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Synergistic Impacts and Optimization of Gas Flow Rate, Concentration of CO_2, and Light Intensity on CO_2 Biofixation in Wastewater Medium by *Chlorella vulgaris*

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***Abstract*—**The synergistic impact and optimization of gas flow rate, concentration of CO_2, and light intensity on CO_2 biofixation rate were investigated using wastewater as a medium to cultivate *Chlorella vulgaris* under different conditions (gas flow rate 1-8 L/min), CO_2 concentration (0.03-7%), and light intensity (150-400 µmol/m^2.s)). Response Surface Methodology and Box-Behnken experimental Design were applied to find optimum values for gas flow rate, CO_2 concentration, and light intensity. The optimum values of the three independent variables (gas flow rate, concentration of CO_2, and light intensity) and desirability were 7.5 L/min, 3.5%, and 400 µmol/m^2.s, and 0.904, respectively. The highest amount of biomass produced and CO_2 biofixation rate at optimum conditions were 5.7 g/L, 1.23 $gL^{-1}d^{-1}$, respectively. The synergistic effect between gas flow rate and concentration of CO_2, and between gas flow rate and light intensity was significant on the three responses, while the effect between CO_2 concentration and light intensity was less significant on CO_2 biofixation rate. The results of this study could be highly helpful when using microalgae for CO_2 biofixation in wastewater treatment.

***Key words*—**Synergistic impact, optimization, CO_2 biofixation, airlift reactor.

I. Introduction

CARBON dioxide (CO_2) mitigation and wastewater treatment have attracted significant attention due to the fact that CO_2 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 CO_2, and treat wastewater [2]. Microalgae fix CO_2 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 CO_2, and aeration rate on CO_2 biofixation as well as the capability of microalgae to remove nutrients from different types of wastewater [4].

CO_2 biofixation using microalgae is affected by the CO_2 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 CO_2 biofixation in a stirrer photobioreactor with 2% CO_2 concentration and a light intensity of 30-300 µmol/m^2.s. Their result was 0.45 $gCO_2.L^{-1}.d^{-1}$ CO_2 biofixation rate at a light intensity of 100 µmol/m^2.s. Cheng et al. [7] investigated the effect of CO_2 concentration in the inlet gas at 0.05% to 1% on CO_2 biofixation using *Chlorella vulgaris* in an air lift reactor with a light intensity range from 23.6 to 236.4 µmol/m^2.s. They obtained 260 mg/l.hr biofixation rate at 1% CO_2 concentration and a light intensity of 157.6 µmol/m^2.s. The impact of gas flow rate on CO_2 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 CO_2 increased from 26.3 to 31.9 $g.m^{-2}.d^{-1}$ with an increased gas flow rate from 50 to 150 m^3/hr. In another study by Yoo et al. [9], the ability of *Botryococcus braunii, Chlorella vulgaris, and Scenedesmus* sp. were investigated for CO_2 biofixation at a light intensity of 150 µmol/m^2.s and 10% CO_2 concentration. They reported that *Scenedesmus* sp. was the most efficient species for CO_2 mitigation due to its having the highest biomass productivity and biofixation rate ability. Despite of considerable researches, optimum conditions for CO_2 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, CO_2 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 CO_2 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, CO_2 biofixation and nutrient removal. In this study, the synergistic impact of the aeration rate (gas flow rate and concentration of CO_2) and the light intensity on the CO_2 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. CO_2-enriched air was bubbled into the photobioreactor through a perforated plate sparger.

B. Calculation of Biomass Productivity and CO_2 Biofixation Rate

Productivity of biomass was calculated (P_X) using:

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

where Px is biomass productivity ($gL^{-1}.D^{-1}$), X_o is initial biomass concentration (gL^{-1}), X_t is concentration of biomass when the cultivation finished (gL^{-1}), and t is the time of cultivation.

CO_2 biofixation rate is calculated as:

$$CO_2\ biofixation\ rate\ R_{CO_2}\ (gL^{-1}d^{-1}) = \%C \times P_x \times \frac{MW_{CO_2}}{MW_c} \quad (2)$$

where $\%C$ is the percentage of carbon measured using an element analyser, P_x is biomass productivity ($gL^{-1}d^{-1}$), MW_{CO_2} is molar mass of CO_2, 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 CO_2 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, CO_2 concentration X_2, and light intensity X_3). To obtain these goals, 15 experiments will be accomplished by varying gas flow rate, concentration of CO_2, and light intensity from 150 to 400 $\mu E/m^2.s$ 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 ($\mu mol/m^2.s$)	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:

$$Y = \beta_o + \sum_{i-1}^{k} \beta_{ii}\, y_i^2 + \sum_{i-1}^{k}\sum_{j-1}^{k} \beta X_i + \varepsilon \quad (3)$$

where Y is the response; i and j are the patterns index numbers; β_o 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:

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

where x_i and X_i are the coded and actual values of the independent factors, X_o 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), CO_2 concentration (0.03-7 %), and light intensity (150-400 $\mu mol/m^2.s$).

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 (R^2) 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} ($gL^{-1}d^{-1}$)	
	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}= CO_2 biofixation rate.

Multiple analysis of regression was used to specify the relationships of the response variable of CO_2 biofixation rate with respect to concentration of CO_2, 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:

$$CO_2\ biofixation\ rate = 0.945 + 0.098375x_1 + 0.04925x_2 + 0.107875x_3 - 0.07375x_1x_2 + 0.106x_1x_3 + 0.00975x_2x_3 + 0.00375x_1^2 - 0.343x_2^2 - 0.05275x_3^2 \quad (5)$$

where x_1, x_2, and x_3 are coded values calculated using (4). The determination coefficient (R^2) for CO_2 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.

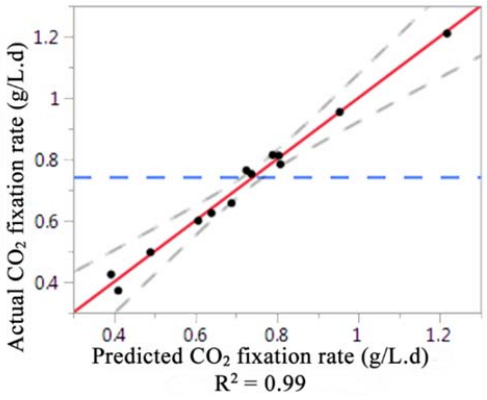


Fig. 1 Experimental and predicted data Comparison of CO_2 bio-fixation rate, (.) experimental values, (_ _ _, — —) confidence bands, and (——) fit line

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*
$x_1.x_2$	-0.07375	0.018498	-3.99	0.0105*
$x_1.x_3$	0.106	0.018498	5.73	0.0023*
$x_2.x_3$	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 (*% CO_2* and *I*), coefficients of quadratic term ($\%CO_2^2$ and I^2), and coefficients of interactive term (*Q. %CO_2* and *Q.I*) significantly influence the CO_2 biofixation rate. In addition, CO_2 biofixation rate and total nitrogen removal efficiency were significantly impacted by gas flow rate, CO_2 concentration, and light intensity with a *p*-value less than 0.05 (Table III). The findings showed that the responses of CO_2 biofixation rate were significantly influenced by the synergistic effect of (*Q. %CO_2* and *Q.I*) with *p*-value < 0.05, while (*% CO_2.I*) synergistic effect was less significant (*p*-value = 0.62) on CO_2 biofixation rate.

C. Box Behnken Design Analysis

The impacts of gas flow rate, concentration of CO_2, and light intensity on CO_2 biofixation rate are shown in Fig. 2. Fig. 2 illustrates response surfaces (3D) and contours (2D) for CO_2 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 CO_2 biofixation rate.

Fig. 2 (a) shows the impact of concentration of CO_2 and gas flow rate on CO_2 biofixation rate. Increasing concentration of CO_2 led to an increase in the rate of CO_2 biofixation due to the microalgae cells consuming CO_2 in order to store and use it during times of nutrient deficiency [20]. Gas aeration rate had a significant influence on CO_2 biofixation rate due to its using aeration as a carbon source improved mixing that enhanced utilization of light by the cells [23]. CO_2 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 CO_2 and light intensity on CO_2 biofixation rate. And CO_2 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 µmol/m^2.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 CO_2, gas flow rate, and light intensity could enhance the CO_2 biofixation and microalgae growth.

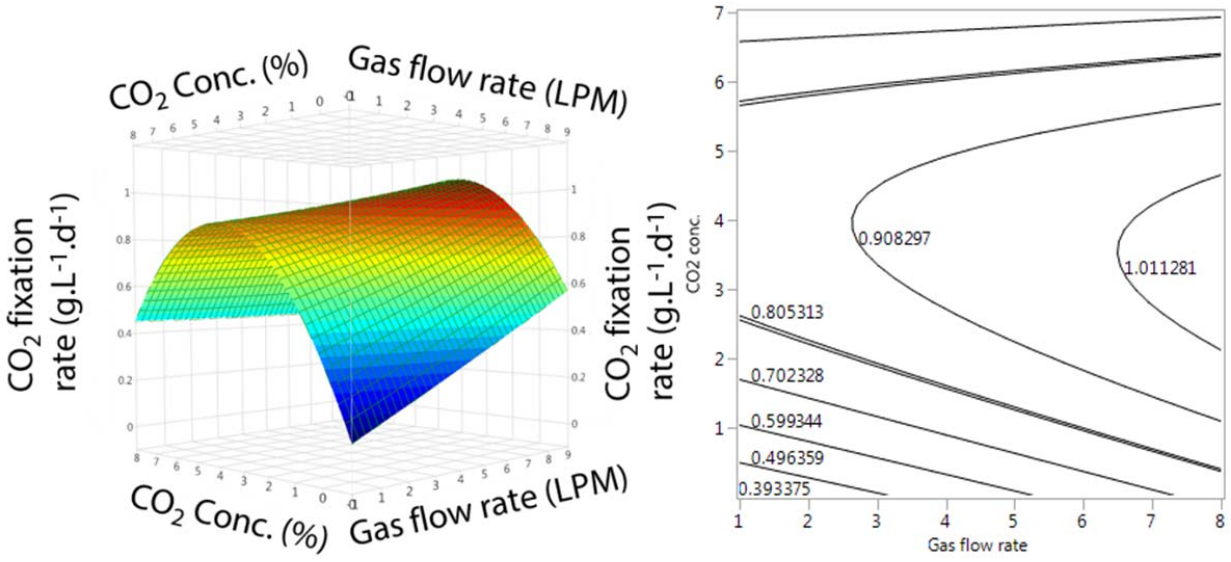
D. Model Validation

Based on the Box Behnken Design results, the optimal

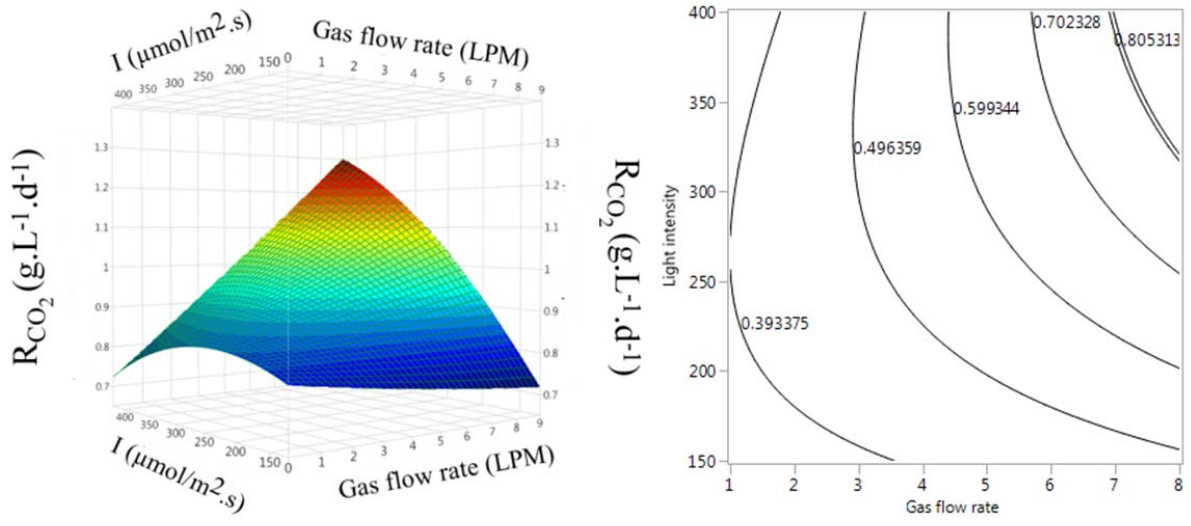
value of gas flow rate, concentration of CO_2, and light intensity were 7.5 L/min, 3.5%, and 400 µmol/m^2.s, respectively at 0.904 desirability. The predicted values of CO_2 biofixation rate based on the optimal value of the three independent factors was 1.186 $g.L^{-1}.d^{-1}$. For model validation 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.

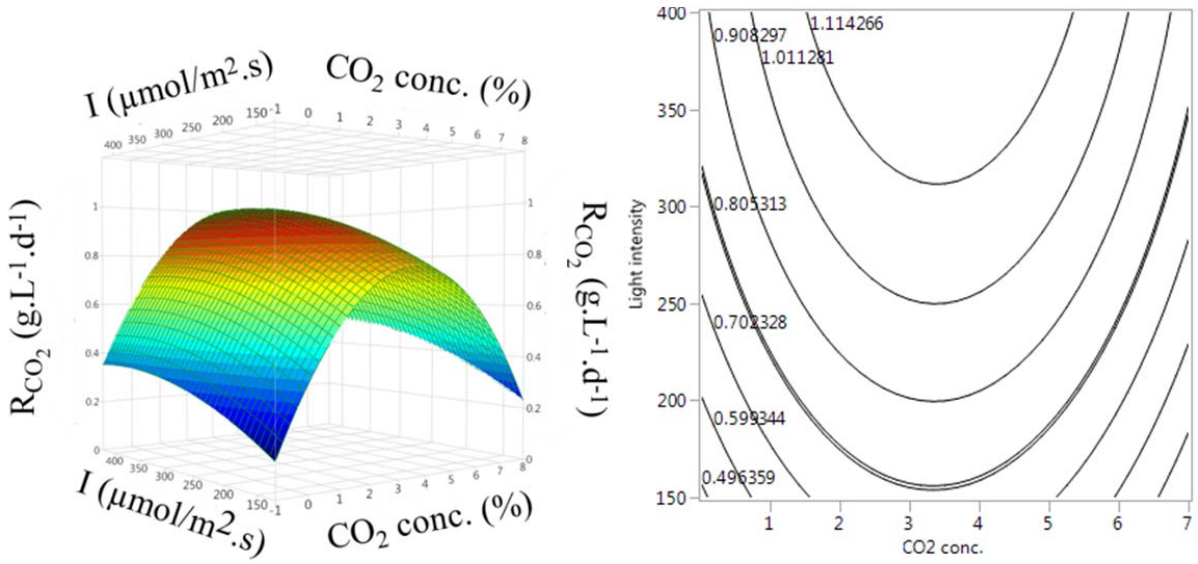
The findings of this study are comparable with those of Shabani [27] who investigated the ability of *Spirulina platensis* and *Chlorella vulgaris* for CO_2 biofixation under different concentrations of CO_2 and salinity levels. They reported values of 4.84 g/L and 0.95 g/L.d for biomass concentration 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 µmol/m^2.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 µmol/m^2.s. The maximum biomass concentration achieved was 2.46 g/L.



(a)



(b)



(c)

Fig. 2 3D response surface for CO_2 bio-fixation, (a) CO_2 conc. and gas flow rate, (b) light intensity and gas flow rate, and (c) light intensity and CO_2 conc

TABLE IV
COMPARISON OF PREDICTED AND EXPERIMENTAL RESPONSE VALUES AT OPTIMUM

Optimal condition		Response	Experimental	predicted	Error (%)
Gas flow rate (L/min)	7.5	R_{CO_2}	1.23	1.19	3.36
CO_2 conc. (%)	3.5	TN removal efficiency	0.839	0.866	3.12
I (µmol/m^2.s)	400	TP removal efficiency	1	0.995	0.5

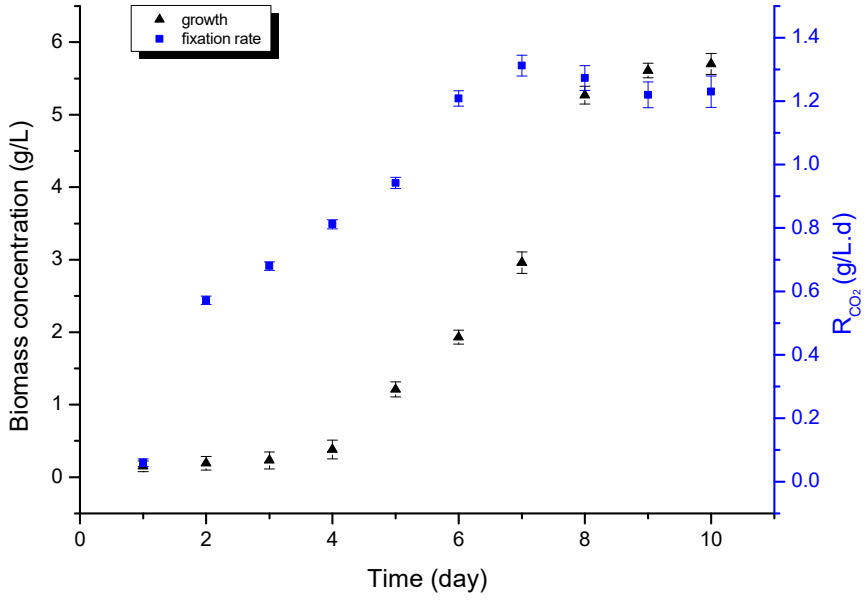


Fig. 3 *Chlorella vulgaris* growth and CO_2 biofixation rate at optimum conditions

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 CO_2 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 CO_2 biofixation rate were 5.7 g/L, 1.23 gL^{-1}d^{-1}, respectively. Optimization was based on

gas flow rate (1-8 L/min), concentration of CO_2 (0.03-7%), and light intensity (150-400 µmol/m^2.s). The analysis using the Box-Behnken Design and Response Surface Methodology showed that the three independent factors (gas flow rate, concentration of CO_2, and light intensity) have a crucial influence on CO_2 biofixation rate while concentration of CO_2 - light intensity was less significant for CO_2 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 CO_2 biofixation and wastewater treatment in controllable environments.

TABLE V
PREVIOUS STUDIES FOR BIOMASS PRODUCTION, CO_2 BIOFIXATION, AND NUTRIENT REMOVAL

Conditions				Microalgae	Biomass conc. (g/L)	R_{CO_2}	reference
Gas flow rate	CO_2 conc. (%)	I	medium				
0.03 L/min	15	4500 lux	Bristol's solution	*Chlorella PY-ZU1*	4.84	0.95	[30]
1.4 L/min	5	-	Municipal WW	*Chlorella vulgaris*	0.94	1.4	[27]
0.25 L/min	0.03-10	60 µmol/m^2.s	Domestic WW	*Scenedesmus* sp.	0.43	0.368	[31]
1 vvm	2.5-15	74 µmol/m^2.s	Bristol's medium	*Scenedesmus obliquus*	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	*Chlorella* sp.	2.46	-	[29]
0.2 L/min	3-10	163.5±9.4 µmol/m^2.s	WW	*Chlorella vulgaris*	1.01	0.127±0.008	[33]
0.5 L/min	10	-	Brewery WW	*Scenedesmus obliquus*	0.95±0.07	-	[34]

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