

# Cultivation of *Scenedesmus dimorphus* for C/N/P removal and lipid production



Xinmiao Xu, Ying Shen<sup>\*</sup>, Jiacheng Chen

College of Mechanical Engineering and Automation, Fuzhou University, Fuzhou, Fujian 350108, China

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## ABSTRACT

**Background:** CO<sub>2</sub> emission, water pollution and petroleum shortage are the issues coming with the development of industry. A cost effective system was constructed to fix the CO<sub>2</sub> in flue gas (15% CO<sub>2</sub>), remove nitrogen and phosphorus from manure wastewater and produce biofuels at the same time. The significant cultivation conditions were selected by Plackett–Burman design, and then optimized with central composite design.

**Results:** Optimum culture condition was predicted at light intensity of 238 μmol·m<sup>-2</sup>·s<sup>-1</sup>, TN of 152 mg·L<sup>-1</sup>, inoculum density of 0.3 g·L<sup>-1</sup>, under which the measured CO<sub>2</sub> fixation rate, total nitrogen and phosphorus removing rate, and lipid content were 638.13 mg·L<sup>-1</sup>·d<sup>-1</sup>, 88.16%, 73.98% and 11.9%, respectively. The lipid content was then enhanced to 24.2% by a nitrogen starvation strategy.

**Conclusion:** A cultivation strategy was suggested to achieve effective C/N/P removal from flue gas and manure wastewater, and meanwhile obtained high lipid content from microalgal biomass.

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## 1. Introduction

Microalgae, a group of organisms that can achieve 3–5% photosynthetic rate, are one of the most promising pathways to ease the greenhouse effect [1]. In general, photosynthetic microalgae have 50% of its weight as carbon and hence 1.83 kg of CO<sub>2</sub> will be needed theoretically to produce a kilogram of biomass [2]. Utilization of concentrated carbon sources such as industrial emissions and manure wastewater to grow microalgae is a cost-effective concept to achieve both CO<sub>2</sub> fixation and wastewater treatment [2,3]. Since the C:N:P rate in wastewater (around 20:8:1) is much lower than algal biomass (around 106:16:1), the addition of CO<sub>2</sub> is one of the effective methods to stimulate algae growth and nutrient uptake. Woertz et al. [4] reported an enhancement of biomass concentration from 317 to 812 mg·L<sup>-1</sup> when CO<sub>2</sub> was added to a semi-continuous culture of a microalgae consortium in a preliminarily clarified wastewater. Several studies also proved the feasibility of remediating power plants with microalgae using either direct industrial flue gas or by using pure gas mixtures that mimic the flue gas composition [5,6,7].

Microalgal species, such as *Scenedesmus* sp., *Chlorococcum littorale*, and *Chlorella* sp. were reported to have great tolerance of high concentration CO<sub>2</sub> [8,9]. For instance, Maeda et al. [6], found a strain of *Chlorella* sp. T-1 which could grow under 100% CO<sub>2</sub>, although the maximum growth rate occurred under a 10% concentration [6]. Generally, the CO<sub>2</sub> fixation rate (CFR) is affected by many factors, such as light intensity, nitrogen concentration, aeration rate, culture period,

pH, inoculum density, and so on [10,11,12]. For instance, Ho et al. [12] found that the specific growth rate increased dramatically with rising light intensity at the beginning of the process (*i.e.*, in the light limited region), and then it gradually leveled off as the light intensity continued to rise (*i.e.*, in the light saturation region). The maximum biomass productivity of 840.56 mg·L<sup>-1</sup>·d<sup>-1</sup> was observed at a light intensity of 420 μmol·m<sup>-2</sup>·s<sup>-1</sup>, with the highest CFR of 1435.90 mg·L<sup>-1</sup>·d<sup>-1</sup> [12].

The objective of this study was to optimize the culture conditions of freshwater alga *Scenedesmus dimorphus* to achieve C/N/P removal from flue gas and manure wastewater, and stimulate lipid accumulation with nitrogen deficiency strategy. Plackett–Burman design and central composite design were applied to screen and optimize the culture conditions, including: light intensity, wastewater concentration (described with total nitrogen concentration (TN)), aeration rate, aeration period, inoculum density, pH, and magnesium (Mg) concentration.

## 2. Materials and methods

### 2.1. Algal strain and subculture

The alga *S. dimorphus* (FACHB-496) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (Wuhan, China). The alga was maintained in Modified Basal medium [13]. The inocula were grown in 250 mL Erlenmeyer flasks, each containing 120 mL medium, and incubated at 26 ± 2°C in an orbital shaker set to 125 rpm. The illumination was provided by 18 W cool white fluorescent light at 60–80 μmol·m<sup>-2</sup>·s<sup>-1</sup>. The cell concentration

<sup>\*</sup> Corresponding author.

E-mail address: yshen@fzu.edu.cn (Y. Shen).

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of the inocula was monitored by Hemocytometer (Cat. No. 0650030, Marienfeld, Germany). Once it reached  $10^6$  cells·mL<sup>-1</sup>, the biomass dry weight was determined by gravimetric method. Subsequently, aliquot inocula were centrifuged to make 75 mL high concentrated algal medium, and then the high concentrated algal medium was added into 1.425 L wastewater to achieve the objective inoculum density.

## 2.2. Cultivation system

To reduce the capital cost, polyethylene bags with diameter of 7 cm and volume of 2 L (each containing 1.5 L medium) were used as cultivation system, as shown in Fig. 1. Solenoid valves were applied to intermittently aerate simulated flue gas (15% CO<sub>2</sub>). Pre-treated manure wastewater was used as culture medium. The manure wastewater was obtained from a piggery farm close to Fuzhou University (Fuzhou, Fujian, China). The wastewater was filtered to remove any large solid particles and then diluted with tap water before feeding it to microalgae. The TN and total phosphorus (TP) in filtered wastewater were  $306.15 \pm 3.90$  mg·L<sup>-1</sup> and  $115.08 \pm 0.82$  mg·L<sup>-1</sup>, respectively. The same batch of wastewater was used throughout the experiments. Based on the nutrient removing rate, each batch of the culture lasted 7-d.

## 2.3. Experimental design

Based on the experience from previous experiments [14] and references [15,16], seven variables were determined to perform Plackett–Burman experiments, including: light intensity, wastewater concentration (described with TN), aeration rate, aeration period, inoculum density, pH, and Mg concentration (MgSO<sub>4</sub> × 7H<sub>2</sub>O), as shown in Table 1. Each taking two levels, in such a way as to minimize the variance of the estimates of these dependencies using a limited number of experiments.

Based on the estimation of Plackett–Burman design, light intensity, TN and inoculum density were determined to be the significant factors ( $p$ -value  $\leq 0.05$ ). The optimal response recommended for central composite design (CCD) was: light intensity of  $240 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , TN of  $150 \text{ mg}\cdot\text{L}^{-1}$ , inoculum density of  $0.3 \text{ g}\cdot\text{L}^{-1}$ , aeration rate of 0.1 vvm, aeration period of 15:450 s:s (on:off), and pH of 8. To avoid heavy metal pollution, MgSO<sub>4</sub> × 7H<sub>2</sub>O, which was not significant on CFR in this case, was not added into the second step of the experiments. As shown in Table 2, 5 coded levels ( $-\alpha$ ,  $-1$ ,  $0$ ,  $+1$ , and  $+\alpha$ ) of light intensity, TN and inoculum density were assessed (the other factors were fixed at the recommended level). A total of 18 experiments were conducted, including 8 fractional factorial designs ( $2^3$ ), 8 star points ( $2 \times 3$ ), and 4 replicates at center points. According

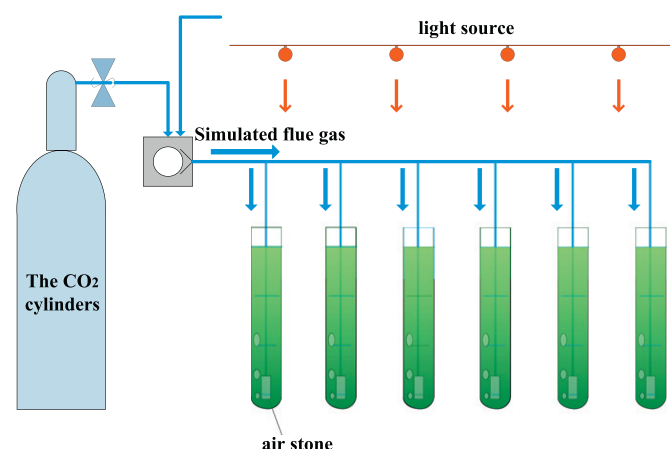


Fig. 1. Schematic of attached algal culture system.

to rotatable design of the response surface, the parameter of  $\alpha$  was chosen to be 1.681. The treatments were carried out in duplicate as independent experiments to take into account the non-adjustable data and the analysis of variance. The results of the CCD were fit with a second-order polynomial equation using a multiple regression technique in [Equation 1]:

$$Y = X_0 + \sum_{i=1}^4 a_i X_i + \sum_{i=1}^4 a_{ii} X_i^2 + \sum_{j=1}^4 \sum_{i < j} a_{ij} X_i X_j \quad [\text{Equation 1}]$$

where  $Y$  is CFR ( $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ );  $X_0$  stands for the model intercept;  $X_1$ ,  $X_2$ , and  $X_3$ , are the levels of light intensity, TN and inoculum density, respectively;  $a_i \dots a_{ij}$  are the regression coefficients. The  $p$ -value of each term was determined to remove insignificant terms. The analysis of variance was carried out through the Design Expert software version 7.0 (Statease, Minneapolis, MN).

The optimal culture condition identified by CCD was validated by duplicate experiments. To increase the lipid content, the validated experiments were continued with a 5-d nitrogen starvation cultivation by replacing 20%, 40%, 60% and 80% of the culture solution with tap water.

## 2.4. Analysis

Biomass dry weight (DW) was measured by filtering aliquot algal sample on pre-weighed glass-fiber filter paper with pore size of  $0.45 \mu\text{m}$  (Q/IEFJ01-1997, Xingya purification material factory, Shanghai, China). The filters were then dried at  $105^\circ\text{C}$  in an oven for 12 h. Algal biomass DW was determined by the difference of the two weights. pH was measured using pH meter (PB-10, Sartorius. Co., Ltd, China) once every day and adjusted with 0.5 M HCl or NaOH solution to maintain the objective pH value. The TN and TP were measured once every day by using Mi-parameter meter (5H-3BA, Lian-hua Tech. Co., Ltd, China) with standard procedure. Lipid extraction was carried out using a BioSpec Model 3110 BX bead-beater (Bartlesville, Oklahoma) for cell disruption (3 min) followed by solvent extraction with  $n$ -hexane. The oils collected after evaporation was dried at  $95^\circ\text{C}$  for 1.5 h before weighing. Lipid content was the percentage of lipid weight to disrupted biomass. To obtain the carbon content, part of the algal biomass was freeze-dried (LGJ-10, Songyuanhuaxing Tech. Co., Ltd, China) for 48 h, and then tested by elemental analysis (vario EL cube, Elementar Analysensysteme GmbH, Germany). The CFR was calculated according to [Equation 2] [17]:

$$\text{CFR} = C_c \frac{M_{\text{CO}_2} (DW_2 - DW_1)}{M_c t} \quad [\text{Equation 2}]$$

where  $C_c$  is the carbon content of *S. dimorphus* ( $44.83 \pm 0.39\%$ ),  $M_{\text{CO}_2}$  is the molecular weight of CO<sub>2</sub> ( $44 \text{ g}\cdot\text{mol}^{-1}$ ),  $M_c$  is the molecular weight of C ( $12 \text{ g}\cdot\text{mol}^{-1}$ ),  $DW_2$  is the biomass DW achieved in the end of the cultivation ( $\text{g}\cdot\text{L}^{-1}$ ),  $DW_1$  is the inoculum density ( $\text{g}\cdot\text{L}^{-1}$ ), and  $t$  is the culture time (d).

## 3. Results and discussion

### 3.1. Optimizing the culture conditions to increase C/N/P removing rate

As shown in Table 1, the CFR, TN and TP removing rate varied from  $303.75 \pm 10.47$  to  $610.13 \pm 13.09$   $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ,  $68.47 \pm 0.54$  to  $92.13 \pm 0.29\%$ , and  $58.82 \pm 0.33$  to  $76.85 \pm 0.44\%$  in Plackett–Burman design, respectively. Since the TN and TP removing rates were relatively high, the CFR was chosen for further optimization. As shown in Table 3, the significance of the seven factors on CFR was analyzed via F-test. Factors evidencing  $p$ -values of less than 0.05 were considered to have significant effects on the response, and therefore

**Table 1**  
Design and responses of Plackett–Burman design.

No	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	CFR (mg·L <sup>-1</sup> ·d <sup>-1</sup> )	TN removing rate (%)	TP removing rate (%)
A1	240	150	0.1	15:450	0.3	9	0.5	602.27 ± 2.095	82.03 ± 1.05	58.82 ± 0.33
A2	160	150	0.3	15:300	0.3	9	4.0	570.85 ± 5.24	84.67 ± 0.56	73.85 ± 0.56
A3	240	100	0.3	15:450	0.1	9	4.0	432.06 ± 23.57	76.67 ± 0.88	69.64 ± 0.78
A4	160	150	0.1	15:450	0.3	7	4.0	610.13 ± 1.309	86.87 ± 0.91	67.37 ± 0.56
A5	160	100	0.3	15:300	0.3	9	0.5	455.63 ± 10.47	69.45 ± 0.34	62.01 ± 0.23
A6	160	100	0.1	15:450	0.1	9	4.0	303.75 ± 10.47	68.47 ± 0.54	67.18 ± 0.45
A7	240	100	0.1	15:300	0.3	7	4.0	479.20 ± 13.09	92.13 ± 0.29	67.18 ± 0.56
A8	240	150	0.1	15:300	0.1	9	0.5	555.14 ± 20.95	88.27 ± 0.58	72.17 ± 0.65
A9	240	150	0.3	15:300	0.1	7	4.0	573.47 ± 18.33	89.93 ± 0.90	76.85 ± 0.44
A10	160	150	0.3	15:450	0.1	7	0.5	447.78 ± 18.33	82.03 ± 1.09	63.44 ± 0.63
A11	240	100	0.3	15:450	0.3	7	0.5	597.03 ± 5.24	85.55 ± 1.00	65.62 ± 0.67
A12	160	100	0.1	15:300	0.1	7	0.5	439.92 ± 15.71	70.78 ± 0.98	62.37 ± 0.89

X<sub>1</sub>: Light intensity, μmol·m<sup>-2</sup>·s<sup>-1</sup>; X<sub>2</sub>: TN, mg·L<sup>-1</sup>; X<sub>3</sub>: Aeration rate, vvm; X<sub>4</sub>: Aeration period, on:off s:s; X<sub>5</sub>: inoculum density, g·L<sup>-1</sup>; X<sub>6</sub>: pH; X<sub>7</sub>: Mg concentration, mM.

light intensity, TN and inoculum density were selected for further optimization studies.

The optimal control of light intensity, TN and inoculum density was conducted by CCD. The design matrix and corresponding experimental data are given in Table 2. The experimental results were fit to a second-order polynomial in [Equation 3]:

$$Y = 610.89 - 27.56 \times X_1 + 12.52 \times X_2 + 19.24 \times X_3 - 27.01 \times X_1 \times X_2 - 35.52 \times X_1 \times X_3 - 30.83 \times X_2 \times X_3 - 64.43 \times X_1^2 - 52.81 \times X_2^2 - 38.48 \times X_3^2$$

[Equation 3]

The significance of the regression model and individual variables were determined at 95% confidence level. As shown in Table 4, the *p*-value of second-order polynomial model is lower than 0.05, which indicates that the model is statistically significant. Based on the *p*-values, the variables of light intensity, inoculum density, as well as the interactions between light intensity and TN, light intensity and inoculum density, TN and inoculum density were significant on CFR.

### 3.2. Interactions between light intensity and TN

The interactions between light intensity and TN on CFR when inoculum density was constant at the center point were shown in Fig. 2. The contour lines indicate that CFR favored medium light intensity and medium TN. The designed point was predicted at light intensity of 238 μmol·m<sup>-2</sup>·s<sup>-1</sup> and TN of 152 mg·L<sup>-1</sup>. Basically,

**Table 2**  
Design and responses of CCD.

No	Coded (real) values			Experimental data CFR (mg·L <sup>-1</sup> ·d <sup>-1</sup> )	TN removing rate (%)	TP removing rate (%)
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>			
B1	-1 (200)	-1 (125)	-1 (0.2)	387.51 ± 17.61	87.35 ± 1.03	68.44 ± 0.47
B2	1 (280)	-1 (125)	-1 (0.2)	446.22 ± 7.07	86.65 ± 1.12	67.50 ± 0.87
B3	-1 (200)	1 (175)	-1 (0.2)	502.59 ± 4.72	90.01 ± 1.09	87.16 ± 0.65
B4	1 (280)	1 (175)	-1 (0.2)	476.76 ± 21.21	83.85 ± 0.98	85.54 ± 0.38
B5	-1 (200)	-1 (125)	1 (0.4)	527.25 ± 22.39	90.02 ± 0.37	56.77 ± 0.67
B6	1 (280)	-1 (125)	1 (0.4)	467.37 ± 11.78	84.97 ± 0.56	49.27 ± 0.54
B7	-1 (200)	1 (175)	1 (0.4)	542.52 ± 30.64	85.58 ± 0.76	84.90 ± 0.19
B8	1 (280)	1 (175)	1 (0.4)	351.11 ± 4.72	86.95 ± 1.05	84.85 ± 0.73
B9	-α (173)	0 (150)	0 (0.3)	465.02 ± 2.34	86.95 ± 0.65	69.48 ± 0.65
B10	α (307)	0 (150)	0 (0.3)	371.07 ± 5.85	85.82 ± 0.45	74.17 ± 0.67
B11	0 (240)	-α (108)	0 (0.3)	413.35 ± 10.6	94.04 ± 0.89	49.15 ± 0.56
B12	0 (240)	α (192)	0 (0.3)	488.50 ± 23.53	81.32 ± 0.76	76.02 ± 0.48
B13	0 (240)	0 (150)	-α (0.13)	435.66 ± 20.03	86.80 ± 0.56	79.22 ± 0.88
B14	0 (240)	0 (150)	α (0.47)	547.22 ± 2.34	86.83 ± 0.37	72.09 ± 0.54
B15	0 (240)	0 (150)	0 (0.3)	612.98 ± 9.39	86.90 ± 0.98	64.96 ± 0.49
B16	0 (240)	0 (150)	0 (0.3)	598.89 ± 2.34	85.63 ± 0.79	62.75 ± 0.70
B17	0 (240)	0 (150)	0 (0.3)	631.77 ± 9.39	86.63 ± 0.47	64.42 ± 0.45
B18	0 (240)	0 (150)	0 (0.3)	603.58 ± 2.34	85.98 ± 0.89	65.88 ± 0.67

X<sub>1</sub>: light intensity, μmol·m<sup>-2</sup>·s<sup>-1</sup>; X<sub>2</sub>: total nitrogen concentration, mg·L<sup>-1</sup>; X<sub>3</sub>: inoculum density, g·L<sup>-1</sup>.

light is the most important factor influencing the growth of a photosynthetic microorganism and represents the main source of energy for *S. dimorphus*. Usually, light intensity around 60 to 120 μmol photons m<sup>-2</sup> s<sup>-1</sup> is preferred by a number of microalgal species [17]. However, the addition of CO<sub>2</sub> may increase the light saturated photosynthetic capacity [18,19]. Soletto et al. [19] reported that the maximum carbon utilization rate over 90% was achieved with photosynthetic efficiency of 7.5% under saturate light intensity of 166 μmol photons m<sup>-2</sup> s<sup>-1</sup> and sufficient CO<sub>2</sub> supplementation (0.44 g·L<sup>-1</sup>·d<sup>-1</sup>) [19]. In the present study, light intensity around 240 μmol photons m<sup>-2</sup> s<sup>-1</sup> was preferred by *S. dimorphus* when 15% (v/v) CO<sub>2</sub> was supplied.

Based on the analysis of variance shown in Table 4, CFR was independent on TN but affected by the interaction of TN and light intensity. Since nitrogen is one of the most significant nutrients for microalgae growth, sufficient nitrogen supplement may stimulate the biomass growth when light has good penetration [12,20]. Solovchenko et al. [21] reported that both biomass productivity and fatty acid content of *Parietochloris incisa* were enhanced by increasing light intensity from 35 to 200 μmol·m<sup>-2</sup>·s<sup>-1</sup> under nitrogen sufficient situation [21]. However, further increasing TN (>175 mg·L<sup>-1</sup>) may prolong the lag period of *S. dimorphus*, which decreased the CFR.

### 3.3. Interactions between light intensity and inoculum density

The interactions between light intensity and inoculum density on CFR when TN was constant at the center point were shown in Fig. 3. The contour lines indicate that CFR favored medium light intensity

**Table 3**  
Analysis of variance of Plackett–Burman design.

Variables	Effect	Coefficient	Standard error	F-value	p-Value
X <sub>1</sub> <sup>a</sup>	85.92	42.96	10.86	15.65	0.0167*
X <sub>2</sub>	97.27	48.64	10.86	20.05	0.0110*
X <sub>3</sub>	12.33	6.16	10.86	0.32	0.6006
X <sub>4</sub>	14.68	7.34	10.86	0.46	0.5362
X <sub>5</sub>	105.88	52.94	10.86	23.76	0.0082*
X <sub>6</sub>	-51.08	-25.54	10.86	5.53	0.0784
X <sub>7</sub>	-48.73	-24.37	10.86	5.03	0.0883
R <sup>2</sup> = 0.9465		Adj R <sup>2</sup> = 0.8530.			

<sup>a</sup> The symbols are the same as those in Table 1.

\* Statistically significant at a probability level of 90%.

and medium inoculum density. The designed point was predicted at light intensity of 238  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and initial concentration of 0.3  $\text{g}\cdot\text{L}^{-1}$ . According to the analysis of variance shown in Table 4, inoculum density was one of the key factors that affected the CFR. When light intensity was constant, increasing inoculum density increased CFR to some extent. The result was in consistency with several researches [22,23,24]. For instance, Lau et al. [22] found that the efficiency of reducing wastewater-borne nutrients by an algal system was directly related to the physiological activity and growth of the *Chlorella* cells which in turn were affected by the inoculum density. It is expected that the higher the algal density, the better the growth and the higher the nutrient removal efficiency. However, high algal density would lead to self-shading, an accumulation of auto-inhibitors, and a reduction in photosynthetic efficiency [23,24]. Therefore, the inoculum density was corresponding to light intensity for optimum CFR. Excessive light may cause light saturation, while excessive inoculum density may lead to self-shading, both decreasing the CFR.

### 3.4. Interactions between TN and inoculum density

The interactions between TN and inoculum density on CFR when light intensity was constant at the center point were shown in Fig. 4. The contour lines show that either increasing inoculum density or TN increased CFR. The designed point was predicted at TN of 152  $\text{mg}\cdot\text{L}^{-1}$  and inoculum density of 0.3  $\text{g}\cdot\text{L}^{-1}$ . As previously explained, when optimum light intensity was applied, either increasing TN or inoculum density may increase the cell density, leading to high CFR.

### 3.5. Validation and lipid enhancing strategy

Based on the second-order polynomial model presented in [Equation 3], the optimum culture condition for maximum CFR was

**Table 4**  
Analysis of variance of second-order polynomial model.

Sources	Sum of squares	Mean square	F-value	p-Value
X <sub>1</sub> <sup>a</sup>	10,374.57	10,374.57	17.14	0.0033**
X <sub>2</sub>	2141.54	2141.54	3.54	0.0968
X <sub>3</sub>	5055.95	5055.95	8.35	0.0202*
X <sub>1</sub> × X <sub>2</sub>	5835.72	5835.72	9.64	0.0146*
X <sub>1</sub> × X <sub>3</sub>	10,094.56	10,094.56	16.67	0.0035**
X <sub>2</sub> × X <sub>3</sub>	7601.46	7601.46	12.56	0.0076*
X <sub>1</sub> <sup>2</sup>	52,513.19	52,513.19	86.74	<0.0001**
X <sub>2</sub> <sup>2</sup>	35,273.75	35,273.75	58.26	<0.0001**
X <sub>3</sub> <sup>2</sup>	18,733.60	18,733.60	30.94	0.0005**
Model	1.186E+5	13,174.74	21.76	0.0001**
Error	634.32	211.44		
Total	1.234E+5			
R <sup>2</sup> = 0.9608		Adj R <sup>2</sup> = 0.9166		

<sup>a</sup> The symbols are the same as those in Table 2.

\* Statistically significant at a probability level of 90%.

\*\* Statistically significant at a probability level of 99%.

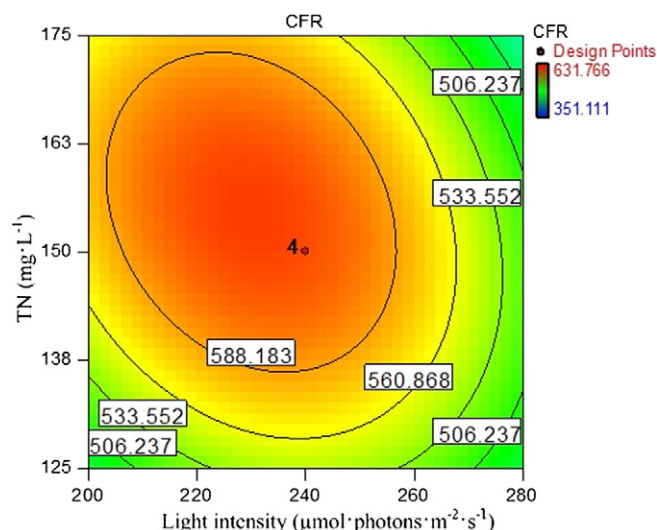


Fig. 2. Interaction of light intensity and TN on CFR.

estimated as follow: light intensity of 238  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , TN of 152  $\text{mg}\cdot\text{L}^{-1}$ , inoculum density of 0.3  $\text{g}\cdot\text{L}^{-1}$ , under which, the predicted CFR is 639.64  $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ . The predicted condition was validated and the measured CFR, TN and TP removing rate, and lipid content were  $638.13 \pm 11.7 \text{ mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ,  $88.16 \pm 0.98\%$ ,  $73.98 \pm 0.56\%$  and  $11.9 \pm 0.68\%$ , respectively. The prediction was close to validation experimental results, indicating that the model can be used to guide and optimize the CO<sub>2</sub> fixation of *S. dimorphus*.

The validated experiments were continued with a nitrogen starvation strategy to increase the lipid content. The maximum lipid content of  $24.2 \pm 0.13\%$  was achieved by replacing 80% of the culture solution with tap water, followed by 18.2 ± 0.60%, 17.7 ± 0.15% and 14.3 ± 0.34% by replacing 60%, 40% and 20% of the culture solution, respectively. Nutrient (especially nitrogen) starvation has so far been the most commonly employed approach for directing metabolic fluxes to lipid biosynthesis of microalgae. In this scenario, microalgae accumulate lipids as a means of storage under nutrient limitation when energy source (i.e., light) and carbon source (i.e., CO<sub>2</sub>) are abundantly available and when the cellular mechanisms for the photo biosynthesis are active [25]. By replacing the culture solution with tap water, both nitrogen concentration and algal density in the medium decreased, which increased the light penetration rate. The stress

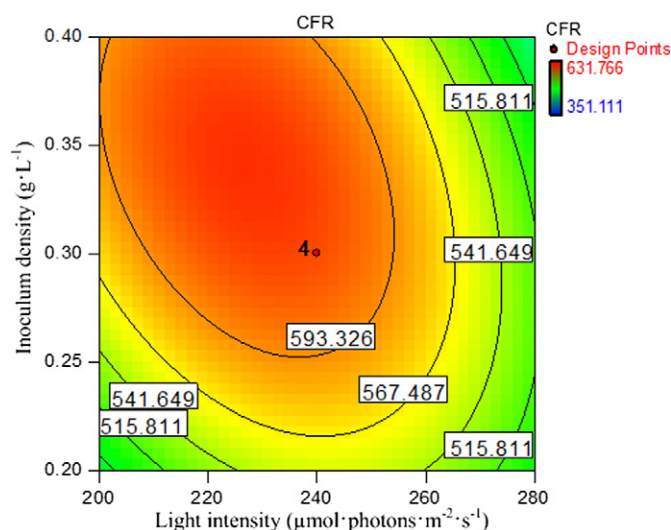


Fig. 3. Interaction of light intensity and inoculum density on CFR.

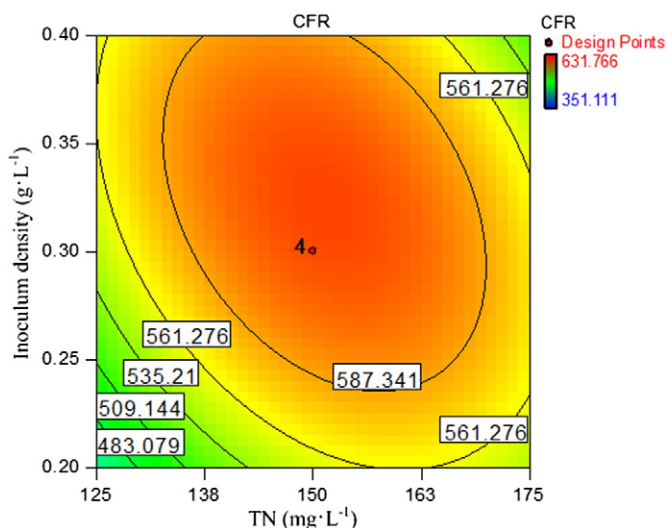


Fig. 4. Interactions of TN and inoculum density on CFR.

culture condition of saturate light, sufficient carbon content and deficient nitrogen doubled the lipid content.

#### 4. Conclusions

To increase the C/N/P removal from flue gas and manure wastewater, Plackett–Burman design and central composite design were applied to optimize the culture conditions for *S. dimorphus*. The optimized light intensity, TN and inoculum density were identified as  $238 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $152 \text{mg}\cdot\text{L}^{-1}$ , and  $0.3 \text{g}\cdot\text{L}^{-1}$ , respectively. Operated under the optimized conditions, the highest CFR was improved to  $638.13 \text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ , and TN and TP removing rate were 88.16% and 73.98%. A 5-d nitrogen starvation strategy with 80% culture medium replaced by tap water enhanced the lipid content from 11.9% to 24.2%.

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