OPTIMIZATION OF GROWTH CONDITION FOR *CHLORELLA VULGARIS* USING RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract

The potential of microalgae as a source of renewable energy has received considerable interest now a days, but if microalgae biofuel production is to be economically viable and sustainable to mitigate the energy crises. As microalgae has group of advantages. So further optimization of growth conditions are needed to make a different sources which provide cost-effective and sustainable means of algal growth for biofuel. This study shows that the optimum environmmental condition i.e. Temperature, pH and light period for the growth of Chlorella vulgaris in respect to produce lipid for biofuel production using response surface methodology (RSM).

Keywords: *Microalgae; Lipid extraction; Chlorella vulgaris; Response surface methodology (RSM), etc.*

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1. INTRODUCTION

Current initiatives clearly specifies to make algal biofuel production at all levels of government and in the private sector in the development of algal biofuels technologies and enterprises. Microalgae are one of the most important bioresources that are currently receiving a lot of attention due to a multiplicity of reasons such as Algal biomass has been widely used as fertilizer [1], food source for both humans and animals [2], biological cells for secondary waste water treatment [3] and bioremediation [4,5]. The world is faced with energy challenges in the near future and it is reported that fossil fuel reserves will be depleted in half a century [6]. This will be an unprecedented vicissitude that will impact negatively on all anthropogenic activities most importantly agriculture, industry and commerce. With this in mind, it is crucial to explore renewable and cost-effective sources of energy for the future sustainibility. It has been estimated that biomass could provide about 25% of global energy requirements and can also be a source of valuable chemicals, pharmaceuticals and food additives [7].

This study shows that the optimum environmmental condition i.e. Temperature, pH and light period for the growth of *Chlorella vulgaris* in respect to produce lipid for biofuel production using response surface methodology (RSM).

2. MATERIALS AND METHODS

2.1 Growth parameters of microalgae

The *Chlorella vulgaris* was acquired from natural fresh water source of Hamirpur (H.P), India. *C. vulgaris* was grown in several environmental conditions using Bold's basal medium at 21 days of time interval as shown in fig.1. The pH of the medium was adjusted with diluted H_2SO_4 and NaOH solutions with the range of 5 to 11 and the pH values were measured with pH meter after sterilization in vertical autoclave.

Fig. 1 *C. vulgaris* grows in culture room.

C. vulgaris sample was cultured at different temperature in range of 10-40 ^{o}C and light period in range to 6-24 LP with light intensity having 6 W/m^2 for a maximum 21 days of experiment time and growth was measured OD (optical density) taken by UV-spectrophotometer (Agilent cary-100) at 680 nm and total lipid Extraction done by Bligh & Dyer method [8]. The initial quanity of microalgae was 0.00492 gm in 10 ml.

2.2 Response surface methodology (RSM)

RSM is a widely accepted statistical technique used to design the experiments. Mainly, RSM follows three major steps consisting of carrying out statistically designed experiments, evaluating the coefficients in a mathematical model with prediction of response and examining the adequacy of the model. It is very useful for modelling and predicting the response of interest affected by a number of input variables with the aim of optimizing the responses. RSM can find the optimal set of experimental parameters that produce a maximum or minimum value of response, and can represent the direct and interactive effects of process parameters through two- and three-dimensional plots. The most popular response surface design (central composite design) is used to formulate the present design of experiments in this study.

2.2.1 Test for significance on individual model coefficients

This test forms the basis for model optimisation by adding or deleting coefficients through backward elimination, forward addition or stepwise elimination/addition/exchange. It involves the determination of the P-value or probability value, usually relating the risk of falsely rejecting a given hypothesis. For example, a “Prob. > F” value on an F-test tells the proportion of time one would expect to get the stated F-value if no factor effects are significant. The “Prob. > F” value determined can be compared with the desired probability or α-level. In general, the lowest order polynomial would be chosen to adequately describe the system.

2.2.2 Test for lack-of-fit

As replicate measurements are available, a test indicating the significance of the replicate error in comparison to the model dependent error can be performed. This test splits the residual or error sum of squares into two portions, one which is due to pure error which is based on the replicate measurements and the other due to lack-of-fit based on the model performance. The test statistic for lack-of-fit is the ratio between the lack-of-fit mean square and the pure error mean square. As previously, this F-test statistic can be used to determine as to whether the lack-of-fit error is significant or otherwise at the desired significance level, α. Insignificant lack-of-fit is desired as significant lack-of-fit indicates that there might be contributions in the response relationship that are not accounted for by the model.

Additionally, checks need to be made in order to determine whether the model actually describes the experimental data [9]. The checks performed here include determining the various coefficient of determination R2. These R2 coefficients have values between 0 and 1. In addition to the above, the adequacy of the model is also investigated by the examination of residuals [10]. The residuals, which are the difference between the respective, observe responses and the predicted responses are examined using the normal probability plots of the residuals and the plots of the residuals versus the predicted response. If the model is adequate, the points on the normal probability plots of the residuals should form a straight line. On the other hand the plots of the residuals versus the predicted response should be structure less, that is, they should contain no obvious patterns.

3. RESULT AND DISCUSSION

3.1 Optimization of *C. vulgaris* growth

All experiments were designed by RSM which is a statistical technique used to design the experiments called design of experiment 7.0 software. Around 22 sets of experiments designed for optimizing the growth of *C. vulgaris* in respect of concentration of lipid extraction at incubation period of 21 days. All experiments were done in triplate form. The result of pridicted values were almost similar in comparision of actual values of the experimental result. This model gave optimized value of all environmental perameters i.e. temperature (30 ^{0}C), pH (8) and light period (14 LP). The result of all optimized combine environmental condition was 1788% of *C. vulgaris* growth increase while it was 1178 % in case of optimized temperature condition, 1461%, in case of optimized pH condition and 1089% in case of optimized light period condition and concentration of total lipid extraction as shown in fig. 2, was found more in case of light period (LP), as shown in fig. 3. The percentage of growth measured in respect of 0.00492gm in 10ml BBM medium.

Fig. 2 Lipid before evaporate

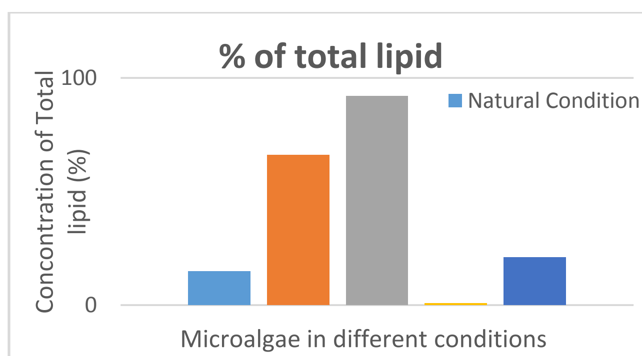

Fig. 3. % of total lipid concentration

3.2 ANOVA (Analysis of variance) for Response Surface Reduced Quadratic Model

The Model F-value of 11.05 implies the model is significant. There is only a 0.03% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, A^2, B^2, C^2 are significant model terms, values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 2.18 implies the Lack of Fit is not significant relative to the pure error. There is a 35.58% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good to the model to fit. Based on Analysis of Variance (ANOVA) for the response surface quadratic model (table no.1), the following statistical tests were performed:

i. Test for significance of the regression model. The p-value (p = 0.0002) is less than 0.05 so it indicates that the model is significant at α = 0.05.

ii. Test for lack-of-fit. The lack-of-fit test compares residual error (from model error) to pure error.

By analysing the ANOVA table it is possible to see that for the full quadratic model, the p-value for lack of fit is 0.3558, which is greater than α = 0.05. We conclude that the model adequately fits the response surface.

Table 1. ANOVA (Analysis of variance) for Response Surface Reduced Quadratic Model

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	2.286E+006	6	3.810E+005	11.05	0.0003 significant
A-Temperature	21320.45	1	21320.45	0.62	0.4470
B-pH	7.209E+005	1	7.209E+005	20.90	0.0006
C-Light Period	22565.62	1	22565.62	0.65	0.4344
A^2	1.527E+006	1	1.527E+006	44.27	< 0.0001
B^2	7.999E+005	1	7.999E+005	23.19	0.0004
C^2	7.274E+005	1	7.274E+005	21.08	0.0006
Residual	4.140E+005	12	34497.13		
Lack of Fit not significant	3.791E+005	10	37911.16	2.18	0.3558
Pure Error	34854.00	2	17427.00		
Cor Total	2.700E+006	18			

3.3 Regression diagnostic was carried out in order to check the model's adequacy:

3.3.1 Normal ProbabilityPlotof residuals is useful to check the assumption that the errors are normally distributed. A check on the plot in fig. 4 reveals that the residuals follow a straight line and so the errors seem to be normally distributed. We clarify that in this case too many parameters are estimated to justify this as a test. We can consider it as a descriptive approach.

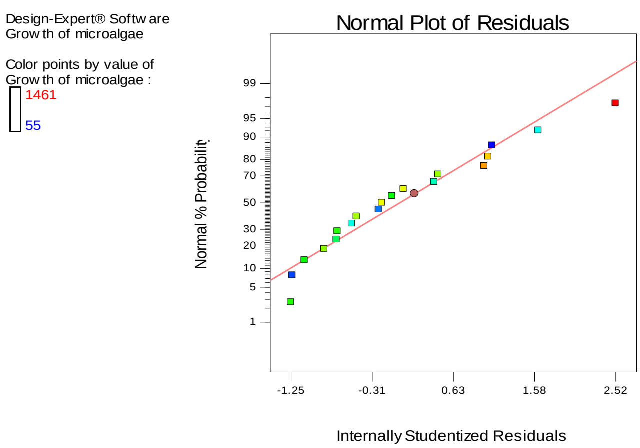

Fig. 4 Normal probability plot of residuals

The plots of the residuals versus the predicted response for growth of microalgae is shown in Fig. 5. And fig. 4 and 5 revealed that they have no obvious pattern and unusual structure also. This implies that the models proposed are adequate and there is no reason to suspect any violation of the independence or constant variance assumption. The Plot of predicted vs. actual values is shown in fig. 6.

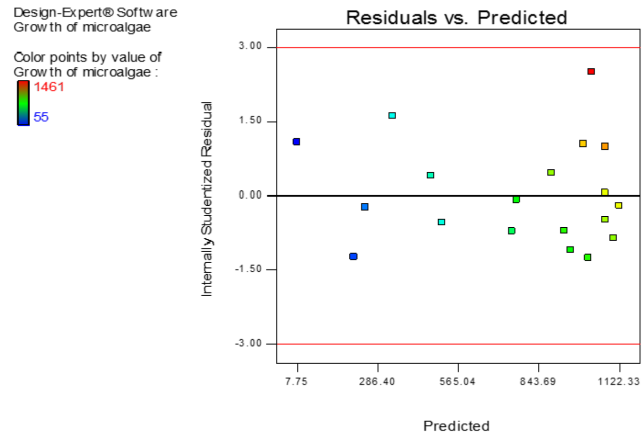

Fig. 5 Plot of residuals vs. predicted response

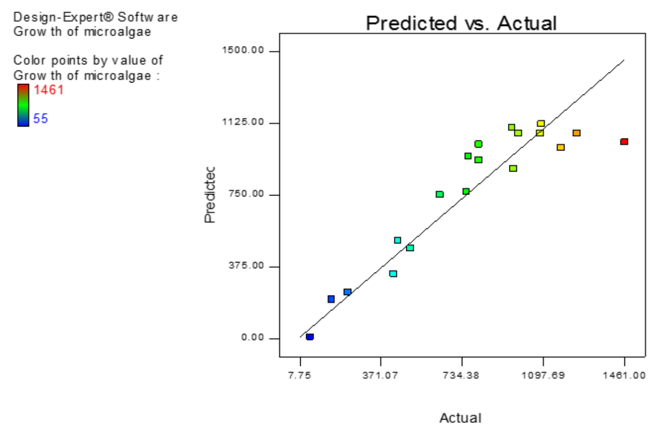

Fig. 6 Plot of predicted vs. actual values

The one factor curve and 3D surface graphs for showing the optimize growth pattern as shown in Fig. 7 (a) to 7 (f) respectively. Both have curvilinear profile in accordance to the quadratic model fitted. Fig. 7 (a) to (c) shows the optimum condition of environmental parameter i.e. temperature, pH and light period respectively. And fig. 7 (d) to (f) shows optimum environmental combine condition.

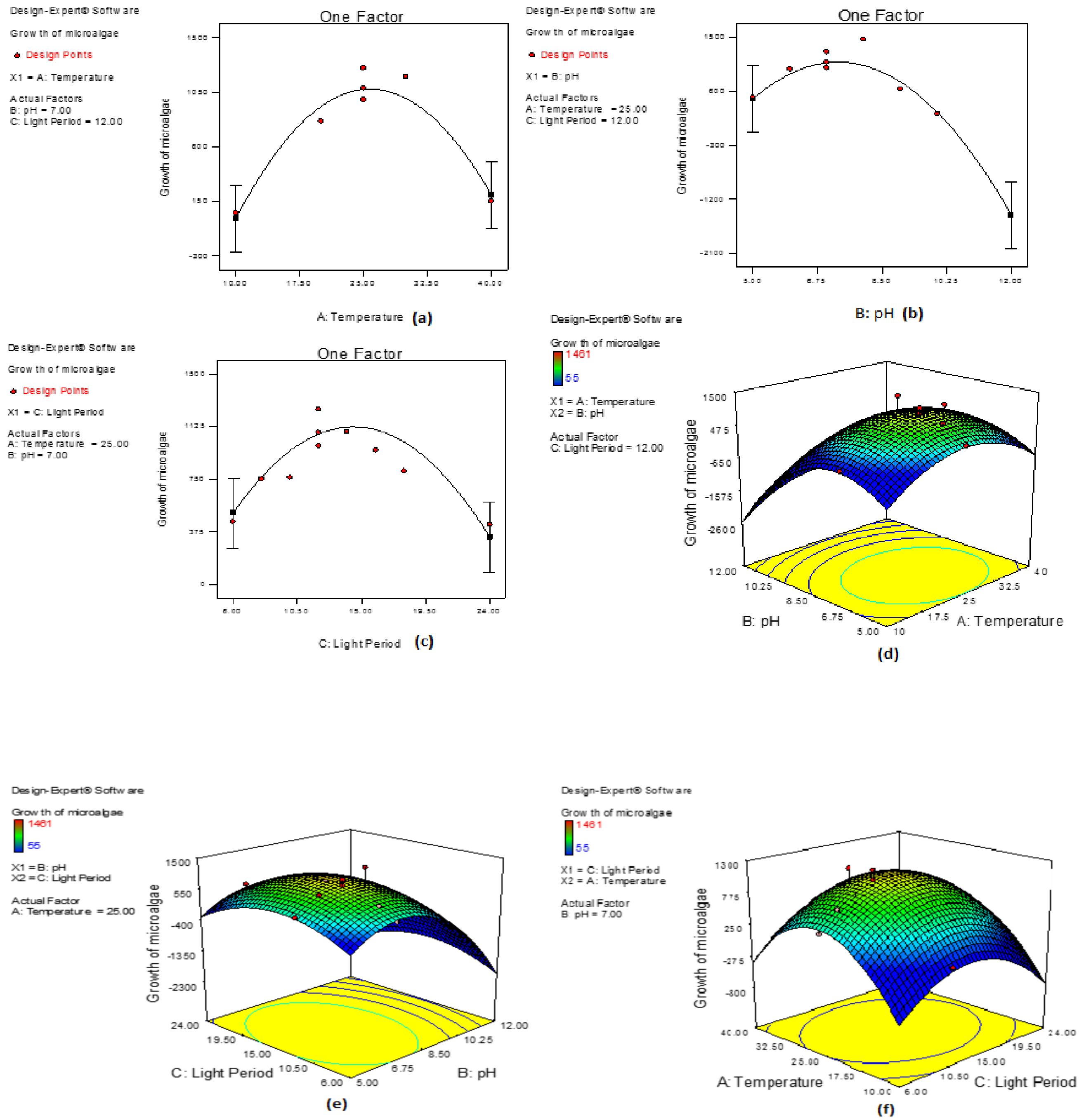

Fig. 7 (a) one factor curve of temperature, (b) one factor curve of pH, (c) one factor curve of light period, (d) 3D surface graphs for showing the optimize growth with having actual factor of light period, (e) 3D surface graphs for showing the optimize growth with having actual factor of temperature and (f) 3D surface graphs for showing the optimize growth with having actual factor of pH.

4. CONCLUSION

The growth of *C. vulgaris* was studied by optimum environmental parameter i.e. temperature (30 ^{0}C), pH (8) and light period (14 LP) using RSM in respect of concentration of lipid. The result of all optimized combine environmental condition was 1788% of *C. vulgaris* growth increase while it was 1178 % in case of optimized temperature condition, 1461%, in case of optimized pH condition and 1089% in case of optimized light period condition and concentration of total lipid extraction as shown in fig. 2, was found more in case of light period (LP), as shown in fig. 3for optimized growth and lipid extraction for biofuel production. The validity and adequacy of the predicted models was successfully verified by significant F-values and insignificant lack-of-fit P-values. The optimized growth of *C. vulgaris* was seen 1788% with all parameters together while optimized growth of *C. vulgaris* in case of temperature was 1178 %, 1461% in case of pH and 1089% in case of light period respectivly.

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