Modified closed flat-plate photobioreactor for optimizing CO2 bio-fixation by the cyanobacterium *Synechococcus elongatus*

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Abstract:

Global warming due to carbon dioxide (CO_2) emissions is one of the most important environmental problems in the current century, therefore, there is an urgent need to search for effective eco-friendly strategies to reduce its risks. Biomass of autotrophic microorganisms has been used to reduce carbon dioxide emissions and to produce useful and economically valuable biomass. In the current study, a new strategy was used to improve the CO_2 bio-fixation ability of the cyanobacterium *S. elongatus*, by modifying the design of the closed flat plate photobioreactor by adding mutually inverted U-shaped baffles to increase the efficiency of CO_2 retention in the aqueous medium, thus increasing gas availability and utilization during photosynthesis. The results of the saturation and stability experiments indicated that optimal saturation occurred after 60–80 minutes and that the gas stability was for about 2 hours. The results of the bio-fixation experiment showed that the presence of fourteen baffles significantly increased the contact time between the trapped carbon dioxide and the culture medium of *S. elongatus*, in addition to preventing the rapid release of carbon dioxide from the aqueous medium, thus increasing the availability of carbon dioxide, which led to increasing the biomass productivity of *S. elongatus*.

Keywords: Biomass, Carbon dioxide emissions, Cyanobacteria, Global warming, Modified flat-plate photobioreactor, *Synechococcus elongatus*.

Introduction

The development of renewable energy and the reduction of greenhouse gas emissions are two complementary elements. Renewable energy sources such as solar, tidal, wind, and biomass have the potentiality to replace current fossil fuel energy sources **(Soman and Shastri, 2015)**. Energy derived from fossil fuels emits CO_2 into the environment at a high rate, as a result, CO_2 is primarily to

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blame for the change of 60–63% in net irradiance between different layers of the atmosphere between 1979 and 2004, and there is growing concerned about the environmental effects of CO_2 emission on the global warming throughout the current century **(Hofmann et al., 2006)**.

There is an urgent need to accelerate the development of CO_2 capture and sequestration technologies to reduce CO_2 emissions from a range of pollution-emitting sources, including industrial flue gas and vehicles used for transportation **(Zhao *et al.*, 2012).** Direct bio-fixation of CO_2 by microalgae is an effective way to reduce emissions. The biomass of autotrophic microorganisms, particularly algae and cyanobacteria was used to consume different amounts of CO_2 gas and convert it into useful and economically important components. Among the cyanobacteria, *Synechococcus elongatus*, is one of the most effective autotrophic organisms for this purpose **(Mortezaeikia *et al.* 2016)**.

Photobioreactor (PBR) technology using microalgae cultivation is an ideal artificial environment to achieve adequate production of microalgae biomass through strict operational control **(Vo *et al.*, 2019)**. The photobioreactor, which is operated in the form of a closed system, successfully meets the requirements for the bio-fixation of CO_2 **(Lee and Han, 2016)**, It provides higher biomass productivity while operational parameters such as temperature, light, and nutrients are strictly controlled, and less CO_2 loss was observed **(Singh and Sharma, 2012)**. Common types of photobioreactors include many designs used for different purposes. Tubular PBR is frequently applied for outdoor applications, but it has some drawbacks, including dissolved oxygen accumulation, elevated temperature, high pH, CO_2, and O_2 gradients, light limits, and high operational costs **(Huang *et al.*, 2017)**. Bubble column PBR, in addition to light limitations, the hydrodynamics of bubbles and the flow regime should be considered when

developing or scaling up. However, bubble column PBR is characterized by heat and mass transfer in a reasonable range, the release of O_2 gas, efficient radial mixing, and appropriate moving of components **(Kumar *et al.*, 2011)**. Airlift PBR, which is based on the rotation of aqueous culturing media through a dark-light regime causing a flashing light effect, which increases the differential in gas retention between the riser region, where the gas is sparged, and the downcomer region where is no gas provided, however, the gas residence time in different two areas may affect other operational factors **(Singh and Sharma, 2012)**, the last two types of PBRs (i.e., bubble columns and airlift) have a small surface-area-to-volume ratio, which makes them ineffective for massive algal biomass productivity (**Mirón *et al.*, 1999)**. In the flat-plate PBR, the polygonal shape of the reactor allows the ability to control the cultivation chamber until reaching the lowest light path and the highest surface-area-to-volume ratio **(Kumar *et al.*, 2011)**. Flat-plate PBR is also characterized by low dissolved oxygen accumulation and high photosynthetic efficiency due to large-illuminated surface area **(Cañedo and Lizárraga, 2016)**.

There is an urgent need to modify and improve the design of the photobioreactors in a manner that is appropriate to solve different problems and challenges, the most important of which is to reduce carbon dioxide emissions. The goal of this study was to improve the design of the flat-plate photobioreactor to increase the holding time (i.e., availability) of the carbon dioxide in aqueous culturing media to increase gas consumption as well as the growth rate of the autotrophic cyanobacterium *Synechococcus elongatus*, these improvements were achieved by adding mutually inverted baffles to capture an appropriate volume of the gas for as long as possible, enabling the cyanobacterium to consume CO_2 more efficiently.

Materials and Methods

1. Cultivation of *S. elongatus*

The cyanobacterium *S. elongatus* was obtained from the Algae Culture Collection at Al-Azhar University (ACCAZ), Cairo, Egypt. The strain was cultivated in a sterilized BG_{11} medium **(Allen 1968; Allen and Stanier 1968; Rippka *et al.*, 1979)**, and the culture was incubated at 27 °C and illuminated by a cool white fluorescent lamp at 5000 Lux for 12/12 h dark-light cycles. BG_{11} medium consists of macronutrient components including NaNO3 (1500 mg L^{-1}), K_2HPO_4 (40 mg L^{-1}), $MgSO_4.7H_2O$ (75 mg L^{-1}), $CaCl_2.7H_2O$ (36 mg L^{-1}), citric acid (6 mg L^{-1}), Na_2CO_3 (20 mg L^{-1}), Na_2EDTA (1 mg L^{-1}), ferric ammonium citrate (6 mg L^{-1}) and micronutrient stock solution (1 ml stock per 1 L of media), the final pH was adjusted to 7.4.

2. Growth measurements (Cell count)

S. elongatus cells (Fig. 1A and B) were counted by hemocytometer and the average of cells was calculated from count counting five small squares and the final cell count (cell ml^{-1}) was calculated using the following equation:

$$\text{Cell count} = \frac{\text{Average cells per small square} \times \text{Dilution factor}}{\text{The volume of a small square (mL)}}$$

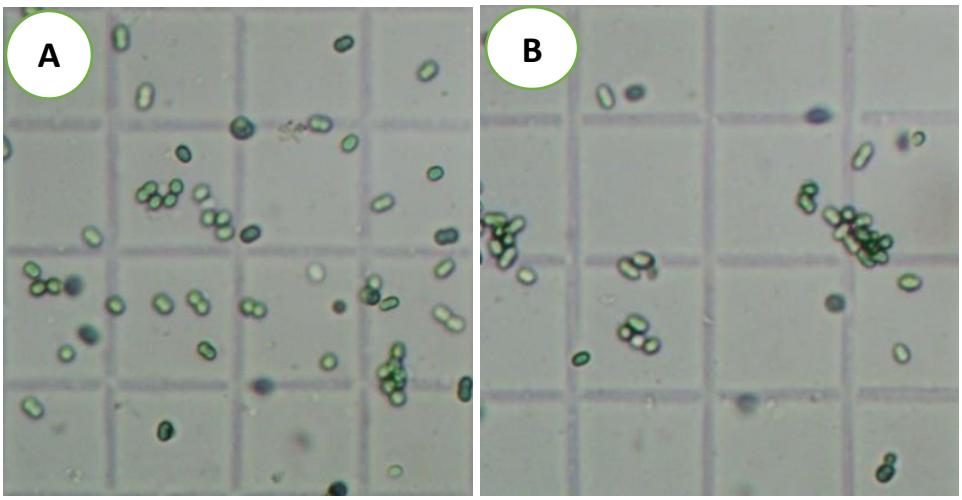

Fig. 1 (A and B): Cell count of the cyanobacterium *S. elongatus* cells

3. Supply of exogenous CO_2 gas

Carbon dioxide gas was obtained from Obour Company for filling gases in a cylinder with a 6 kg weight. The gas was bubbled at a flow rate of 0.25 L min^{-1}. *pH* was measured periodically every hour and adjusted to an optimal level of 8.0 when necessary.

4. Determination of CO_2 content

The CO_2 content was determined by the acid-base titrimetric method (**APHA, 2017)** using phenolphthalein indicator at an endpoint of pH 8.3. In the case of acid titrant, the CO_2 content was calculated as the following:

$$\text{mg } CO_2\ (L^{-1}) = \frac{A \times N \times 44000}{\text{mL sample}}$$

where, A = mL titrant, and N = normality of NaOH.

While the CO_2 content was calculated in the case of alkaline titrant using the following equations:

A. Bicarbonate alkalinity

$$HCO_3^{-} \text{ as mg } CaCO_3\ L^{-1} = \frac{T - 5.0 \times 10^{(pH-10)}}{1 + 0.94 \times 10^{(pH-10)}} \quad \ldots \text{ (A)}$$

where, T = total alkalinity, mg $CaCO_3$ L^{-1}.

B. Carbonate alkalinity

$$CO_3^{2-} \text{ as mg } CaCO_3\ L^{-1} = 0.94 \times A \times 10^{(pH-10)} \quad \ldots \text{ (B)}$$

C. Free carbon dioxide

$$\text{mg } CO_2\ L^{-1} = 2.0 \times A \times 10^{(6-pH)} \quad \ldots \text{ (C)}$$

D. Total carbon dioxide

$$\text{mg total } CO_2\ L^{-1} = C + 0.44\ (2A + B)$$

5. Modified flat-plate PBR: design and setup

The modified flat-plate PBR (Fig. 2) was made of 6 mm thick glass panels. The dimensions of the polygonal culturing chamber were 35 cm high, 55 cm wide, and 10 cm deep. The culturing chamber was covered with an airtight glass plate. The culturing chamber and the cover had slots for the source of carbon dioxide, gas exhaust, thermostat, fresh media supplement, and culture sampling. The CO_2 cylinder was connected to a gas humidifier unit which contained an air pump to distribute the gas to the culturing chamber through gas spargers to achieve maximum CO_2 dissolution. All components and units were connected by silicone hoses and a 0.22 µm membrane air filter before the culturing chamber.

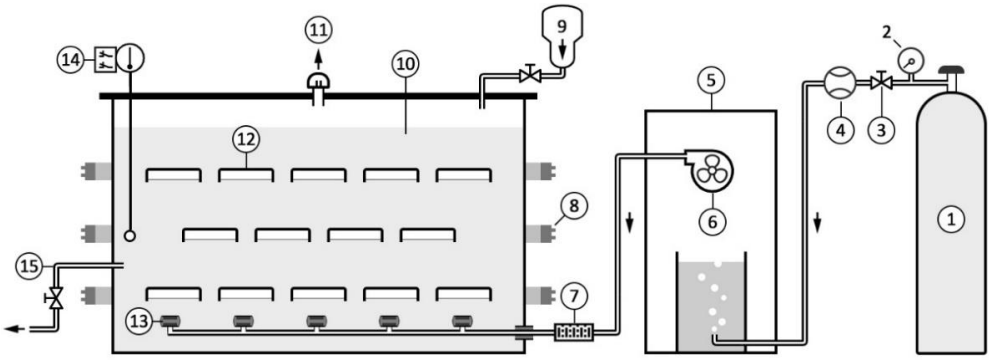

Fig. 2: Schematic diagram of the modified flat-plate PBR showing the external CO_2 flow and distribution of the 14 inverted U-shaped baffles used for CO_2 capture. (1) compressed CO_2 gas cylinder, (2) pressure gauge, (3) flue gas valve, (4) gas flow rate meter, (5) CO_2 gas humidifier unit, (6) air pump, (7) a 0.22 μm membrane air filter, (8) artificial cool-white LED light, (9) sterile fresh medium inlet, (10) front view of the culturing chamber, (11) gas exhaust, (12) inverted U-shaped baffle, (13) gas sparger, (14) thermostat connected to a temperature sensor, and (15) sampler outlet. Arrows indicating the flow path of CO_2 gas and liquid BG_{11} medium.

6. Experimental Design

A total of six culturing chambers (two replicates for each treatment group) were used (Fig. 3A and B). The inverted baffles were distributed as follows: group "A", each culturing chamber has 14 inverted baffles distributed alternatively in three lines (i.e., 5, 4, and 5 baffles) to hold about 2000 ml volume of CO_2; group "B", each chamber has 7 baffles distributed in two lines (i.e., 4 and 3 baffles) to hold about 1000 ml volume of CO_2; and group "C" with no baffles as

a control to exclude the effect of the baffles. All chambers were illuminated by artificial cool-white LED lights with a dark-light cycle.

It was necessary first to determine the changes in the CO_2 content based on the number of baffles, so the experiment went through three stages, stage I to determine the saturation time, stage II to determine the gas stability time in the aqueous media, and stage III to determine the CO_2 bio-fixation ability by *S. elongatus.*

6.1. Stage I: CO_2 saturation time

The objective of this experiment was to determine the time at which the carbon dioxide gas reaches the saturation state in the medium and its relation to the number of baffles. The hypothesis was that the higher the number of baffles, the faster the saturation state to due increasing the contact time between the trapped gas and the aqueous media. The carbon dioxide content was measured at intervals of 1, 5, 10, 20, 30, 40, 60, 80, 120, 150, and 180 minutes within each group (i.e., 14, 7, and no baffles).

6.2. Stage II: CO_2 stability time

This experiment aimed to determine the stability period of carbon dioxide gas in the aqueous medium after reaching the saturation state, depending on the number of baffles. We hypothesized that the higher the number of baffles, the longer the stability period due to preventing the gas from being released quickly from the aqueous media. After reaching the saturation state the gas pumping stopped and the carbon dioxide content was measured at 1, 2, 3, 4, 5, and 6 hrs.

6.3. Stage III: Effect of baffles on CO_2 bio-fixation

After studying the saturation and stability of CO_2, the effects of baffles on the algal growth and bio-fixation of CO_2 were studied. Equal amounts of *S. elongatus* culture were inoculated into 12 L of freshly prepared BG_{11} medium (Fig. 3C) per chamber within each treatment. the conditions were adjusted according to the previous optimization experiments. Cell count (cell ml^{-1}) and total CO_2 content (mg L^{-1}) were measured daily for 10 days.

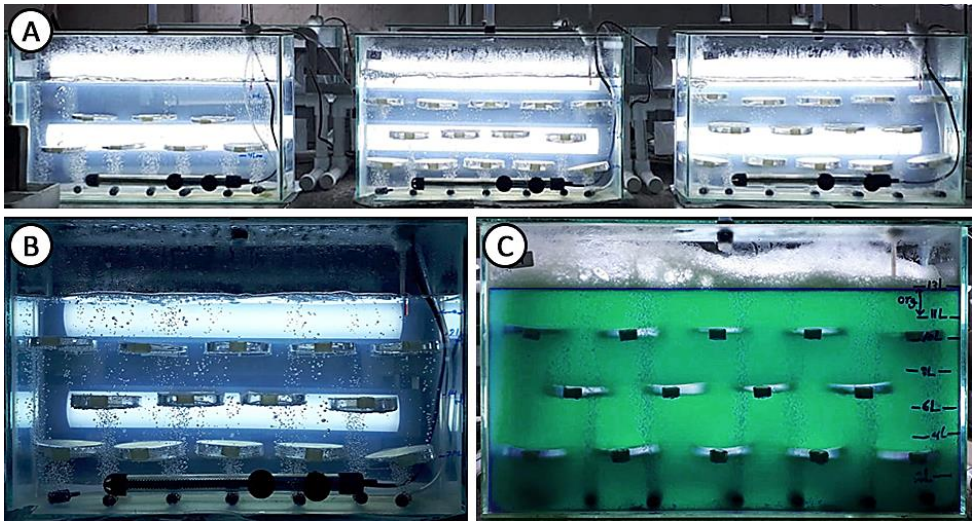

Fig. 3: Modified flat-plate PBR. (A) PBRs arrangement and setup, (B) a closer view illustrating the internal structure, and (C) a running experiment.

7. Statistical analysis

All CO_2 saturation, stability, and bio-fixation experiments followed a typical two-way experimental design with one fixed (number of baffles) and one repeated measure (RM: time) factor. Two-way Analysis of Variance (ANOVA) with a one-repeated measure factor was applied to determine the significance of main and interaction effects within and between factors. Tukey's post hoc test was applied for multiple pairwise comparisons between levels within and between factors. The significance was tested at $\alpha = 0.05$. In the CO_2 stability experiment, the strength and direction of gas loss between factors were determined by generating a linear regression equation associated with the coefficient of determination (R^2) to determine the fit of the regression model to the data using a rating of 0 to 1. The Minitab® 18.1 statistical software package was used for the statistical data analyses.

Results and Discussion

1. CO_2 saturation time

The results (table 1 and Fig. 4) showed a significant difference in the main and interaction effects of baffles number and time. In the 14-baffle chambers, the gas saturation occurred after approximately 60 min, and a maximum value of 370.5 mg L^{-1} of CO_2 content was recorded after 80 min, while in the case of 7-baffle chambers the gas saturation occurred at 356 mg L^{-1} of CO_2 content after 120 min. In the chambers with no baffles, the saturation occurred after 60 min but with a significantly lower CO_2 content at 231.4 mg L^{-1}. The

results revealed that the presence of baffles increases the contact time between the trapped gas and aqueous media which leads to quickly reaching a state of saturation with a higher content of carbon dioxide compared to the absence of baffles.

Table 1: Two-way ANOVA with one RM factor for testing the significant differences in CO_2 saturation time between baffles number (fixed factor) and time of exposure to CO_2 (RM factor).

Source of Variation	DF	SS	MS	F	*p*-Value
Baffles Number (A)	2	133691	66845.4	1255.27	<0.001
Subject (Baffles Number)	3	159.755	53.252		
Exposure Time (B)	10	701940	701940	451.191	<0.001
A × B	20	61286.3	3064.31	19.6970	<0.001
Residual	30	4667.25	155.575		
Total	65	901744	138730		

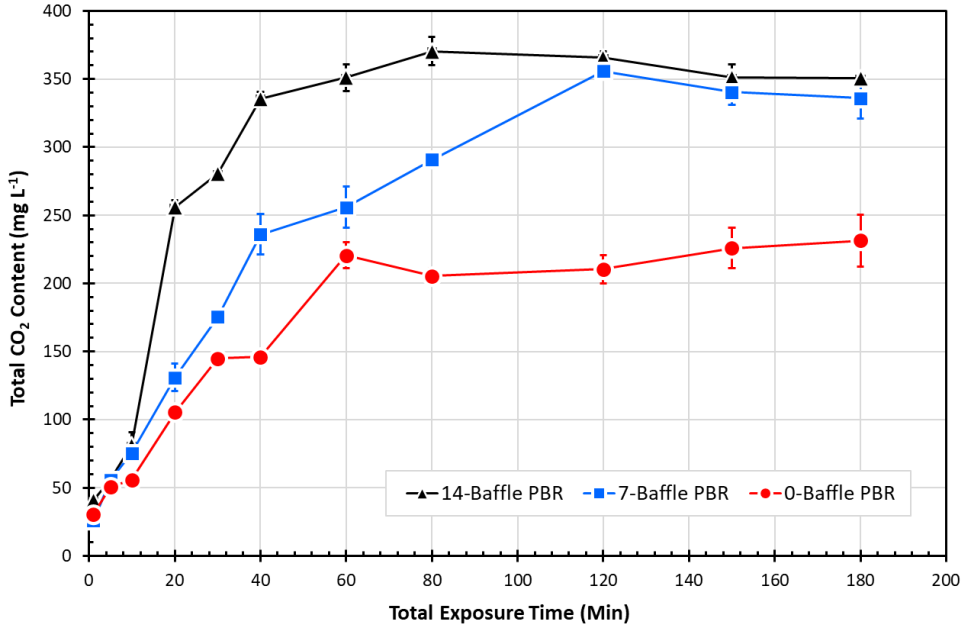

Fig. 4: Results of total CO_2 content (mg L^{-1}) at different numbers of baffles and different exposure time (min) to determine the time to reach the gas saturation state. Error bars represent the Standard Error (±SE).

2. CO_2 stability time

The results (table 2 and Fig. 5) showed that, in general, the presence of baffles significantly reduced the rate and quantity of gas loss (i.e., increased the time of gas availability) in the aqueous medium, while gas loss increased in the chambers with no baffles. The slope of the regression line (Fig. 4) showed a lower rate of CO_2 loss in the chambers with 14 baffles (-29.99; $R^2 = 0.96$) compared with 7-baffles (-52.0; $R^2 = 0.97$) and no baffles (-40.57; $R^2 = 0.99$) chambers.

Table 2: Two-way ANOVA with one RM factor for testing the significant differences in CO_2 stability period between baffles number (fixed factor) and time of resting (RM factor) after reaching the CO_2 saturation.

Source of Variation	DF	SS	MS	F	*p*-Value
Baffles Number (A)	2	130403	65201.7	138.34	0.001
Subject (Baffles Number)	3	1413.95	471.316		
Resting Time (B)	5	177737	35547.5	95.286	<0.001
A × B	10	108320	1083.20	2.9040	0.031
Residual	15	5595.91	373.06		
Total	35	325983	9313.79		

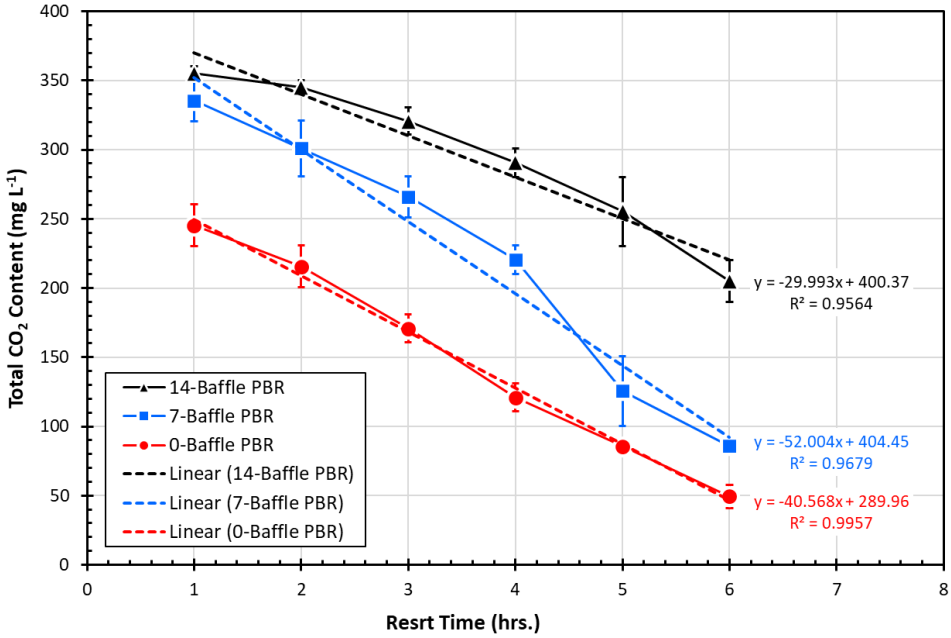

Fig. 5: Results of total CO_2 content (mg L^{-1}) at different numbers of baffles and different resting time (hrs.) to determine the stability period of CO_2 gas in the aqueous medium after reaching the saturation state. Error bars represent the Standard Error (±SE). The dashed line represents the linear regression fitting.

3. Effect of baffles on CO_2 bio-fixation

The baffles significantly affected the growth of *S. elongatus* (Table 3 and Fig. 6) and CO_2 bio-fixation (Table 4 and Fig. 6). The presence of 14 baffles accelerated the growth rate of *S. elongatus*, reaching the start of the stationary phase with high growth rates after five days, in which the number of cells was recorded up to 2133×10^4 cell ml^{-1}, and reached the highest level at 2435×10^4 cell ml^{-1} after seven days. In the 7-baffles chambers, *S. elongatus* reached the start of the stationary phase after eight days and recorded the maximum growth at 2425×10^4 cell ml^{-1} after nine days. The absence of baffles significantly reduced the growth of *S. elongatus*, where the maximum growth at 1462×10^4 cell ml^{-1} was recorded after ten days.

It is known that an increase in the growth rates of autotrophs leads to an increase in carbon dioxide consumption during photosynthesis. However, in the case of 14-baffles chambers, although growth rates were high after five days, the presence of these baffles kept the amount of carbon dioxide at higher levels, due to increasing the contact time between the trapped gas and the *S. elongatus* culture and reduced the rate of gas loss to make it available for longer periods when compared to the 7-baffles and 0-baffles chambers. (Fig. 5).

Table 3: Two-way ANOVA with one RM factor for testing the significant differences in cell count (cell ml^{-1}) between baffles number (fixed factor) and time of incubation (RM factor).

Source of Variation	DF	SS	MS	F	*p*-Value
Baffles Number (A)	2	6927711.5	3463855.8	460.52	<0.001
Subject (Baffles Number)	3	22564.955	7521.652		
Time (B)	10	34060527	3406052.7	166.47	<0.001
A × B	20	3984270.2	199213.51	9.7370	<0.001
Residual	30	613808.55	20460.285		
Total	65	45608882	701675.11		

Table 4: Two-way ANOVA with one RM factor for testing the significant differences in total CO_2 content (mg L^{-1}) between baffles number (fixed factor) and time of incubation (RM factor).

Source of Variation	DF	SS	MS	F	*p*-Value
Baffles Number (A)	2	179589.74	89794.871	397.69	<0.001
Subject (Baffles Number)	3	677.37	225.79		
Time (B)	10	144926.47	14492.647	162.49	<0.001
A × B	20	9789.062	489.453	5.4880	<0.001
Residual	30	2675.771	89.192		
Total	65	337658.41	5194.745		

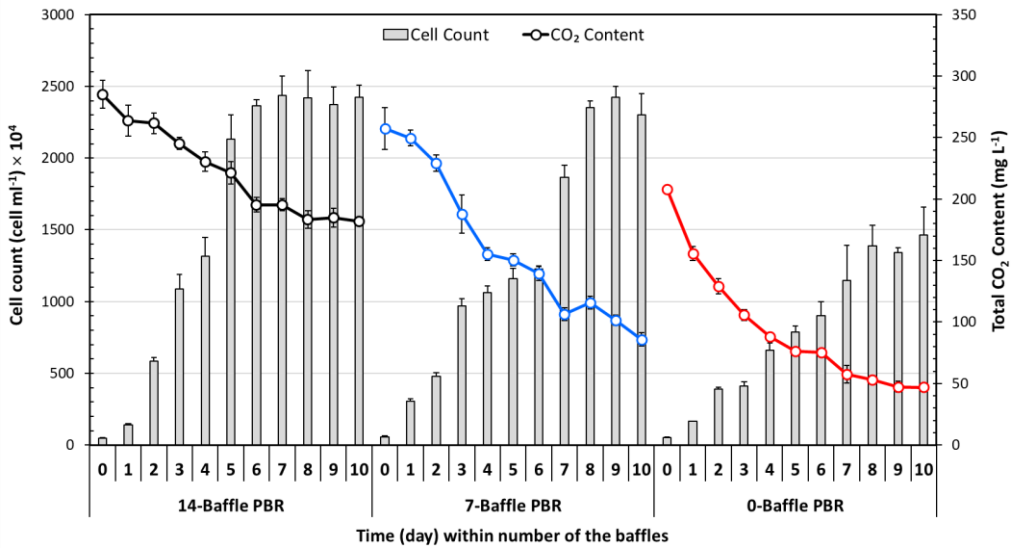

Fig. 6: Results of cell count (cell ml^{-1}) and associated total CO_2 content (mg L^{-1}) at different numbers of baffles and different incubation time (days), and their effects on the growth and bio-fixation ability of *S. elongatus*. Error bars represent the Standard Error (±SE).

Autotrophic microorganisms have a high ability to utilize carbon dioxide and convert it into useful energy **(Gao *et al.*, 2010; Phukan *et al.*, 2011; Cheng *et al.*, 2013; Raeesossadati *et al.*, 2014)**. This study aimed to improve carbon

dioxide bio-fixation by growing the cyanobacterium *S. elongatus* in a modified flat-plate closed photobioreactor as an eco-friendly solution to reduce carbon dioxide emissions. Flat-plate PBR has been widely used for microalgae culturing due to its advantages of large surface-area-to-volume ratio, efficient light distribution, simple structure, flexibility of use, and easy biomass harvesting **(Sierra *et al.*, 2008; Xu *et al.*, 2009; Wang and You, 2013).**

Several studies were concerned with improving the efficiency of Flat-plate PBR by modifying the design for use in different purposes. **Degen *et al.*, (2001)** proposed a new PBR design that uses baffles to induce regular light cycling. The results revealed that the productivity in the improved baffled reactor increased by 1.7 times which means that baffles can significantly enhance the growth of microalgae. **Wang *et al.*, (2014)** added horizontal baffles to a flat-plate photobioreactor to improve flow, mixing, and light utilization efficiency. The results showed that horizontal baffles improved the algal biomass productivity and uniform growth conditions inside the photobioreactor. **Guo *et al.*, (2020)** applied alternatively permuted conical baffles in the raceway pond (an open PBR) to enhance the flashing light frequency and CO_2 fixation rate by creating a vortex movement. The results indicated significant increases in the light fraction during the light/dark cycle, and an increase in the rate of carbon dioxide fixation.

From the previously mentioned studies, the benefits of including baffles in the designs of PBRs were uniform growth conditions, improve illumination cycling, and CO_2 fixation rate due to the constant uniform turbulent force caused by the presence of baffles. In the current study, we used a novel strategy to improve the bio-fixation of CO_2 by modifying the design of the closed flat-plate PBR to increase its efficiency in retaining carbon dioxide gas as long as possible in the liquid medium and thus increasing the gas availability and utilization during

photosynthesis. The modification was made by adding mutually inverted U-shape baffles to the PBR and arranging it in many rows to capture an appropriate volume of the CO_2 gas to enable *S. elongatus* to consume carbon dioxide more efficiently. The effects of adding baffles went through three stages, including the determination of saturation time, the gas stability time, and the CO_2 bio-fixation ability by *S. elongatus*.

The results of the saturation and stability experiments indicated that optimal saturation occurred after 60-80 minutes and that the gas stabilization was for about 2 hours, therefore, in the bio-fixation experiment, to obtain the highest efficacy of the baffles, the CO_2 gas was pumped into the chambers (during the illumination periods) for 80 minutes, then the pumping stopped for 2 hours, repeatedly. The results of the bio-fixation experiment showed that the presence of 14 baffles increased the contact time between the trapped carbon dioxide and the *S. elongatus* culturing media, in addition to preventing the rapid release of CO_2 gas from the aqueous medium, and thus increasing the availability of carbon dioxide gas, which led to increasing the growth of *S. elongatus*.

Conclusion

The main objective of this study was to improve the efficiency of the closed flat plate PBR in retaining carbon dioxide for as long as possible so that the autotrophic microorganisms can use it in a higher quantity during photosynthesis. In this study, the properties of CO_2 gas in aqueous media were studied based on the effect of mutually inverted U-shape baffles added to closed flat plate PBR. The results illustrated that the presence of the baffles led to a significant increase

in the gas concentration, an increase in its stability period, and an increase in its consumption, which subsequently led to a significant increase in biomass productivity. This study recommends adding mutually inverted U-shape baffles to the closed flat plate PBR to increase its efficiency in reducing carbon dioxide emissions from vehicle exhaust and plant output.

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تحسين التثبيت الحيوي لثاني أكسيد الكربون بواسطة البكتيريا الخضراء المزرقة سينيكوكوكس ايلونجاتس بواسطة مفاعل حيوي ضوئي – مُعدل – من النوع المغلق

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يعد الاحتباس الحراري بسبب انبعاثات ثاني أكسيد الكربون أحد أهم المشاكل البيئية في القرن الحالي، لذلك هناك حاجة ملحة للبحث عن استراتيجيات فعالة صديقة للبيئة لتقليل مخاطرها. تم استخدام الكتلة الحيوية للكائنات الدقيقة ذاتية التغذية لتقليل انبعاثات ثاني أكسيد الكربون وإنتاج كتلة حيوية مفيدة وذات قيمة اقتصادية. في الدراسة الحالية، تم استخدام استراتيجية جديدة لتحسين قدرة التثبيت الحيوي لثاني أكسيد الكربون في البكتيريا الخضراء المزرقة *سينيكوكوكس ايلونجاتس*، عن طريق تعديل تصميم المفاعل الحيوي الضوئي ذي اللوحة المسطحة المغلقة بإضافة حواجز على شكل (U) مقلوبة ومتبادلة لزيادة كفاءة حجز غاز ثاني أكسيد الكربون في الوسط السائل، وبالتالي زيادة توافر الغاز واستخدامه أثناء عملية التمثيل الضوئي. أشارت نتائج تجارب التشبع والثبات إلى أن التشبع الأمثل حدث بعد 60-80 دقيقة وأن استقرار الغاز كان لمدة ساعتين تقريباً. أظهرت نتائج تجربة التثبيت الحيوي للغاز أن وجود أربعة عشر حاجزاً زاد بشكل كبير من وقت التلامس بين ثاني أكسيد الكربون المحجوز ووسط استزراع *سينيكوكوكس ايلونجاتس*، بالإضافة إلى منع الفقد السريع لثاني أكسيد الكربون من الوسط السائل، وبالتالي زيادة توافر ثاني أكسيد الكربون، مما أدى إلى زيادة إنتاجية الكتلة الحيوية في *سينيكوكوكس ايلونجاتس*.