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**Article**

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# Investigation of biomass productivity from *Nannochloropsis gaditana* via response surface methodology using MATLAB

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## Abstract

This study aimed to investigate biomass productivity to produce bioethanol from *Nannochloropsis gaditana* grown in Johnson's medium including different concentrations of macronutrients. For this study, we prepared various concentrations of  $\text{KNO}_3$  (0.1–2 g/L),  $\text{NaHCO}_3$  (5–50 g/L) and  $\text{KH}_2\text{PO}_4$  (0.05–0.8 g/L). In addition, central composite design (CCD) was used to optimize biomass productivity for acquiring the maximum biomass productivity. It was found that biomass concentration may vary with macronutrient concentrations. According to the experimental results, the maximum biomass concentration was found in 0.5, 20 and 0.8 g/L of  $\text{KNO}_3$ ,  $\text{NaHCO}_3$  and  $\text{KH}_2\text{PO}_4$ , respectively. The maximum experimental biomass yield of 2.04 g/L was obtained at these optimum conditions. These results showed that *Nannochloropsis gaditana* can be used for bio-ethanol production after optimization of macronutrient concentration.

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**Keywords:** Biomass production; Central composite design; Macronutrients; *Nannochloropsis gaditana*; Quadratic model

## 1. Introduction

Algae can be categorized as macroalgae and microalgae. Microalgae are unicellular structures and can be classified as prokaryotic and eukaryotic microorganisms. Microalgae are used for pharmaceutical, cosmetic, food and biofuel industry. They can grow fast and comprise of huge amount of biomass. They can be used for maintaining of renewable energy. There are various sources of biofuel such as biodiesel, bio-ethanol and bio-butanol [1,2]. To procedure, disruption methods can be settled to microalgae biomass for obtaining bioethanol from microalgae. Slurry of samples is reacted with microorganisms to gain bio-ethanol, include derivatives of carbohydrates such as cellulose and starch bounded various chemical structures. Starch has different chemical structures and functions compare to other carbohydrate derivative molecules. The high cost of microalgae cultivation limits effective bio-fuel production so optimum component concentration is important for bio-fuel production Onay [3]. One of these macronutrients, N has critical role for metabolism of microalgae. It gets involved in protein metabolism by means of formation

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of amino acids. C is other macronutrient and it is responsible for generation of macromolecules. P is the last in our study and it contributes to ATP formation in metabolism. The purpose of this study is to optimize culture media composition for maximum biomass productivity using central composite design CCD [4–6]. Response Surface Methodology (RSM) can mimic living cell processes in silico medium for regulations of microalgal metabolism [7,8]. RSM is a statistical technique and it is used to design experiment, build model, predict optimum conditions [9,10].

## 2. Materials and methods

### 2.1. Experimental studies of microalgae

Culture of microalgae was acquired from Yuzuncu Yil University (YYU)-microalgae collection. *N. gaditana* DEE003 was grown at  $24 \pm 2$  °C and Johnson's medium was modified. They were followed at 680 nm with OD under different concentrations of macronutrients with KNO<sub>3</sub> (0.1–2 g/L), NaHCO<sub>3</sub> (5–50 g/L) and KH<sub>2</sub>PO<sub>4</sub> (0.05–0.8 g/L).

### 2.2. Harvesting of microalgae

The algal biomass was centrifuged until it accumulated bottom of the tube. Then, samples were lyophilized and waited in deep-freeze for experiments. All experiments were performed with three biological replicates.

### 2.3. Response surface methodology

RSM is one of the statistical techniques. It serves to design experiments, determine the effect of variables and build models. In addition, RSM finds deserved experiment set up for expected response within the specified range. The empirical model was improved to correlate the response to the process and is approximated by second order quadratic polynomial equation as represented by Eq. (1) to find out the effect of parameter interactions.

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

where Y represents the response (dependent variable),  $\beta_0$  indicates the intercept. In addition,  $\beta_i$  is the linear effect,  $\beta_{ii}$  is the square effect,  $\beta_{ij}$  is the interaction effect,  $n$  is the number of variables,  $X_i$  and  $X_j$  are the independent (input) variables. Quadratic response surfaces can be called as simple models. These models are used to obtain both a maximum and minimum without making additional assumptions about the form of the response. The experimental design was executed using 2<sup>3</sup> full-factorial designs which need more runs than necessary to calculate model parameters exactly. CCD was performed to find the interaction of different medium variables such as KH<sub>2</sub>PO<sub>4</sub> (X1), NaHCO<sub>3</sub> (X2) and KNO<sub>3</sub> (X3) on biomass yield from *Nannochloropsis gaditana*. In addition, the regression model equations and processing parameters from the experiments can be determined by using CCD. The CCD experimental design was applied on our paper to determine deserved experimental media to produce maximum biomass. CCD experimental design was developed using MATLAB software package (MATLAB & SIMULINK, R2015a). Table 1 displays the ranges of the chosen process parameters.

**Table 1.** The levels of factors for the experimental design.

Reactant	−2 level	−1 level	Zero level	1 level	2 level
X <sub>1</sub> : KH <sub>2</sub> PO <sub>4</sub>	0.05	0.1	0.2	0.4	0.8
X <sub>2</sub> : NaHCO <sub>3</sub>	5	10	20	30	50
X <sub>3</sub> : KNO <sub>3</sub>	0.1	0.25	0.5	1	2

### 2.4. Factorial design of experiments

We optimized biomass productivity using central composite design (CCD) also called as Box–Wilson design. CCD is suitable for adjusting full quadratic model to obtain the maximum biomass productivity. The circumscribed CCD was applied on reactant levels to plan the experiment design. MATLAB software package (MATLAB &

SIMULINK, R2015a) was used for this work. The concentrations of macronutrients such as  $\text{KH}_2\text{PO}_4$  (X1),  $\text{NaHCO}_3$  (X2), and  $\text{KNO}_3$  (X3) were determined as independent experimental factors, and their levels were coded as  $-2$  (low),  $-1$ ,  $0$  (central point),  $+1$  and  $+2$  (high) and shown in Table 1. X1 may vary from 0.05 to 0.8 g/L with zero level at 0.2 g/L. X2 concentration may vary from 5 to 50 g/L with zero level at 20 g/L and X3 may vary from 0.1 to 2 with zero level at 0.5 g/L. Each set of input conditions (reactants) defined an experimental run.

After the experiments were performed, the data were collected (preferably in triplicates) to obtain the experimental output. Subsequently the RSM model was developed that may endorse and fit into the experimental data.

### 3. Results and discussion

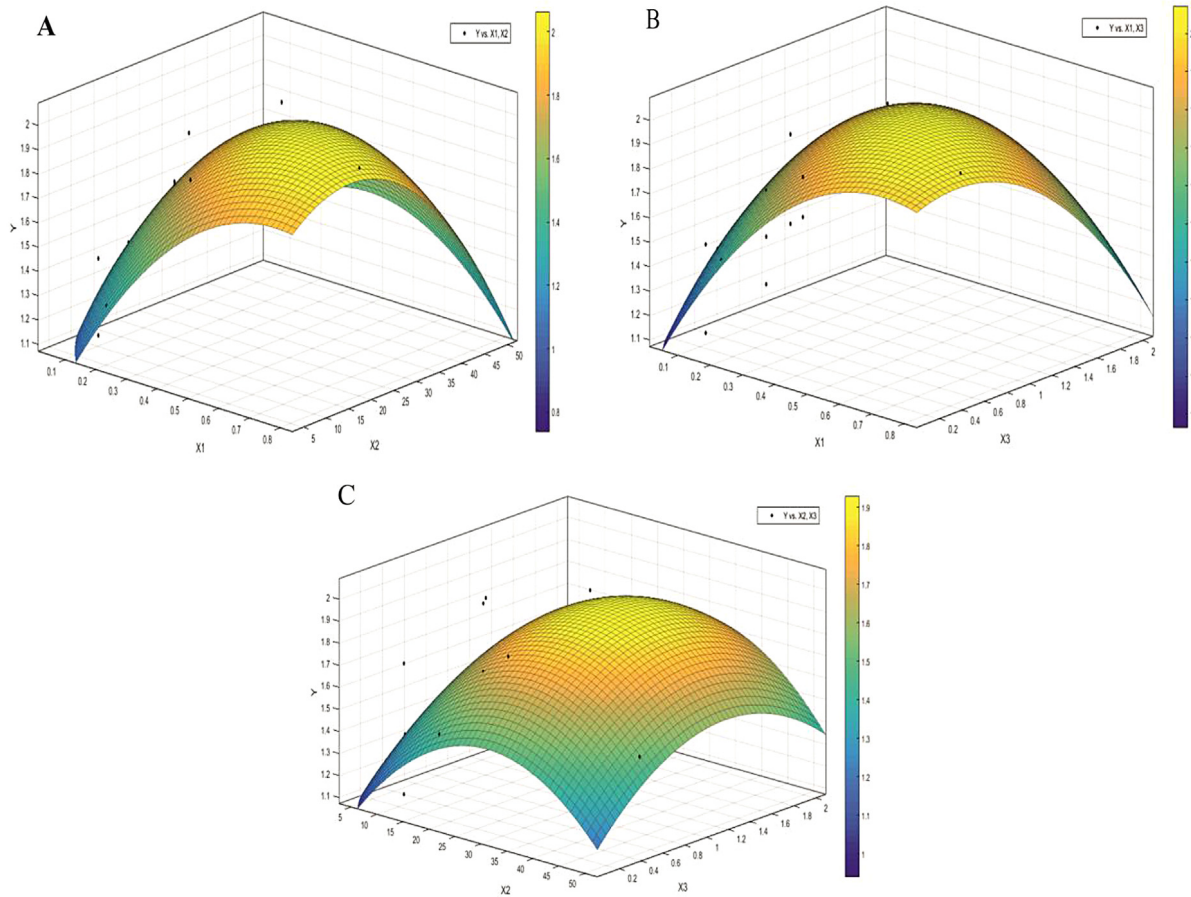
CCD of RSM in actual units for optimization of biomass production was showed in Table 2.

**Table 2.** CCD of RSM in actual units for optimization of biomass production from *Nannochloropsis gaditana*.

Exp. order	Process parameters			Biomass yield (Y), g/L	
	$\text{KH}_2\text{PO}_4$ (X1), [g/L]	$\text{NaHCO}_3$ (X2), [g/L]	$\text{KNO}_3$ (X3), [g/L]	Experimental	RSM predicted
1	0.1	10	0.25	1.141	1.119
2	0.1	10	1	1.336	1.433
3	0.1	30	0.25	1.469	1.483
4	0.1	30	1	1.744	1.800
5	0.4	10	0.25	1.643	1.714
6	0.4	10	1	1.831	1.877
7	0.4	30	0.25	1.849	1.879
8	0.4	30	1	1.962	2.046
9	0.05	20	0.5	1.329	1.403
10	0.8	20	0.5	2.042	2.007
11	0.2	5	0.5	1.375	1.315
12	0.2	50	0.5	1.539	1.511
13	0.2	20	0.1	1.353	1.485
14	0.2	20	2	1.757	1.713
15	0.2	20	0.5	1.734	1.698
16	0.2	20	0.5	1.748	1.698
17	0.2	20	0.5	1.743	1.698
18	0.2	20	0.5	1.739	1.698
19	0.2	20	0.5	1.744	1.698
20	0.2	20	0.5	1.737	1.698
21	0.2	20	0.5	1.739	1.698
22	0.2	20	0.5	1.736	1.698
23	0.2	20	0.5	1.742	1.698
24	0.2	20	0.5	1.747	1.698

According to our results, the maximum biomass concentration of 2.04 g/L was found in 0.5, 20 and 0.8 g/L of  $\text{KNO}_3$ ,  $\text{NaHCO}_3$  and  $\text{KH}_2\text{PO}_4$ , respectively. The RSM model estimated biomass yield was found in Table 2. The mean squared error (MSE) with a value of 0.0555, rsquare ( $R^2$ ) with a value of 0.9297 (the R-square statistic) and adjrsquare with a value of 0.8845 (the adjusted square statistic) were the coefficients of determination of the RSM model developed in this study. Mean squared error can be termed as the variance of the residuals, the lower values of which indicate better fit. The R-square value is one minus the ratio of the error sum of squares to the total sum of squares. In addition, the  $R^2$  value is the measure of the goodness of fit of the model. In this case it is 0.9297, which means that 92.97% of the total variation in the observed response value could be given by the model, or by experimental parameters and their interactions. The rest 7.03% can be attributed to the experimentation and other errors. When the terms of the model can be adjusted to get a better goodness of fit the corresponding  $R^2$  is termed as the adjusted  $R^2$ . The value of adjusted  $R^2$  was less than the  $R^2$  for the model. The regression model acquired by statistical analysis of experimental biomass yield can be written in Eq. (2) form as follows:

$$\begin{aligned} \text{Biomass (g/L)} = & 0.2266 + 3.4536 \times \text{KH}_2\text{PO}_4 + 0.0497 \times \text{NaHCO}_3 \\ & + 0.8280 \times \text{KNO}_3 - 0.0331 \times \text{KH}_2\text{PO}_4 \\ & \times \text{NaHCO}_3 - 0.6735 \times \text{KH}_2\text{PO}_4 \times \text{KNO}_3 + 0.0002 \times \text{NaHCO}_3 \end{aligned}$$



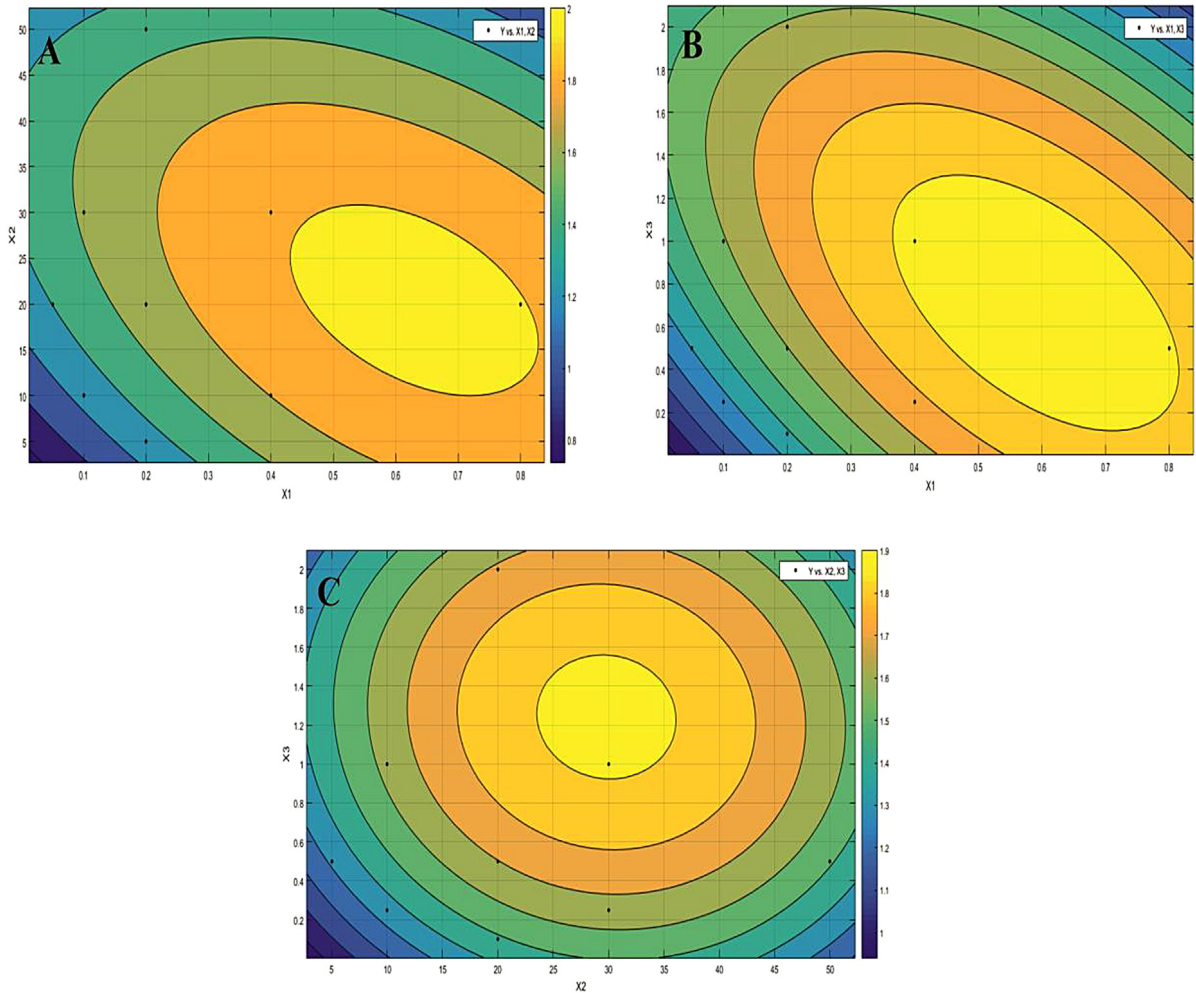
**Fig. 1.** The response surface plot of (A)  $\text{KH}_2\text{PO}_4$  ( $X_1$ ) and  $\text{NaHCO}_3$  ( $X_2$ ), (B)  $\text{KH}_2\text{PO}_4$  ( $X_1$ ) and  $\text{KNO}_3$  ( $X_3$ ), (C)  $\text{NaHCO}_3$  ( $X_2$ ) and  $\text{KNO}_3$  ( $X_3$ ) g/L concentration for biomass production.

$$\begin{aligned} & \times \text{KNO}_3 - 1.9413 \times \text{KH}_2\text{PO}_4 \times \text{KH}_2\text{PO}_4 \\ & - 0.0007 \times \text{NaHCO}_3 \times \text{NaHCO}_3 - 0.2754 \times \text{KNO}_3 \times \text{KNO}_3 \end{aligned} \quad (2)$$

The standard quadratic equation with constant, linear, interaction, and squared terms of the RSM model were given in Eq. (2). The response surface plots in Fig. 1 showed the interaction effect of important process variable combinations on biomass yield.

Fig. 1(A) shows the interaction effect of  $\text{KH}_2\text{PO}_4$  and  $\text{NaHCO}_3$  g/L concentration on biomass yield. All surface plots displayed that as the level of variables increased, biomass production increased up to a certain level. Biomass concentration showed a non-linear effect with the increase of  $\text{KH}_2\text{PO}_4$  from 0.05 to 0.8 g/L, and with the increase of  $\text{NaHCO}_3$  from 5 to 50 g/L. Both phosphate and carbon has increased biomass yield, when phosphate and carbon increased from their lower level to middle level.  $X_1$  and  $X_2$  has decreased biomass yield, when they increased from their middle level to higher level. Fig. 1(B) represented that Biomass concentration showed a non-linear effect with the increase of  $\text{KH}_2\text{PO}_4$  from 0.05 to 0.8 g/L, and with the increase of  $\text{KNO}_3$  from 0.1 to 2 g/L under constant  $\text{NaHCO}_3$  g/L concentration. Phosphate and nitrogen has decreased biomass yield, when  $X_1$  and  $X_3$  increased from their middle level to higher level. Fig. 1(C) surface plot displayed that both high and low concentrations of both  $\text{NaHCO}_3$  and  $\text{KNO}_3$  g/L concentration had negative effect on biomass production. Both carbon and nitrogen has decreased biomass yield, when they increased from their middle level to higher level. Both  $X_2$  and  $X_3$  has increased biomass yield, when  $X_2$  and  $X_3$  increased from their lower level to middle level.





**Fig. 2.** The contour plot of (A)  $\text{KH}_2\text{PO}_4$  (X1) and  $\text{NaHCO}_3$  (X2), (B)  $\text{KH}_2\text{PO}_4$  (X1) and  $\text{KNO}_3$  (X3), (C)  $\text{NaHCO}_3$  (X2) and  $\text{KNO}_3$  (X3) g/L concentration for biomass production.

The contour plots in Fig. 2 showed the relationships between biomass and different experimental conditions. Fig. 2(A) supports the regular and spherical shape of the contour plot that there was no interaction effect on biomass yield by  $\text{KH}_2\text{PO}_4$  and  $\text{NaHCO}_3$ . Fig. 2(B) shows that there was no interaction between  $\text{KH}_2\text{PO}_4$  and  $\text{KNO}_3$  on biomass yield. The regular and spherical shape of the contour plot in Fig. 2(C) define that there was no interaction relationship between biomass and carbon, nitrogen. All contour plots represented that they did not have relationship between parameters and biomass yield in detail.

#### 4. Conclusion

The regression model carried out with the statistical analysis of the experimental biomass yield was obtained in this study. The model is very forceful to determine the influence of various process parameters on biomass production from *Nannochloropsis gaditana* grown in Johnson's medium including different concentrations of macronutrients. Twenty four experimental combinations were detected by CCD and RSM was used to analyze the experimental data. The experimental biomass yield of 2.04 g/L (the maximum biomass concentration) was found in 0.5, 20 and 0.8 g/L of  $\text{KNO}_3$ ,  $\text{NaHCO}_3$  and  $\text{KH}_2\text{PO}_4$  g/L respectively. These results showed that *Nannochloropsis gaditana* can be used for biomass production after optimization of macronutrient concentration. The developed RSM model gained advantage from the chemical consumption and the cost of biomass production.

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