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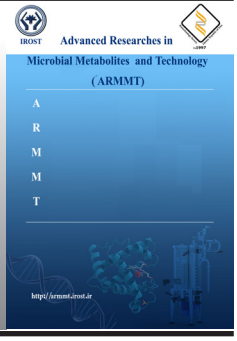

Statistical optimization of medium for biomass production of a local Iranian microalgae *Picochlorum* sp. RCC486 by response surface methodology

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Abstract

Microalgae biomass production and optimization is necessary to overcome food security problems and address environmental topics as well as high-value compounds production. In this investigation the influence of three culture media (BBM, BG-11 and Guillard f/2) on the growth and biomass production of *Picochlorum* sp. RCC486 microalgae was evaluated. One-way ANOVA with Tukey's HSD and Fisher's LSD tests were used to test the ef-fect of different culture media on optical density. Then maximum optical density and biomass production by *Picochlorum* sp. RCC486 was evaluated using response surface methodology. Lastly, Historical data design (HDD) of response surface methodology was used to find the optimum response, which yielded the highest biomass production. It was found that microalgal growth in Guillard f/2 medium reached its highest cell count in the considered time period, and the microalgae presented the highest biomass production in comparison with the other two culture media (BBM and BG-11). Additionally, biomass productivity (58 mg $L^{-1}d^{-1}$), specific growth rate (0.681d^{-1}), dry weight (1.516 g L^{-1}), and cell density(52.9 $\times 10^6$ cells mL^{-1}) were also the highest in comparison with the other two culture media. Using the HDD, the predicted optical density and biomass production were found to be close to their experimental values of 1.50313 and 1.976gL^{-1}, respectively, while the nitrogen source and phosphorus source were achieved in concentrations of 1345.55 mg L^{-1} and 6.983 mg L^{-1}, respectively. Under the optimum condition, the biomass yield was 1.961 gL^{-1}, which is 0.76% less than the predicted value. Also, the study reported that the biomass production of *Picochlorum* sp. RCC486 improved from 1.516 g L^{-1} in the defined Guillard f/2 medium to 1.961 g L^{-1} in the modified Guillard f/2 medium resulting in a 22.69% increase. Thus, the *Picochlorum* sp. RCC486 biomass content can be enhanced by optimizing nitrogen and phosphorus concentrations.

1. Introduction

Over the past few years, microalgae have become widely used for human consumption as a health food and a high value source for production of biochemical agents. These photosynthetic microorganisms are the first known organisms to produce oxygen on the planet. The

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valuable compounds with antimicrobial, anti-inflammatory, antiviral, cytotoxic and antioxidant activity are produced by microalgal biomass (De La Noue & De Pauw, 1988; Pulz & Gross, 2004).

The vast range of microalgae biological properties have been attributed to different valu-able compounds. Microalgae, as new source of valuable chemical substances, contain second-ary metabolites and diverse of biologically ac-tive molecules which can have a variety of food and pharmaceutical applications (Simić et al., 2012). The rate of microalgae growth shows that microalgae are able to thrive in a wide range of culture medium conditions, and manipulation of cultivation conditions could induce metabolite accumulation (Rios et al., 2015). Thus, optimiz-ing culture conditions to achieve higher content biomass in a short period of time could be a pri-mary objective, especially for isolated marine-water microalgae strains (Yang et al., 2012).

In experimental design some techniques are critical to determine key nutrients required for algal growth. Almost 50% of microalgae biomass contains carbon derived from CO_2 via pho-tosynthetic process (Neumara et al., 2014). Two main groups of physical and chemical param-eters, such as nutrient medium, should be considered in developing an optimal culturing for a microalgae biomass (Imamoglu et al., 2007; Wong et al., 2017). Availability of nutritional factors, such as nitrogen and phosphorus, in cul-ture media could affect the quantity and quality of microalgae biomass (Raja et al., 2014).

Response surface methodology (RSM) as a reliable statistical technique of Design of Exper-iments (DOE) is applied to predict the relationship between independent variable (s) and the response (s). Applying the RSM to a few appropriate experimental data can determine the regression model and also has the capability to optimize the response function and predict future responses. Several design experimental types of RSM, such as historical data design (HDD), miscellaneous, optimal, one-factor, central com-posite and Box–Behnken, are provided by Design Expert software. Selecting which design to use depends upon on the number of factors specified. HDD or one-factor design can be ap-plied for modeling in situations with one design factor (Jeirani et al., 2013). RSM, as a very useful statistical optimization tool, is applied extensively in optimization of medium composition. This technique can overcome the time-consum-ing and low capability process with a limited capability in the search for a global optimal condition with independent factors interactions (Cui et al., 2006).

A local microalgae sample identified as *Picochlorum* sp. RCC486 has been collected and isolated from the Persian Gulf coast. This study investigated the effects of three culture media on the biomass production with the goal of achieving higher microalgae growth rates and high content biomass in *Picochlorum* sp. RCC486 in closed systems. In our experiment, one way analysis of variance (ANOVA) was applied to optimize culture conditions and the HDDof RSM was adopted to investigate the effect of medium components (i.e. phosphorus and nitro-gen) on biomass production.

2. Materials and methods

2.1. Microalgea strain

The microalga strain, *Picochlorum* sp. RCC486, was isolated from the Persian Gulf of Iran. The identification was done by 18S rRNA sequencing and deposited in the Persian Type Culture Collection (PTCC) under accession NO. 6032. The isolation and identification of *Picochlorum* sp. RCC486 has been reported in our previous paper (Haji Abolhasani et al., 2018)

2.2. Test medium

Stock culture of *Picochlorum* sp. RCC486 was grown photoautorophically in three culture media, BBM, BG-11, and Guillard f/2, (Table 1). As it has been shown, the ratio of nitrogen to phos-phorus is different in the three mediums.

Table 1. Nutrient Composition of Different Culture Media: BBM, BG-11, and Guillard f/2

Constituents	BBM (mg L^{-1})	BG-11 (mg L^{-1})	Guillard f/2 (mg L^{-1})
$NaNO_3$	250	1500	750
K_2HPO_4	100	40	-
KH_2PO_4	150	-	-
$NaH_2PO_4.H_2O$	-	-	5
$Na_2SiO_3.9H_2O$	-	-	300
$Fe(NH_4)_3(C_6H_5O_7)_2$	-	6	-
$CaCl_2.2H_2O$	25	30	-
$CoCl_2.6H_2O$	-	-	10
$MnCl_2.4H_2O$	1.44	2	180
NaCl	25	-	-
Na_2CO_3	-	19	-
Na_2EDTA	50	0.7	4.36
$Na_2MoO_4.2H_2O$	0.71	0.4	6.3
H_3BO_3	11.4	3	-
$MgSO_4.7H_2O$	75	8	-
$ZnSO_4.7H_2O$	8.82	0.2	22
$FeSO_4.7H_2O$	0.08	-	-
$CuSO_4.5H_2O$	1.57	0.1	9.8
$FeCl_3.6H_2O$	-	-	3.15
$Co(NO_3)_2.6H_2O$	0.49	0.05	-
Citric acid monohydrate	-	7	-
vitamin B1 (thiamine hydrochloride)	100	-	200
vitamin B12(cyanocobalamin)	0. 25	-	1
vitamin H(biotin)	0. 15	-	0.1

Culture medium pH was adjusted to 6.8 (BBM), 8.0 (Guillard f/2) and 7.5 (BG-11) and the mixtures were sterilized at 121 ºC for 15 min at 15l bs. Vitamin solutions were sterilized with a membrane Filters of 0.2 µm porosity and added after sterilization of the culture.

2.3. Growth conditions

The microalgal strain was cultivated axenically as batch cultures in 250 ml erlenmeyer flasks with 150 mL of each of the three culture medium at initial counts of 10×10^6 cell mL^{-1}. The exponentially growing algal cultures were transferred into fresh sterile medium [10% (v/v) of inoculums] to produce the biomass. The inoculated media were shaked at 25 °C and 110 rpm and was illuminated by cool white fluorescent light at an intensity of 2700 lux in 16:8 h light-dark cycles. To determine biomass (g L^{-1}), at the end of the logarithmic phase each sample was centrifuged for 20 minutes at 1500 ×g, washed twice with distilled water, and then centrifuged again. After freeze-drying the wet biomass was stored at -20 °C for further experiments. The optical density (OD) was measured daily using on a microplate reader (Epoch, BioTek, America) at 620 nm. The productivity of the biomass was determined by OD measurement of the cells. The specific growth rates were determined from a logarithmic plots slope of the growth curves monitored by measuring the time-dependent OD. For determination of dry weight (DW), 10 mL aliquots of the cell suspension were harvested from each culture, filtered through filter paper (Whatman GF/F), and washed with distilled water. Finally, the filters containing the algae were oven-dried for 24h at 80°C, cooled in a desiccator, and weighed on analytical balance. DW was estimated from the difference between the initial and final weight. The number of cells was obtained by counting in a standard hemacytometer with neubauer ruling using an optical microscope. Cell size and shape were determined with an optical microscope (Nikon H550s) coupled with a digital camera for image acquisition (Rios et al., 2015). All experiments were performed under the same growth condi-

tions and were conducted three times.

2.4. Analysis of data

In this study the data analysis process consisted of two stages. The first stage was comprised of hypothesis testing with one-way ANOVA to indicate the influence of different culture media on OD (Sow, 2014). The second stage included historical data design (HDD). A historical data of response surface methodology (RSM) was used to find the optimum response, which yielded the highest biomass production. In HDD, the design points are defined by using all or some of the available experimental data. In comparison to one-factor RSM design, there is no limitation on the number of design factors in HDDof RSM (Jeirani et al., 2013).

2.5. Statistical data analysis

The goal of the present study was be to examine the effect of different culture media on OD. The independent variable was different culture media and the dependent variable was OD.

One-way analysis of variance (ANOVA) with Tukey's HSD and Fisher's LSD tests were applied to examine the significant differences among the growth parameters, OD was obtained for the different culture media. The Analysis of Variance one-way ANOVA was performed using minitab 17. The significance level (α) for all statistical tests was 0.05.

2.6. Statistical optimization of the different media components by RSM

Statistical optimization of the different medium components was carried out by using HDD, where two critical nutritional factors were chosen and their optimum levels as well as interactive effects were examined. This optimization was attempted with a view to enhance the productivity of *Picochlorum* sp. RCC486 biomass. Based upon our literature review and preliminary studies, three culture medium with different concentrations of nitrogen and phosphorus were selected for the cultivation of *Picochlorum* sp. RCC486. The RSM was employed to evaluate the first and higher-order main effects of each of the mentioned factors and in order to analyze the impact of the factor interactions to further optimize which concentrations favor maximum biomass (Dhull et al., 2014). Expert1 software version 10.0.1.0 (Stat-Ease Inc., Minneapolis, USA) was applied to conduct RSM in this study. A HDD matrix resulting from 12 experimental runs was generated (Table 2).

Table 2. Historical Data Design Matrix with Experimental Values of OD for *Picochlorum* sp. RCC486

Runs	Factor1 A: N amount	Factor2 B: P amount	Response1 OD
1	300	100	0.626
2	250	250	0.719
3	250	250	0.726
4	1500	40	0.803
5	300	100	0.653
6	750	5	1.058
7	250	250	0.764
8	750	5	1.086
9	1500	40	0.84
10	1500	40	0.882
11	750	5	1.09
12	300	100	0.686

3. Results and discussion

3.1. Growth analysis

Fig. 1 shows the *Picochlorum sp.* RCC486 cells with an amplification of 100x. The cells are circular and non-flagellated with an average 4-6 µm in diameter.

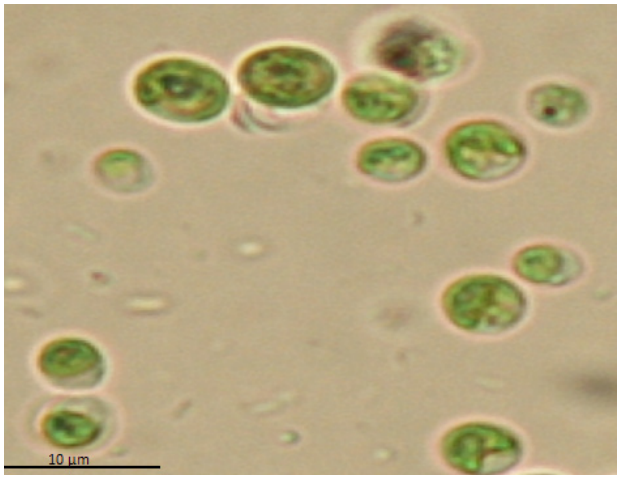


Fig.1 *Picochlorum sp.* RCC486 cells in Guillard f/2 medium on day 12 by optical microscope 1000x.

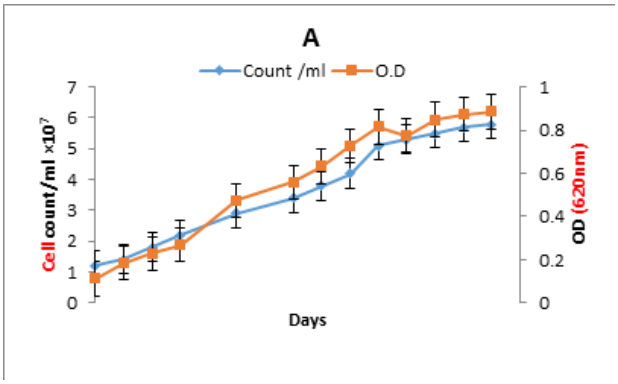
The analysis of different parameters like pH, biomass productivity (g $L^{-1}d^{-1}$), specific growth rate (μd^{-1}), dry weight (DW) (g L^{-1}), and cell density ($\times 10^7$ cells mL^{-1}) were compared throughout 12 days of cultivation in each of the three culture media. The results were recorded separately and documented in Table 3. Also, the cell count mL^{-1} and OD over time of *Picochlorum* grown are presented in Fig. 2.

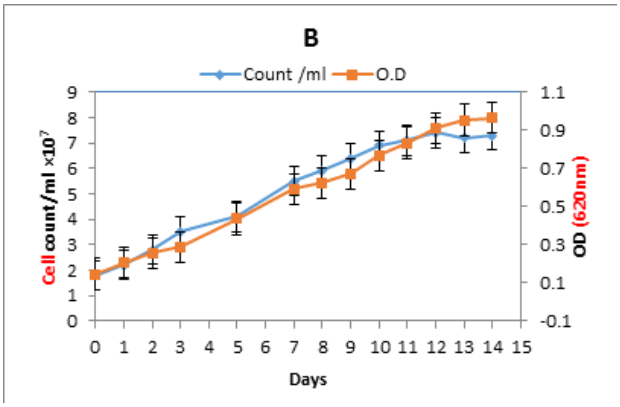
As shown in Fig. 2, microalgae growth rate was the highest in the Guillard f/2 medium in comparison with the other two culture media. Maximum cell concentration in the Guillard f/2 medium was 52.9 $\times 10^6$ cells mL^{-1}, with a maximum growth rate (μ_{max}) of 0.681 d^{-1}. The BG-11 and BBM medium cell concentration in day 12 of cultivation were 44.8 and 33.2 $\times 10^6$ cells mL^{-1}, with a maximum growth rate (μ_{max}) of 0.547 and 0.435 d^{-1}, respectively. Thus, cultivation in Guillard f/2 should be considered for process scale-up since biomass production and growth rate in this medium increased due to a difference in the ratio of concentration of nitrogen and phosphorus (Rios, et al., 2015).

The choice of a suitable culture medium is one of the major targets in the development of algal biomass production as both algal productivity and secondary metabolites are affected by the composition of the culture medium. According to our results, the growth process of *Picochlorum* sp. RCC486 is obviously dependent on the culture medium composition. The quality and quantity of the algal biomass could be optimized and improved by modifying the concentrations of the culture medium components such as phosphorus and nitrogen. Other process variables of growth conditions include pH, CO_2, agitation and intensity, and periodicity of light affect the biomass production (Dahmen et al., 2013; Coelho et al., 2013).

Table 3. Effect of different culture media on growth of *Picochlorum* sp. RCC486

Culture media	pH	Biomass productivity (g $L^{-1}d^{-1}$)	Specific growth rate (μ d^{-1})	Dry weight (g L^{-1})	Cell density ($\times 10^7$ cells mL^{-1})
BBM	6.8	0.036±0.05	0.435	1.279	3.32
BG-11	7.5	0.044±0.03	0.547	1.347	4.48
Guillard f/2	8.0	0.058±0.01	0.681	1.516	5.29





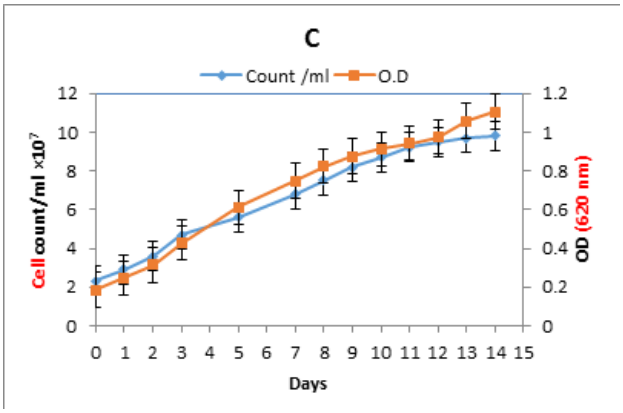


Fig.2 Effect of different media on the growth of *Picochlorum sp.* RCC486,
(A) BBM, (B) BG-11, and (C) Guillard f/2.

3.2. One-way analysis of variance (ANOVA) of the experimental results

ANOVA followed by the Tukey test (T-test) and Fisher's test (F-test) were conducted to determne the influence of different culture media on optical density. The F-value represents the ratio (a fraction) of the regression mean square and real error mean. The influence of controlled factors on each tested model is indicated by the F-value. The results of the One-way ANOVA presented in Tables 4 and 5 show an F-value for biomass production of 114.22 and a p-value of 0.000, indicating the significance of the factor on responces. The large F-value represents that the variation in the square of the responses can be illustrated by the regression equation. If the p-value is less than the chosen significance level (α<0.05) it denote that the model and the associated terms are statistically significant. The fitness of the model and sufficiency are determined by computing the coefficient of determination (R^2) and adjusted-R^2. The calculated value of R^2 for the biomass production was 0.9772, which indicates that 97.72% of experimental data was well-suited. The adjusted R-Squared value of 0.96.86% supports the R-Squared value (97.72%), which confirms the high correlation between biomass production and type of culture media. The very small p-value (0.000) and high coefficient of determination in the One-way ANOVA analysis of the regression model signifies an significant and adequate relationship between the response and input (Swamy et al., 2014).

Table 4. Analysis of variance (ANOVA) for the experimental results of **One-way ANOVA**

Source	Sum of squares	df	Mean square	F value	p-Value
Type of media	0.280305	3	0.093435	114.22	0.000
Error	0.006544	8	0.000818		
Total	0.286849	11			

R-Squared=97.72%; **Adj R-Squared**=96.86%;
Std.Dev.=0.0286

Table 5. Tukey method for **one-way ANOVA**

Level	N	Mean	St Dev	Individual 95% CIs
BBM	3	0.7267	0.0086	(- - * -)
BG-11	3	0.8403	0.0120	(- - * - -)
Guillard f/2	3	1.0580	0.0547	(- * - -)

0.75 0.90 1.05 1.20

[a] For Mean Based on Pooled St Dev

3.3. Optimization culture conditions for biomass production by *Picochlorum* sp. RCC486 using RSM

According to the results presented in Table 2, increasing the nitrogen concentrations from 250 mg L^{-1} to 1500 mg L^{-1} is associated with a decrease in phosphorus from 250 mgL^{-1} to 5 mgL^{-1}, the OD clearly increased (p-value<0.001). In addition, high nitrogen associated with low phosphorus concentrations had a positive effect on OD in *Picochlorum* sp. RCC486. Therefore the amount of nitrogen and phosphorus were the important variables impacting OD, and thus were chosen for further optimization by the HDD.

The HDD was applied to establish a proper model for the optimization of the biomass production with two independent variables (nitro-

gen and phosphorus). The experimental design, presented in Table 2, was applied to analyze the optimum conditions and explore the influence of the independent variables on the biomass production from *Picochlorum* sp, RCC486. According to the results the predicted values achieved using the model fitting technique in the Design Expert software version 10.0.1.0 correlated well with the experimental real values. Interpretation of the model summary statistics displayed that the two-factor interaction (2FI) model had maximum predicted R-squared and adjusted R squared values. Therefore, further analysis of the data was carried out by the 2FI model.

The significance of the variable is evaluated by the p-value (p-value<0.05). The coefficient estimate is adjusted to assess the effect of the variable (Yang et al., 2014). The ANOVA result of the biomass production, such as p value, standard deviation, R^2, and predicted residual sum of square (PRESS) values, confirmed the adequacy of the regression model as a significant model for biomass production.

The analysis of variance (ANOVA), a powerful statistical technique, was used to test the statistical significance of the ratio of mean square variation due to regression and mean square residual error. If the F-value is high enough to indicate statistical significance differences then the associated p-value is used (Dhull et al., 2014).

The correlation value was the basis for the evaluation of quality of the model. Tables 6 and 7 show the analysis of variance for the HDD results of the experiments..

R^2 and F-value were calculated to check the fit of the model. The results showed R^2 was 0.9783 which indicates that in HDDup to 97.83% of the data could be explained by this model; therefore, the proposed model was reasonable. The high R^2 value points out the good agreement between the predicted growth uptake and experimental resultsin this model.

The model's F-value of 120.42 indicates the significancy of the model, a p-value lower than 0.0001 further supports the fitness of the model to these data. The analysis of R_{adj}^2 and R_{pred}^2 indicated that the R_{pred}^2 of 0.9702 showed good agreement with the R_{adj}^2 of 0.9513. We can conclude that the model explained the data well.

The coefficient of variation (CV) is a measure demonstrating the standard deviation as a percentage of the mean, so better reproducibility could be accessible by smaller values of CV. A CV of less than 10 conveys that the model was reproducible.

Based on our resultsthe low 3.50 value of CV %, demonstrates the greater reliability of the experiments. The analysis of this study proved the appropriate selection of the model form for explaining of the relationship between the factors and the response. For this model the PRESS, which is an evaluation of how a particular model fits each point in the design, was 0.015.

Based on results of the experiments, HDD was used to further confirm the optimum concentrations of nitrogen and phosphorus to maximize biomass production. Among the 12 experiments, the twelfth experiment (nitrogen and phosphorus concentrations of 1345.55 mg L^{-1} and 6.983 mg L^{-1}, respectively) offered the highest OD, while the first experiment (nitrogen and phosphorus concentrations of 849.297 mg L^{-1} and 10.833 mg L^{-}1, respectively) provided the lowest OD.

Through the use of multiple regression analysis on the data above, the equation for biomass production was established as follows:

Biomass = +0.46649 + 8.77249E-004 × N amount + 4.94603E-003 × P amount – 1.89757E-005 × N amount × P amount

Based on this result, the maximum biomass of *Picochlorum* sp. RCC486 was influenced when the concentration of nitrogen and phosphorus reached 1345.55 mg L^{-1} and 6.983 mg L^{-1}, respectively, in the medium. By applying this optimized medium composition through statistical experimental designs, the *Picochlorum* sp. RCC486 biomass was improved from1.516 gL^{-1}

to1.961 gL^{-1} and OD from 1.09384 to 1.50313 in a 12 day batch culture.

Table 6. Analysis of Variance (ANOVA) for the Experimental Results of Historical Data Design

Source	Sum of squares	df	Mean square	F value	p-Value Prob > F	Remarks
Model	0.30	3	0.10	120.42	0.0001 >	significant
A: N amount	0.17	1	0.17	202.01	0.0001 >	
B: P amount	0.21	1	0.21	250.51	0.0001 >	
AB	0.18	1	0.18	211.67	0.0001 >	
Pure Error	6.711E-003	8	8.389E-004			
Cor. total	0.31	11				

N amount: Nitrogen amount, P amount: phosphorus amount

Table 7. Analysis of Variance (ANOVA) for the Fitted Model

Source of variation	Coefficient
R-Squared	0.9783
Adj R-Squared	0.9702
Pred R-Squared	0.9513
Adeq Precision	25.295
Std. Dev.	0.029
Mean	0.83
C.V.%	3.50
PRESS	0.0115

3.4. Adequacy of the models

To ensure that adequate approximation to the real values is done, the fitted model should be verified.A response surface model without adequate analysis and optimization may give disingenuous results. As can be deduced from Fig 3 diagnostic plots, such as predicted versus experimental values, enable us to display the correlation between experimental and predicted values and judge the model's adequacy. As seen in Fig. 3, the calculated actual value is compared to the predicted value computed from the study models. The data are displayed on this plot as a collection of points close to the straight line that signify sufficient agreement between the model data and the actual data. The results suggest that the models used in the process of biomass production from *Picochlorum* sp. RCC486 were able to spot out operating conditions for selective concentrations of nitrogen and phosphorus.

The data were investigated to verifying the normality of the residuals. The normal distribution of the residuals was obtained via a normal probability plot. The residual provide the variation between the experimental value and the value that is taken from the theoretical model. A small residual value represents that the prediction of the model is highly accurate. A normal probability plot of the residuals was constructed to check the normality assumption (Fig. 4). It can be observed in Fig. 4 that the data points on the plot are found close to the straight line. As with normal data, a minimal spread and scattering of the data and led us to conclude that the data was normally distributed.

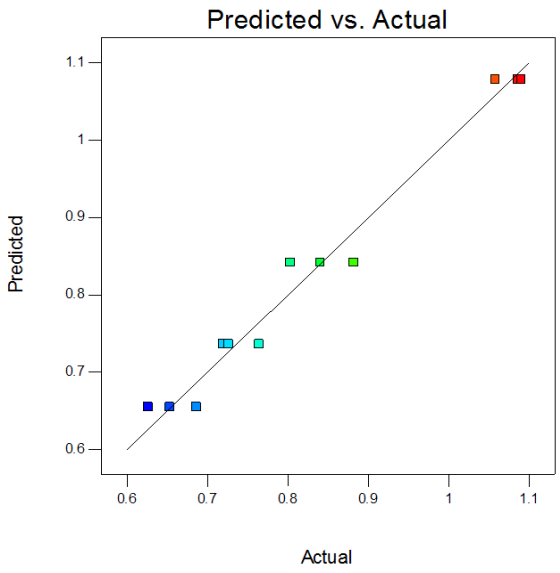


Fig. 3 Comparison between predicted and experimental values

OD in 620 nm.

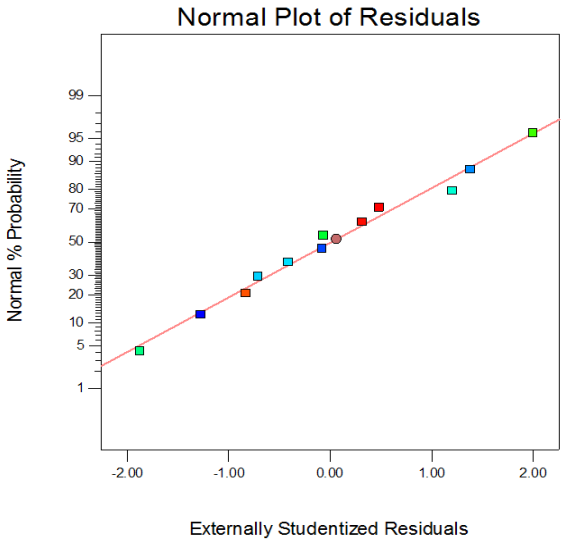


Fig. 4 Normal probability plot of studentized residuals in a 12 day batch cultur.

3.5. Interactions among the factors

The interactions between two parameters (nitrogen and phosphorus) and OD were revealed by response contour and surface plots (Fig. 5). The effects of nitrogen and phosphorus individually and their mutual interaction on OD could be observed in Fig. 4. Varying nitrogen concentration and phosphorus concentration mutual interactions had a significant effect on OD value. The surface plots and contour obtained as a function of nitrogen concentration versus phosphorus concentration indicate that OD increased with the increase of the nitrogen amount and a decrease of the phosphorus amount. The interactive effect of both parameters point out that OD increased gradually with the increase in the level of nitrogen concentration associated with the decrease in phosphorus concentration. A similar fact was noticed in the surface plot with the same concentration of variables. Accordingly, the optimum conditions for maximum production of biomass could be achieved by increasing the concentration of nitrogen (up to 1345.55mg L^{-1}) which promoted the OD (OD=1.503), but it should be noted that further increase in this concentration decreased the OD. Also, a decrease in the concentration of phosphorus promoted the OD up to a concentration of 6.98 mg L^{-1}, but it also decreased with a further decrease or increase in its concentration.

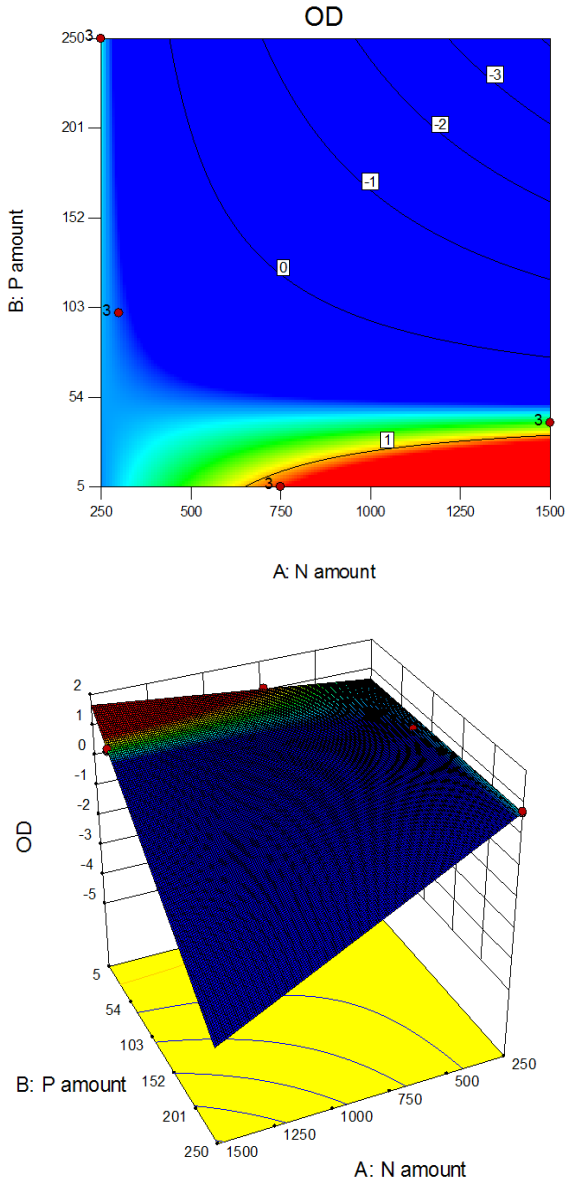
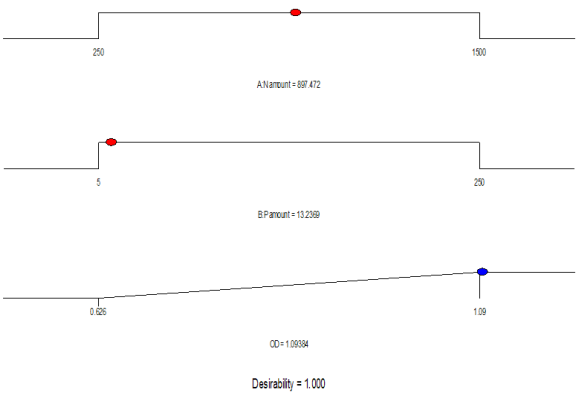


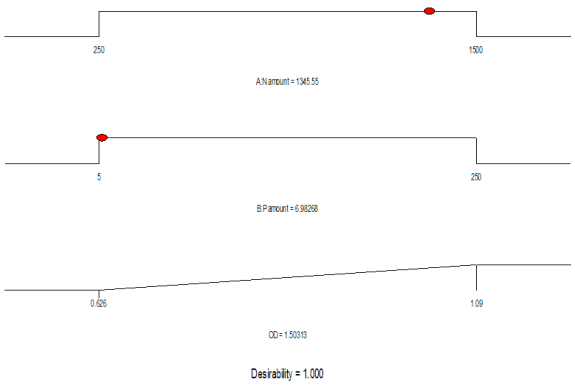
Fig.5 Contour and surface plots for OD values in 620nm from culture of *Picochlorum* sp. RCC486 showing the interaction between concentrations of nitrogen amount and phosphorus amount. All values are expressed in terms of mg L^{-1}.

3.6. Selection of optimum conditions

Optimum conditions for the biomass production were deduced to get maximum biomass production. Two-factor interaction (2FI) models were used for each response to acquire specified

optimum conditions in this study. The Desirability function method was applied to optimize the independent variables. After determining the essentials for each response, a combination of factor levels was explored to optimize a set of responses together. To optimize the biomass production each response Yi (i = 1, 2,., m) was converted into a dimensionless desirability scale which explains a partial desirability function (di), then combining the discrete desirability values to achieve the global desirability function (D), and finally maximizing the D and identifying the optimal factor settings. The desirability function scale operates between 0 (least desirable or completely undesirable response) and 1 (most desirable or fully desired response). To optimize OD from *Picochlorum* sp. RCC486 the following parameters (1) N amount as nitrogen source (from 250 mg L^{-1} to 1500 mg L^{-1}) and (2) P amount as phosphate source (from 250 mg L^{-1} to 5 mg L^{-1}) were set for maximum desirability. The optimized variables were obtained using the methodology of the desired function. This demonstrated that OD in the N concentration (1345.55 mg L^{-1})and P concentration (6.98 mg L^{-1}) reached 1.50313, with an overall desirability value of 1.000. The ramp desirability, which was established from optimum points through numerical optimization, is presented in Fig. 6. The optimum points of the model were confirmed by at least three iterations of the experiments.





Figs. 6 Desirability ramp for optical density (OD in 620nm) optimization.

4. Conclusion

The response surface methodology was employed to optimize nitrogen and phosphorus concentrations in a Guillard f/2 medium for maximal OD and biomass production. The validity of the model and optimum concentrations of the variables were confirmed with a high degree of accuracy. By the use of the desirability function, it was possible to maximize the cell growth and biomass content simultaneously. These results prove that response surface methodology is useful to enhance the biomass production of *Picochlorum* sp. RCC486.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by

any of the authors.

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References

[1] Cui F.J., Li Y., Xu Z.H., Xu H.Y., Sun K., Tao W.Y. (2006). Optimization of the medium composition for production of mycelial biomass and exo-polymer by Grifola frondosa GF9801 using response surface methodology. Bioresour Technol., 97(10), 1209-16.

[2] Dahmen I., Chtourou H., Jebali A., Daassi D., F. Karray F., Hassairi I., Sayadi S., Abdelkafi S., Dhouib A. (2013). Optimisation of the critical medium components for better growth of Picochlorum sp. and the role of stressful environments for higher lipid production. J Sci Food Agric, 94, 1628–38.

[3] De La Noue J., de Pauw N. (1988). The Potential of Microalgal Biotechnology. A Review of Production and Uses of Microalgae. Biotechnol Adv, 6, 725-70.

[4] Dhull N. P., Gupta K., Soni R., Rahi D. K., Soni S. K. (2014). Statistical Optimization of Medium Components for Biomass Production of Chlorella pyrenoidosa under Autotrophic Conditions and Evaluation of Its Biochemical Composition under Stress Conditions. IJBB, 8(8), 941-8.

[5] Haji Abolhasani M., Safavi M., Goodarz M. T., Kassaee S. M., Azin M. (2018). Identification and anti-cancer activity in 2D and 3D cell culture evaluation of an Iranian isolated marine microalgae Picochlorum sp. RCC486. DARU J Pharm Sci., DOI 10.1007/ s40199-018-0213-5

[6] Imamoglu E., Sukan F. V., dalay M. C.(2007). Effect of Different Culture Media and Light Intensities on Growth of Haematococcus pluvialis. IJNES, 1(3), 05-09.

[7] Jeirani Z., Jan B. M., Ali B. S., Noor I. M., See C. H., Saphanuchart W. (2013). Prediction of water and oil percolation thresholds of a microemulsion by modeling of dynamic viscosity using response surface methodology. J. I. E. C., 19, 554–560.

[8] Neumaral L. S. H., Amanda P. P., Donato A. G. A., Lidia M. P. M. (2014). Enhancement of Cell Growth and Lipid Content of a Freshwater Microalga Scenedesmus sp. by Optimizing Nitrogen, Phosphorus and Vitamin Concentrations for Biodiesel Production. Nat Sci, 6, 1044-54.

[9] Raja1 R., Shanmugam H., Ganesan V., Carvalho I. S. (2014). Biomass from Microalgae: An Overview. J. Oceanogr. Mar. Sci., 2(1), 1-7.

[10] Rios L. F., Klein B. C., Jr L. F.L., Maciel M. R. W., Filho R.M. (2015). Influence of Culture Medium on Desmodesmus sp. Growth and Lipid Accumulation for Biodiesel Production. Chem Eng Trans, 43, 601-6.

[11] Pulz O., Gross W. (2004). Valuable products from biotechnology of microalgae, Appl Microbiol Biotechnol., 65(6), 635-48.

[12] Sadera W. A., McNary S. W. (2011). Comparing student success between developmental math courses offered online, blended, and face-to-face. JIOL, 10(3), 128-40.

[13] Simić S., Kosanić M., Ranković B. (2012). Evaluation of In Vitro Antioxidant and Antimicrobial Activities of Green Microalgae Trentepohlia umbrina. Not Bot Horti Agrobo,. 40(2), 86-91.

[14] Sow M. T. (2014). Using ANOVA to Examine the Relationship between Safety & Security and Human Development. JIBE. 2(4), 101-106.

[15] Swamy G. J., Sangamithra. A., Chandrasekar. V. (2014). Response surface modeling and process optimization of aqueous extraction of natural pigments from Beta vulgaris using Box-Behnken design of experiments. Dyes Pigments, 111, 64-74.

[16] Wong Y.K., Ho Y.H., Ho k. C., Leung H.M., Yung K. K.L. (2017). Growth Medium Screening for Chlorella vulgaris Growth and Lipid Production. J Aquac Mar Biol, 6(1), 1-10.

[17] Yang F., Long L., Sun X., Wu H., Li T., Xiang W. (2014). Optimization of Medium Using Response Surface Methodology for Lipid Production by Scenedesmus sp. Mar. Drugs, 12, 1245-57.