - X_0) / (t - t_0)$ where X_t and X_0 are dry weight (g/L) at t and t_0 respectively. Maximum productivity was designated P_{max} ($\text{g L}^{-1} \text{d}^{-1}$).

III. Results and discussion

A. Evaluation of cell biomass

Cell density is easily determined through optical density method compared to direct cell counting and dry weight [16]. Therefore, the relationship between optical density and cell

dry weight was established using linear regression. Optical density precisely predicted cell dry weight ($R^2 = 0.978$; $p < 0.001$). Hence, the values of optical density were used to calculate the related *Characium* sp. in each experiment according the regression established in this study.

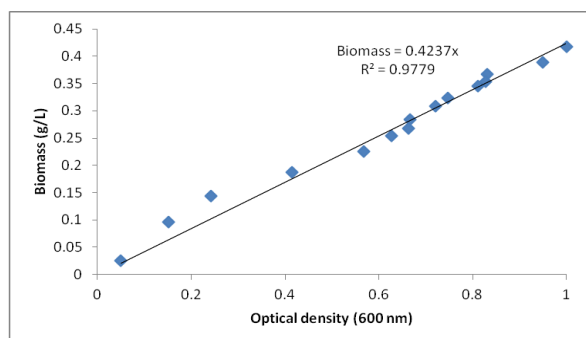


Figure 2. Calibration curve and equation for optical density at 600 nm versus the biomass of *Characium* sp.

B. Effect of CO₂ on cell growth and CO₂ uptake

CO₂ concentration is a limiting factor in photosynthesis. Thus, the concentration should be higher than a certain value to meet carbon need for algal growth and not exceed an upper limit to prevent unnecessary CO₂ loss [17]. To investigate the effect of CO₂ concentration on growth, *Characium* sp. was cultured for 21 days at room temperature and 150 μmol m⁻² s⁻¹ and aerated with different concentrations of CO₂ at 0.1 vvm. Cultures were sampled at the beginning of stationary phase. Specific growth rate was calculated from the logarithmic phase in each experiment.

As the cells reached the stationary phase, the biomass accumulated in 5%, 10% and 15% CO₂ aeration were 0.650 g L⁻¹, 0.458 g L⁻¹, and 0.498 g L⁻¹ respectively (Figure 3). *Characium* sp. recorded the highest specific growth rate of 0.263 d⁻¹ when aerated with 5% CO₂. The specific growth rate dropped to 0.191 d⁻¹ when culture was aerated with 10% CO₂ followed with 0.181 d⁻¹ when the culture was aerated with 15% CO₂.

Characium sp. recorded the highest biomass productivity of 0.048 g L⁻¹ d⁻¹ in 5% CO₂ followed by 0.038 g L⁻¹ d⁻¹ in 10% CO₂. The lowest productivity is 0.028 g L⁻¹ d⁻¹ in 15% CO₂ (Table 1).

Characium sp. illustrated the maximum rate of CO₂ fixation of 1.222 g L⁻¹ d⁻¹ in 5% CO₂ enriched medium followed by 0.936 g L⁻¹ d⁻¹ in 15% CO₂ enriched medium and 0.861 g L⁻¹ d⁻¹ in 10% CO₂ enriched medium. Sydney et al. [18] reported that CO₂ fixation rate for *C. vulgaris* LEB-104, *B. braunii* SAG-30.81, *S. platensis* LEB-104, and *D. tertiolecta* SAG-13.86 were 0.251, 0.497, 0.318, and 0.272 g L⁻¹ d⁻¹ respectively in 5% CO₂.

TABLE 1. Specific growth rate of *Characium* sp. at different CO₂ concentrations.

Algae sp.	CO ₂ (%)	Specific growth rate (μ _{max})	Biomass productivity (g L ⁻¹ d ⁻¹)	P _{CO₂} (g L ⁻¹ d ⁻¹) ^a
<i>Characium</i> sp.	5	0.263	0.048	1.222
	10	0.191	0.038	0.861
	15	0.181	0.028	0.936

a Calculated from biomass productivity [2]

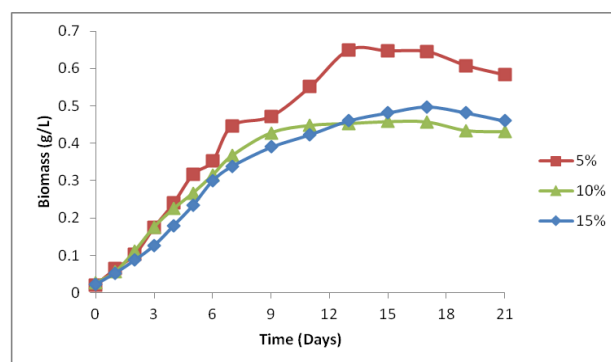


Figure 3. Effects of different concentrations of CO₂ aeration on the growth of *Characium* sp.

C. Effect of CO₂ on chlorophyll content

Chlorophyll *a* is a principal pigment in photosynthesis thereby acts as a precursor to microalgal growth rate. *Characium* sp. scored the highest chlorophyll *a* with 2.82 μg/mL in 15% CO₂ enriched medium followed by 2.412 μg/mL in 10% enriched medium. *Characium* sp. marked the lowest chlorophyll *a* content with 2.284 μg/mL in 5% CO₂ enriched medium (Table 2). Each culture exhibited a decline in chlorophyll *a* reading after reaching exponential phase.

TABLE 2. Chlorophyll *a* and lipid contents of *Characium* sp. at different CO₂ concentrations.

Algae sp.	CO ₂ (%)	Chlorophyll <i>a</i> (μg/mL)	Lipid (μg/mL)
<i>Characium</i> sp.	5	2.284	4.90
	10	2.412	6.44
	15	2.820	18.63

D. Effect of CO₂ on lipid content

Characium recorded the highest lipid content of 18.63 µg/mL in 15% CO₂ enriched medium. The content dropped significantly to 6.44 µg/mL in 10% CO₂ followed by 4.9 µg/mL in 5% CO₂. For each run, the lipid content tended to decline for the species.

IV. Conclusion

Characium sp. could tolerate at least 15% CO₂ level. Therefore, it can be regarded as candidate for biological CO₂ fixation. The greatest mass of CO₂ that was reduced occurred at 5% CO₂ with 1.222 g L⁻¹ d⁻¹. Still, other limiting factors such as light intensity and CO₂ retention time need to be further investigated to obtain optimal CO₂ uptake and growth rates.

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Characium sp. presented the highest CO₂ fixation rate in 5% CO₂ with 0.542 g h⁻¹ and can tolerate CO₂ up to 15% or higher.