

Synergistic Impacts and Optimization of Gas Flow Rate, Concentration of CO₂, and Light Intensity on CO₂ Biofixation in Wastewater Medium by *Chlorella vulgaris*

Ahmed Arkoazi, Hussein Znad, Ranjeet Utikar

Abstract—The synergistic impact and optimization of gas flow rate, concentration of CO₂, and light intensity on CO₂ biofixation rate were investigated using wastewater as a medium to cultivate *Chlorella vulgaris* under different conditions (gas flow rate 1-8 L/min), CO₂ concentration (0.03-7%), and light intensity (150-400 μmol/m².s)). Response Surface Methodology and Box-Behnken experimental Design were applied to find optimum values for gas flow rate, CO₂ concentration, and light intensity. The optimum values of the three independent variables (gas flow rate, concentration of CO₂, and light intensity) and desirability were 7.5 L/min, 3.5%, and 400 μmol/m².s, and 0.904, respectively. The highest amount of biomass produced and CO₂ biofixation rate at optimum conditions were 5.7 g/L, 1.23 gL⁻¹d⁻¹, respectively. The synergistic effect between gas flow rate and concentration of CO₂, and between gas flow rate and light intensity was significant on the three responses, while the effect between CO₂ concentration and light intensity was less significant on CO₂ biofixation rate. The results of this study could be highly helpful when using microalgae for CO₂ biofixation in wastewater treatment.

Key words—Synergistic impact, optimization, CO₂ biofixation, airlift reactor.

I. INTRODUCTION

CARBON dioxide (CO₂) mitigation and wastewater treatment have attracted significant attention due to the fact that CO₂ is one of the major causes of global warming. In addition, nutrients (nitrogen and phosphorous) discharge without a control lead to serious issues in aquatic ecosystems related to water resources pollution [1]. Biotechnology based on the use of microalgae has been studied extensively due to their capability to generate valuable products, mitigate CO₂, and treat wastewater [2]. Microalgae fix CO₂ more efficiently compared with terrestrial plants [3] and grow by absorbing the nutrients in wastewater even at high concentrations of the nutrient [1]. Several studies have investigated the impact of light intensity, concentration of CO₂, and aeration rate on CO₂ biofixation as well as the capability of microalgae to remove nutrients from different types of wastewater [4].

CO₂ biofixation using microalgae is affected by the CO₂ concentration in the injected gas to the photobioreactor, light intensity, microalgae species, and photobioreactor

configuration [5]. Naderi et al. [6] studied the effect of light intensity on *Chlorella vulgaris* growth and CO₂ biofixation in a stirrer photobioreactor with 2% CO₂ concentration and a light intensity of 30-300 μmol/m².s. Their result was 0.45 gCO₂.L⁻¹.d⁻¹ CO₂ biofixation rate at a light intensity of 100 μmol/m².s. Cheng et al. [7] investigated the effect of CO₂ concentration in the inlet gas at 0.05% to 1% on CO₂ biofixation using *Chlorella vulgaris* in an air lift reactor with a light intensity range from 23.6 to 236.4 μmol/m².s. They obtained 260 mg/l.hr biofixation rate at 1% CO₂ concentration and a light intensity of 157.6 μmol/m².s. The impact of gas flow rate on CO₂ biofixation rate in a raceway pond was studied by Cheng et al. [8]. They found that gas flow rate had a significant influence on *Nannochloropsis oculata* growth and the biofixation rate of CO₂ increased from 26.3 to 31.9 g.m⁻².d⁻¹ with an increased gas flow rate from 50 to 150 m³/hr. In another study by Yoo et al. [9], the ability of *Botryococcus braunii*, *Chlorella vulgaris*, and *Scenedesmus* sp. were investigated for CO₂ biofixation at a light intensity of 150 μmol/m².s and 10% CO₂ concentration. They reported that *Scenedesmus* sp. was the most efficient species for CO₂ mitigation due to its having the highest biomass productivity and biofixation rate ability. Despite of considerable researches, optimum conditions for CO₂ biofixation have not yet been adequately established.

Algal wastewater treatment is achieved through the cultivation of microalgae by utilizing nutrients (nitrogen and phosphorus) and other pollutants in the wastewater. Wastewater treatment using microalgae offers advantages such as reduced heavy metal concentration, CO₂ emission through biofixation and processing costs, as well as generation of valuable products [10]. Microalgae can efficiently remove pollutants, toxic compounds, and accumulated heavy metals from industrial and municipal wastewater to produce a biomass that is used for production of biofuel [11]. Several studies [12]-[17] have investigated the capability of microalgae for wastewater treatment.

Gas aeration is an important parameter that influences photobioreactor hydrodynamics, mixing performance and cell exposure to light, and prevents gradients of nutrients concentration inside the photobioreactor. Hence, aeration rate (gas flow rate and CO₂ concentration) is a crucial parameter for enhancing microalgae growth [18]. High aeration rate has shown unfavourable influence on the process cost, especially

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on large-scale biomass production process [19]. Moreover, there is a need to identify the optimum level of aeration sufficient for microalgae growth, CO₂ biofixation and nutrient removal. In this study, the synergistic impact of the aeration rate (gas flow rate and concentration of CO₂) and the light intensity on the CO₂ biofixation in wastewater medium using *Chlorella vulgaris* was investigated and optimized by applying Response Surface Methodology and Box-Behnken Design.

II. METHODS AND MATERIALS

A. Microalgae and Conditions of Culture

Chlorella vulgaris (strain: CCAP 211/11B, CS-42) was used in this study. The microalgae cells were grown in an MLA medium [20]. The algal cells were cultivated in primary wastewater for 10 days in a draft tube airlift photobioreactor, 100 cm in height and 10 cm in diameter, with a draft tube height of 60 cm and a diameter of 6 cm. CO₂-enriched air was bubbled into the photobioreactor through a perforated plate sparger.

B. Calculation of Biomass Productivity and CO₂ Biofixation Rate

Productivity of biomass was calculated (P_x) using:

$$P_x = \frac{X_t - X_0}{t} \quad (1)$$

where P_x is biomass productivity (g.L⁻¹.D⁻¹), X_0 is initial biomass concentration (g.L⁻¹), X_t is concentration of biomass when the cultivation finished (g.L⁻¹), and t is the time of cultivation.

CO₂ biofixation rate is calculated as:

$$CO_2 \text{ biofixation rate } R_{CO_2} \text{ (g.L}^{-1}\text{d}^{-1}\text{)} = \%C \times P_x \times \frac{MW_{CO_2}}{MW_C} \quad (2)$$

where %C is the percentage of carbon measured using an element analyser, P_x is biomass productivity (g.L⁻¹.d⁻¹), MW_{CO_2} is molar mass of CO₂, and MW_C is molar mass of carbon.

C. Design of Experiments and Regression Analysis

Box Behnken Design was utilized for optimizing the performance of microalgae cultivation based on CO₂ biofixation. A matrix of three-parameter with multi-level was employed to investigate the synergistic influence among the three parameters (gas flow rate X_1 , CO₂ concentration X_2 , and light intensity X_3). To obtain these goals, 15 experiments will be accomplished by varying gas flow rate, concentration of CO₂, and light intensity from 150 to 400 μE/m².s as shown in Table I.

TABLE I
EXPERIMENTAL RANGE OF INDEPENDENT VARIABLES

Independent variable	Symbol	Range and level		
		-1	0	+1
Gas flow rate Q (L/min)	X_1	1	4.5	8
CO ₂ concentration (%)	X_2	0.03	3.5	7
Light intensity I (μmol/m ² .s)	X_3	150	275	400

The variables' ranges were selected depending on a literature search [21], [22], [7], [4].

The quadratic model utilized to estimate the maximum response is expressed as:

$$Y = \beta_0 + \sum_{i=1}^k \beta_{ii} y_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where Y is the response; i and j are the patterns index numbers; β_0 is the constant coefficient; β_i , β_{ii} and β_{ij} are the regression coefficients for linear, quadratic, and interaction impact, respectively; x_i and x_j are the investigated factors, and the coded parameters are determined as:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (4)$$

where x_i and X_i are the coded and actual values of the independent factors, X_0 is the value of actual factor at the centre point, and ΔX_i is the value of step change. The design of multi-level experiments (minimum and maximum) used ranges of independent variables: gas flow rate (1-8 L/min), CO₂ concentration (0.03-7 %), and light intensity (150-400 μmol/m².s).

A set of 15 experiments, were randomly carried out in triplicate to minimize the influence of errors to identify the ten terms of the 2nd order equation (3). JMP software (SAS version 14.0.0) was employed to complete the analysis of regression and to draw the 3D graphs and the contours. The factors variability was expressed as the multiple terms of calculating (R²) values, and the equation of model was employed for predicting the optimum point and to determine the interaction among the parameters within the identified boundary conditions of experiments.

III. RESULTS AND DISCUSSION

A. Multiple Regression Analyses

TABLE II
ACTUAL LEVELS OF BOX BEHNKEN DESIGN MATRIX WITH EXPERIMENTAL AND PREDICTED RESPONSE VALUES

Run	Independent variables			R_{CO_2} (g.L ⁻¹ .d ⁻¹)	
	X_1	X_2	X_3	Experimental	Predicted
1	1	0.03	275	0.425	0.393
2	1	7	275	0.625	0.6395
3	8	0.03	275	0.752	0.737
4	8	7	275	0.657	0.688
5	4.5	0.03	150	0.372	0.41
6	4.5	0.03	400	0.6	0.607
7	4.5	7	150	0.497	0.489
8	4.5	7	400	0.764	0.725
9	1	3.5	150	0.812	0.804
10	8	3.5	150	0.814	0.789
11	1	3.5	400	0.784	0.808
12	8	3.5	400	1.21	1.217
13	4.5	3.5	275	0.954	0.954
14	4.5	3.5	275	0.954	0.954
15	4.5	3.5	275	0.954	0.954

R_{CO_2} = CO₂ biofixation rate.

Multiple analysis of regression was used to specify the relationships of the response variable of CO₂ biofixation rate with respect to concentration of CO₂, gas flow rate, and light intensity, which generated polynomial equations of second-order from Box Behnken Design matrix of experimental data as shown in Table II.

The results from the regression formulae were:

$$\text{CO}_2 \text{ biofixation rate} = 0.945 + 0.098375x_1 + 0.04925x_2 + 0.107875x_3 - 0.07375x_1x_2 + 0.106x_1x_3 + 0.00975x_2x_3 + 0.00375x_1^2 - 0.343x_2^2 - 0.05275x_3^2 \quad (5)$$

where x_1 , x_2 , and x_3 are coded values calculated using (4). The determination coefficient (R^2) for CO₂ biofixation rate was 0.99. Therefore, polynomial equation (5) can appropriately represent the parameters and responses relationship. The predicted response from (5) was in line with the data achieved experimentally as shown in Fig. 1.

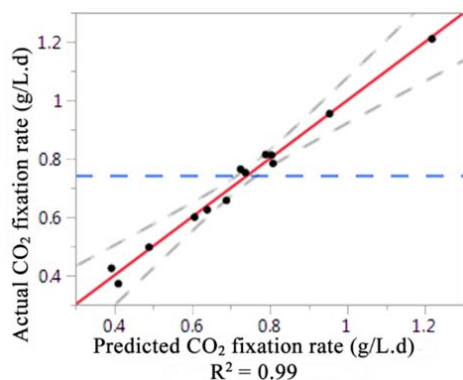


Fig. 1 Experimental and predicted data Comparison of CO₂ biofixation rate, (.) experimental values, (---) confidence bands, and (—) fit line

B. Analysis of Variance (ANOVA)

ANOVA was applied to determine the significance of each term (linear, interactive, and quadratic) of the second-order polynomial models as shown in Table III. The P -value indicates the importance of each term (i.e. $p < 0.05$ indicates that the term is significant).

TABLE III
ANOVA ANALYSIS FROM BOX BEHNKEN DESIGN

Term	Estimate	Std Error	T-Value	P-Value
CO ₂ biofixation rate				
Intercept	0.954	0.021359	-	-
x_1	0.098375	0.01308	7.52	0.0007*
x_2	0.04925	0.01308	3.77	0.0131*
x_3	0.107875	0.01308	8.25	0.0004*
$x_1 \cdot x_2$	-0.07375	0.018498	-3.99	0.0105*
$x_1 \cdot x_3$	0.106	0.018498	5.73	0.0023*
$x_2 \cdot x_3$	0.00975	0.018498	0.53	0.6207
x_1^2	0.00375	0.019253	0.19	0.8532
x_2^2	-0.343	0.019253	-17.82	<.0001*
x_3^2	-0.05275	0.019253	-2.74	0.0408*

Table III shows that the linear term coefficients ($\% \text{CO}_2$ and I), coefficients of quadratic term ($\% \text{CO}_2^2$ and I^2), and coefficients of interactive term (Q , $\% \text{CO}_2$ and $Q \cdot I$) significantly influence the CO₂ biofixation rate. In addition, CO₂ biofixation rate and total nitrogen removal efficiency were significantly impacted by gas flow rate, CO₂ concentration, and light intensity with a p -value less than 0.05 (Table III). The findings showed that the responses of CO₂ biofixation rate were significantly influenced by the synergistic effect of (Q , $\% \text{CO}_2$ and $Q \cdot I$) with p -value < 0.05 , while ($\% \text{CO}_2 \cdot I$) synergistic effect was less significant (p -value = 0.62) on CO₂ biofixation rate.

C. Box Behnken Design Analysis

The impacts of gas flow rate, concentration of CO₂, and light intensity on CO₂ biofixation rate are shown in Fig. 2. Fig. 2 illustrates response surfaces (3D) and contours (2D) for CO₂ biofixation rate. These graphs clearly show the interaction impacts of all three variables on the responses. In Fig. 2, one factor was fixed at level zero while changing the other two factors according to the experiential ranges. Increasing corresponding variables led to enhance responses to a certain level after which the responses decreased, although there was an increase in corresponding variables. This behaviour was aligned with the results of the ANOVA shown in Table III, which demonstrated a significant influence of the quadratic terms of all three parameters on CO₂ biofixation rate.

Fig. 2 (a) shows the impact of concentration of CO₂ and gas flow rate on CO₂ biofixation rate. Increasing concentration of CO₂ led to an increase in the rate of CO₂ biofixation due to the microalgae cells consuming CO₂ in order to store and use it during times of nutrient deficiency [20]. Gas aeration rate had a significant influence on CO₂ biofixation rate due to its using aeration as a carbon source improved mixing that enhanced utilization of light by the cells [23]. CO₂ biofixation rate increased with increased gas aeration rate for all gas flow rates used in this study. However, Han et al. [24] reported that a gas flow rate higher than 9 L/min caused damage to cells due to higher turbulence. Fig. 2 (b) illustrates the influence of concentration of CO₂ and light intensity on CO₂ biofixation rate. And CO₂ biofixation rate increased with increased light intensity until it reached an optimal value. Cheah et al. [25] concluded that the photosynthetic process was enhanced by using low and moderate light intensity due to photoinhibition by higher high light intensity. Ho et al. [26] studied the optimization and characterization of the production of carbohydrate using *Chlorella vulgaris*. They found that the optimal light intensity was 450 $\mu\text{mol}/\text{m}^2 \cdot \text{s}$ and that any further increase in light intensity led to a decrease in biomass production. Present findings are in agreement with these previous findings. The present findings show that synergistic effect of concentration of CO₂, gas flow rate, and light intensity could enhance the CO₂ biofixation and microalgae growth.

D. Model Validation

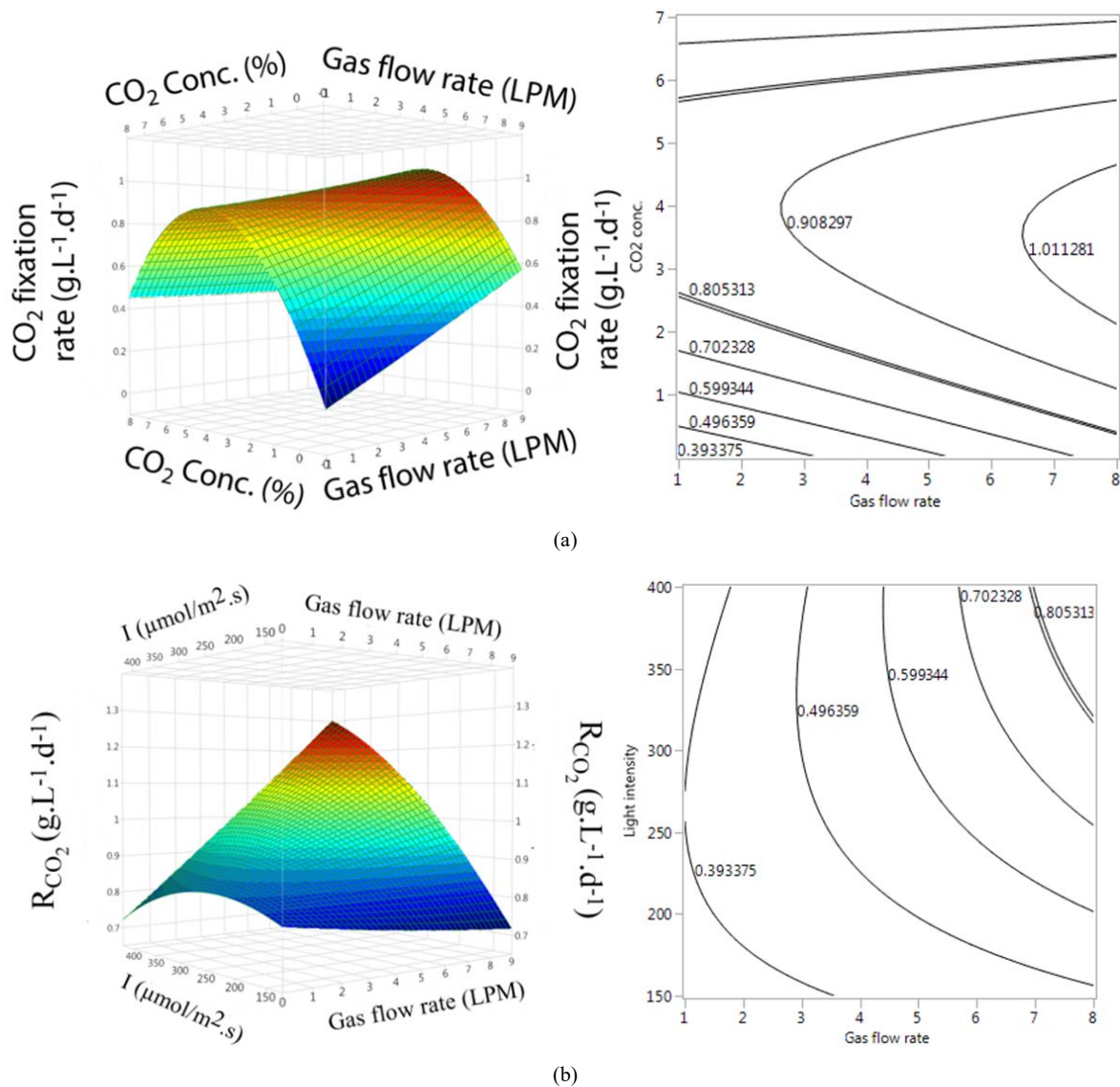
Based on the Box Behnken Design results, the optimal

value of gas flow rate, concentration of CO_2 , and light intensity were 7.5 L/min, 3.5%, and $400 \mu\text{mol}/\text{m}^2\cdot\text{s}$, respectively at 0.904 desirability. The predicted values of CO_2 biofixation rate based on the optimal value of the three independent factors was $1.186 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. For model validation and accuracy, a typical experiment was carried out in triplicate under the optimal parameters of independent factors. The results were compared with the model predicted values as shown in Table IV.

Fig. 3 shows the growth curve for *Chlorella vulgaris* and CO_2 biofixation rate. The adaption phase was observed from the beginning of the cultivation experiment until Day 4. The *Chlorella vulgaris* started to grow exponentially up until Day 8, after which it entered the stationary phase. The highest biomass concentration was 5.7 g/L and 1.23 g/L.d of CO_2 biofixation rate.

The findings of this study are comparable with those of

Shabani [27] who investigated the ability of *Spirulina platensis* and *Chlorella vulgaris* for CO_2 biofixation under different concentrations of CO_2 and salinity levels. They reported values of 4.84 g/L and 0.95 g/L.d for biomass concentration and CO_2 biofixation rate, respectively. Assunção et al. [28] studied the growth of three different microalgae cells under different CO_2 concentrations (2.5-15%), light intensity of $74 \mu\text{mol}/\text{m}^2\cdot\text{s}$ and gas flow rate of 1 vvm. They stated that higher biomass concentration and biofixation rate were achieved by cultivation of *Scenedesmus obliquus* under 15% CO_2 concentration. Kuo et al. [29] investigated the growth performance of *Chlorella* sp. in wastewater and boiler flue gas under different gas flow rates from 0.05 to 0.3 vvm, concentration of CO_2 of 2% and light intensity of $300 \mu\text{mol}/\text{m}^2\cdot\text{s}$. The maximum biomass concentration achieved was 2.46 g/L.



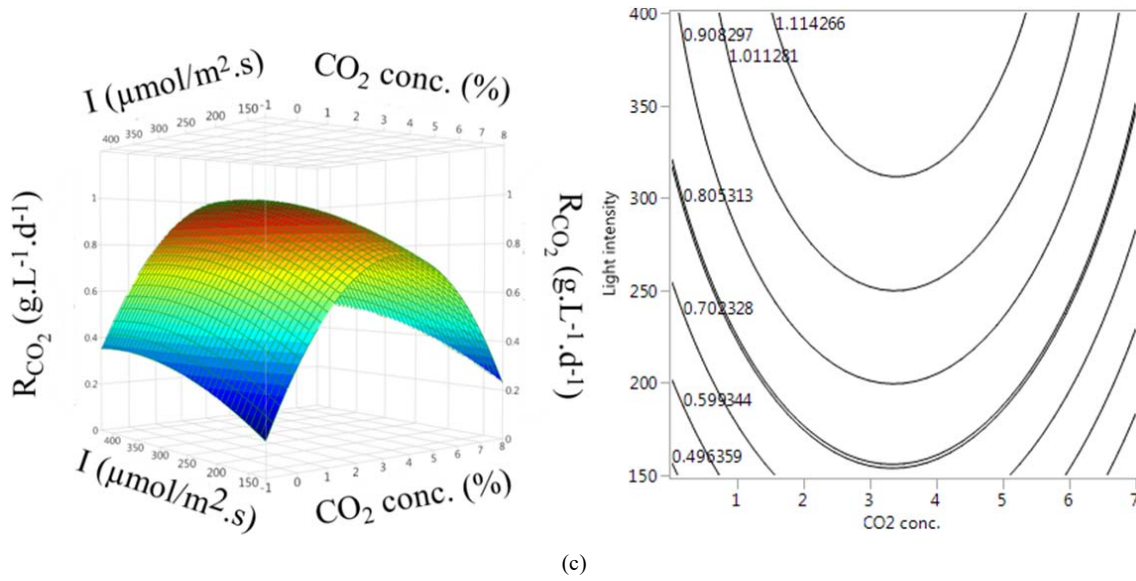


Fig. 2 3D response surface for CO₂ bio-fixation, (a) CO₂ conc. and gas flow rate, (b) light intensity and gas flow rate, and (c) light intensity and CO₂ conc

TABLE IV
COMPARISON OF PREDICTED AND EXPERIMENTAL RESPONSE VALUES AT OPTIMUM

Optimal condition	Response	Experimental	predicted	Error (%)
Gas flow rate (L/min)	R_{CO_2}	1.23	1.19	3.36
CO ₂ conc. (%)	TN removal efficiency	0.839	0.866	3.12
I (μmol/m ² .s)	TP removal efficiency	1	0.995	0.5

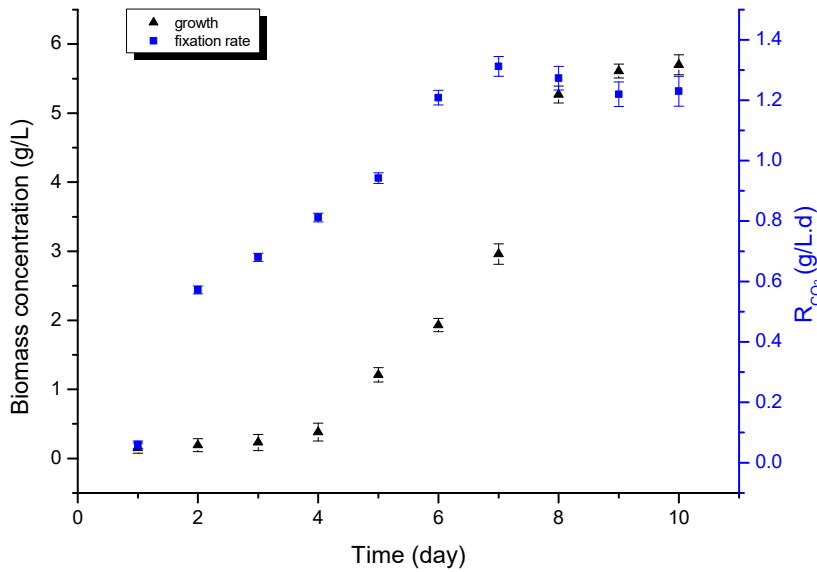


Fig. 3 *Chlorella vulgaris* growth and CO₂ biofixation rate at optimum conditions

The essential reasons for the different results are likely to be due to the different photobioreactor geometry, gas flow rate, and different light intensity used.

IV. CONCLUSIONS

In this study, the optimal values for CO₂ biofixation rate in wastewater as a medium for *Chlorella vulgaris* were predicted

by applying Response Surface Methodology and validated by laboratory experiments. No significant difference was observed between the predicted and experimental values that were achieved by validation experiments, confirming the accuracy of the models. Under optimum conditions, the maximum biomass produced and CO₂ biofixation rate were 5.7 g/L, 1.23 gL⁻¹d⁻¹, respectively. Optimization was based on

gas flow rate (1-8 L/min), concentration of CO₂ (0.03-7%), and light intensity (150-400 μmol/m².s). The analysis using the Box-Behnken Design and Response Surface Methodology showed that the three independent factors (gas flow rate, concentration of CO₂, and light intensity) have a crucial influence on CO₂ biofixation rate while concentration of CO₂ -

light intensity was less significant for CO₂ biofixation. As a result, these strategies could be a good reference for the enhancement of biomass production through microalgae cultivation and its application to large-scale of CO₂ biofixation and wastewater treatment in controllable environments.

TABLE V
PREVIOUS STUDIES FOR BIOMASS PRODUCTION, CO₂ BIOFIXATION, AND NUTRIENT REMOVAL

Conditions		I medium		Microalgae	Biomass conc. (g/L)	R _{CO₂}	reference
Gas flow rate	CO ₂ conc. (%)						
0.03 L/min	15	4500 lux	Bristol's solution	<i>Chlorella PY-ZU1</i>	4.84	0.95	[30]
1.4 L/min	5	-	Municipal WW	<i>Chlorella vulgaris</i>	0.94	1.4	[27]
0.25 L/min	0.03-10	60 μmol/m ² .s	Domestic WW	<i>Scenedesmus</i> sp.	0.43	0.368	[31]
1 vvm	2.5-15	74 μmol/m ² .s	Bristol's medium	<i>Scenedesmus obliquus</i>	2.51	0.47	[28]
0.3 L/min	0.04-30	450 μmol/m ² .s	Modified medium	Tetraselmis	0.72	0.111	[32]
0.05-0.3 vvm	2	300 μmol/m ² .s	Aquaculture WW	<i>Chlorella</i> sp.	2.46	-	[29]
0.2 L/min	3-10	163.5±9.4 μmol/m ² .s	WW	<i>Chlorella vulgaris</i>	1.01	0.127±0.008	[33]
0.5 L/min	10	-	Brewery WW	<i>Scenedesmus obliquus</i>	0.95±0.07	-	[34]

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