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RESEARCH ARTICLE

Application of response surface methodology for optimization of growth and lipids in *Scenedesmus abundans* using batch culture system

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Owing to an increased demand for fuel and depletion of fossil fuel, an alternative source such as algae is currently being exploited for biofuel production. One such potential microalgal candidate for biofuel production is *Scenedesmus abundans*. The aim of this study was to optimize the growth and lipid content of *Scenedesmus abundans* in a batch culture system to extend its applicability to the commercial production of biodiesel. Optimization of growth parameters such as culture time, concentration of inoculum, and concentration of sodium bicarbonate was performed by Central Composite Design-Response Surface Methodology (CCD-RSM) to achieve the maximum production of biomass as well as biofuel yield. CCD-RSM concluded that 30 days, 10% of inoculum and 8 g/L of sodium carbonate are optimum working parameters to achieve a tentative maximum biomass yield of 0.0391 g/L per day and a maximum lipid content of 26.28 % dcw. It was observed that the model prediction values satisfactorily matched with the investigational values within ± 2% for both growth rate and lipid content. Moreover, the individual and synergistic effects of the operational parameters on growth rate and lipid content were analyzed. The obtained experimental data were fitted to Lineweaver-Burk, Langmuir Hanes, Eadie and Eddie Hofstee Plots. Among this model, the Langmuir Hanes method was found to be a better fit with a coefficient of regression of 0.9888.Consequently, the results of the study support *Scenedesmus abundans* to be an apt natural source for biodiesel production.

INTRODUCTION

The ever-increasing demand for fuel owing to extensive utilization has led to the depletion of energy resources, thereby making the recovery of energy from algae inevitable besides merely treating them to extract biofuel[1]. It is apparent that the source will influence the cost in cases of demand being higher than the estimated value, arisen the need to identify an alternative fuel. Biofuel is an alternative viable energy source, which can substantiate an eco-friendly approach to circumvent the issues over energy tragedy and environmental damages such as emission of greenhouse gases, implicated in fossil fuel usage[2].

Biofuel is garnering attention since the past decade while lipids from biosources have been identified as potential producers of biodiesel.[3-6] An alga is the most preferred alternative biological source for biofuel production due to simple and cheap cultivation techniques, efficient yield and its perennial nature[7, 8].

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Despite these elite features, commercial cultivation of algae experiences several hassles due to lack of basic studies to adopt the new techniques and environmental conditions. The present-day research widely focuses on conventional methods of algal cultivation, influence of various factors on algal growth and betterment of metabolic activity to stimulate lipid production.

Microalgae are unicellular or simple multi-cellular photosynthetic microorganisms. Due to their simplest structure, microalgae possess the high-potential abilities such as, high growth rates and photosynthetic efficiencies to provide biofuel in an economical and environmentally sustainable manner[9-12]. Prior studies identified that the biomass yield of microalgae could be 50 times more than that of switch-grass, which is the fastest growing terrestrial plant. Generally, microalgae have optimum range of compositions such as 6 % to 52 % of proteins, 7 % to 23 % of lipids, and 5 % to 23 %.of carbohydrates are species dependent. Moreover, the protein content in algae species has a significantly higher C/N ratio of 10.2[13, 14]. Open pond cultivation of microalgae involves carbon dioxide (CO_2) utilization, which enriches the yield of biomass e.g., one kilogram of dry algal biomass utilizes about 1.83 kg of CO_2[15, 16].

Exhaustive research contemplates the isolation and cultivation of a local strain, extraction and purification of biofuel to develop an industrially viable economical production technique. Yet, it is recommended to optimize the production of biofuel from existing sources with respect to various influencing parameters and induce stress in the culture system so as to enrich the secretion of fatty acids, resulting in an increased yield of biofuel. For instance, *Scenedesmus* species were grown up in the batch column reactor, subjected to distinct influenced environmental conditions such as light, carbon dioxide (CO_2), temperature and the results elucidated a maximum growth rate of 0.47 per hour [17]. Shovon Mandal et al[18]., analyzed the growth response of *Scenedesmus obliquus,* which was cultivated under a distinctive range of culture conditions, and the operating parameters were optimized by RSM. A maximum yield of lipid, 61.3 % was observed in eighth day culture with 0.04, 0.03, and 1.0 gL^{-1} of nitrate, phosphate, and sodium thiosulphate respectively in the culture medium. Penglin Li et al[19]., conducted experiments in *Chlorella* species, grown up in the presence of the rice straw hydrolyzes as carbon source, and it was found that the maximum biomass concentration of 2.83 g/L was attained in 48 hours, and the lipid content was achieved as high as 56.3 %. Barghbani et al[20]., carried outgrowth experiments in *Chlorella vulgaris* and studied the effects of several parameters such as sodium chloride, sodium bicarbonate, iron, light, and temperature, which were optimized by the Taguchi's method. Results demonstrated a maximum biomass production of 3.56 g of dry matter at the optimum condition of 10 g/L sodium chloride, absence of sodium bicarbonate and 18 µ mol/L irons at 30 ± 2 °C. The motive of the present study is to activate the metabolic facility in microalgae by inducing the controlling parameters such as carbon source, culturing period, and concentration of inoculum in the culture system. Incorporation of CO_2 in growing culture has some disadvantages such as penetration and improper mixing of gases in the culture system results in failure of the experiments. Hence, the most suited carbon source for algae is inorganic carbon i.e., Bicarbonates. Algal could able to well grown up in highly alkaline conditions[21]. Therefore, Bicarbonates are supplied in the form solid and liquid state of carbon source was employed in this study.

Optimization of growth parameters plays the most thriving criterion to achieve the maximum production of biomass as well as biofuel. The optimization tool used in this study was design expert, having the several methods such as a Taguchi' method, One-level factor, RSM, etc. Response surface methodology (RSM) is ideally suited for most of the optimization studies as it requires only a fewer amount of data to identify the optimum response of the process. The recent study by Farshid Vejahati et al[22]., exploited RSM to optimize the working parameters while investigating the Canadian oil sand coke for entrained flow gasification. In this study, improvements in biomass yield and lipid content of *Scenedesmus abundans* were achieved by optimizing the operational parameters for a batch culture system using RSM. Hence, the experimental data on substrate utilization and species growth were fitted in disparate forms of curve fitting kinetic models i.e., Lineweaver-Burk, Langmuir Hanes, Eadie and Eddie Hofstee Plots are used to identify the kinetic parameters and also find the best fitted model for batch culture system.

Materials and Methods

Microalgae Collection

Ideal strain selection process plays a crucial role in biofuel production; based upon preliminary studies, the microalgae *Scenedesmus abundans* was selected for this study. *Scenedesmus abundans was* procured from National Chemical Laboratory, Pune, India. Initially, the strain was subjected to sub-culturing techniques and a known quantity of microalgae culture was regularly maintained in the culture room., Subsequent sub-culturing were done every two weeks to maintain the activity of the fresh algal culture and the culture system was continuously illuminated by the artificial fluorescent lamp in the range of 2927.07 W/m^2 with dark/light period of 12:12 hours.

Experimental Procedure

Known quantities of nutrients, vitamins and buffers were dissolved in distilled water for media preparation, followed by sterilization and the medium was kept in an open atmosphere for 24 hours to facilitate carbon dioxide incorporation in growth medium. In this study, M8 medium was used as the growth medium, due to the lesser number of components required for medium preparation, the composition is shown in Table 1[23]. A known quantity of M8 medium was subjected to an input of 2 to 10 % exponential phase inoculum culture; 2 to 30 days culture time; and 0 to 10 g/L sodium bicarbonate as demanded by Central Composite Design (CCD) in an Erlenmeyer flask to improve biomass concentration and lipid productivity. Table 2 shows the optimum ranges of the aforementioned independent variables. Preparation of medium, inoculation of algal culture and maintenance of culture system were carried out under aseptic conditions, and these experiments were performed in atmospheric temperature. The RSM design tool was used to optimize the independent variables such as culture time, inoculum concentration and sodium bicarbonate. The culture system was constantly illuminated by artificial light at the dark/light ratio of 12:12 hours per day for photosynthesis reaction and monitored at regular intervals with simultaneous measurements of growth rate and lipid content. 50 ml of culture was withdrawn and centrifuged at 5000 rpm for 10 minutes, and the biomasses were dried at 105˚C until constant weights (approximately two hours) were obtained. Lipid contents were estimated by Bligh and Dyer method[24, 25].

Methodology

Response Surface Methodology (RSM) is primarily used for developing mathematical and statistical models to elaborate the relationships among the several independent variables and one or more dependent variables. In this study, CCD was utilized to set a designed experiment to get an optimal response. CCD mainly created a two-level factorial design, augmented with center points and axial points. Regular central composite designs have five

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levels for each factor, although this can be modified by choosing an alpha value 1.0, a face-centered CCFD. The face-centered design has only three levels for each factor mainly developed for estimating a quadratic model. It replicated Centre point provides excellent prediction capability near the center of the design space[26]. The reason for chosen CCFD model in this investigation was sodium bicarbonate concentration maintained at 0 to 10 g/L. Centre composite rotatable design (CCRD) model shown a combination of influencing factor falls down to negative value, it is not applicable in experimentation part. Therefore, CCFD was used in this investigation. Entirely, RSM has exploited the individual and interaction effects on the operating parameters and responses were determined.

Results and Discussion

As compared to other simulation models, RSM model is an uncomplicated method to optimize and estimate the operating parameters, and it is possible to optimize with less number of data from the process. RSM model derived in this study was validated by the examination of the individual responses such as growth rate and lipid contents are influenced by independent variables, i.e., culture time, inoculum concentration and sodium bicarbonate. The experimental data were fitted to the second-order polynomial model as below

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} + \beta_{44} X_{42} \quad (1)$$

Where, Y was the response in coded units, β_0 was a constant, β_1, β_2, β_3 and β_4 were the regression coefficients for linear effects, β_{11} , β_{22} , β_{33} and β_{44} were the quadratic coefficients and β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} were the interaction coefficients, and X_1, X_2, X_3 and X_4 were the parameters considered[27, 28]. The model proposed for each response by RSM can be applied to predict the valuest at ease for any combination of the parameters considered in the experimental domain[29]. In this study, the growth rate and lipid content of *Scenedesmus abundans* were controlled by independent variables, i.e., culture time, inoculum concentration, and sodium bicarbonate whereas the dependent output variables were the maximum growth rate and lipid content. The obtained results are shown in Table 3, which describes the combination of explanatory variables and the corresponding response variables.

Response on Growth Rate of *Scenedesmus abundans*

The final quadratic equation obtained for the growth rate of *Scenedesmus abundans* is given below in equation (2).

Growth rate of *Scenedesmus abundans* (g/L/day), $Y_1 = (0.037875 - (4.00297 \times 10^{-6} A) - (9.71294 \times 10^{-5} B) + (7.95287 \times 10^{-5} C) + (1.34286 \times 10^{-6} AB) + (2 \times 10^{-6} AC) - (5.36 \times 10^{-6} BC) + (7.07236 \times 10^{-7} A^2) + (9.41364 \times 10^{-6} B^2) - (5.04727 \times 10^{-6} C^2))$ (2)

The individual influences of *A*, *B*, and *C* and the combined effects of *AB*, *BC*, and AC were having significance in both actual and predicted response data. The graphical analyses of the entire effects and interaction plots were clearly presented. In order to obtain a better response surface result for the growth rate of *Scenedesmus abundans*, a slight modification has been done in the model response equation of growth rate. The growth rate was more or less showing the same response for both the predicted and modified models. The absolute average deviation for predicted model was 0.006 %, and for the customized model it was 1.2382 %. The root mean square deviation for modified and predicted model was 1.7 % respectively had shown in Table 4. Modified Model for the *Scenedesmus abundans* considering the growth rate as its response variable is as follows[30].

Proposed response model for Growth rate of *Scenedesmus abundans* (g/L/day), $Y_1 = (0.037875 - (4.00297 \times 10^{-6} A) - (9.71294 \times 10^{-5} B) + (7.95287 \times 10^{-5} C) + (1.34286 \times 10^{-6} AB) + (2 \times 10^{-6} AC) - (5.36 \times 10^{-6} BC))$ (3)

Influence of Culture time on Growth Rate

The effect of culture time on the growth of *Scenedesmus abundans* was investigated at three different intervals such as 2, 16 and 30 days. It was observed that the growth rate has increased as culture time prolonged with the depletion of nutrients in culture media. This is because the newly cultured algae cells require a certain time period to adapt to the culture environment. The proportional change in growth rate of *Scenedesmus abundans with* culture time is depicted in Fig. 1. It was noted that the growth rate was higher on the 30th day, taking into account of all the parameters that have inflicted. Usually algal cells are made up of multi- cellular membranes which are highly permeable in nature to allow water than solutes or ions. Water can move across the membranes by osmosis. The solutes available in the medium were salts, organic and inorganic molecules which are dispersed in the distilled water (medium). The solvent is capable of penetrating into the cellular membrane which is consumed by algae cells for the growth[31]. It results in the form of cell division and biomass yield increased during growth period. The time taken for establish cell generation and cell stability is slightly longer process i.e., *Scenedesmus abundans* uptake the nutrients in the medium results in depletion after a certain period and it slow down the reproduction rate ended in steady state was investigated.

Influence of Inoculum Concentration on Growth Rate

In the uncarbonated condition, biomass yield of *Scenedesmus abundans* was 0.791 g/L for 2% inoculum concentration and 1.023 g/L for 10 % inoculum concentration indicating optimum inoculum concentration did not greatly influencing the biomass yield. Hence, the system operated with the presence of 10 g/L of sodium bicarbonate in 2 % and 10 % of inoculums concentration

revealed 1.908 g/L of biomass yield obtained in 30th day. Since, the variation in inoculum concentration doesn't have much more influence effect on biomass yield for carbonated growth study. The result concludes that increase the inoculum ratio in lab level batch culture system doesn't have higher yield in biomass for carbonated microalgae system.

Influence of Sodium Bicarbonate on Growth Rate

The effect of sodium bicarbonate on the growth rate of *Scenedesmus abundans* is portrayed in Fig. 2 and 3. It was so obvious that an increase in the amount of sodium bicarbonate ended in high yields of biomass due to the natural high requirement of a carbon source by the algal cells. Carbon source regulates the activity of the dependent cell growth and broadens the mutation foundation in the algal cells. Naturally-occurring CO_2 in air is not sufficient for algal growth at a large-scale level; requiring an artificial source such as sodium bicarbonate, or sodium thiosulphate.

Effects of Culture time and Inoculum Concentration on Growth Rate

The growth rate of *Scenedesmus abundans* increased with an increase in culture time. But, the combination effect of culture time and inoculum concentration didn't significantly influence the growth rate of the study species were shown in Fig. 1. The maximum growth rate of *Scenedesmus abundans* was 0.0391 g/L per day for inoculum concentrations of 2 % and 10 %, and the corresponding culture time was 30 days.

Effects of Inoculum Concentration and Sodium Bicarbonate on Growth Rate

Fig. 2 verifies that the increase in inoculum concentration slightly improved the growth rate while sodium bicarbonate was highly considerable for an increase in the growth rate of culture. The maximum growth rate of *Scenedesmus abundans* was 0.0391 g/L per day, which occurred in the presence of immense sodium bicarbonate (10 g/L) with both the base inoculum concentration (2 %) and also the lofty inoculum concentration (10 %). High biomass production occurred in both low and high concentrations of initial inoculum with an increase in bicarbonate, because the growth of algae mainly depends on the carbon source for their metabolic activities, mitosis and meiosis processes, and sparing little response to the initial number of cells.

Effects of Culture time and Sodium Bicarbonate on Growth Rate

The maximum growth rate of *Scenedesmus abundans* was found to be 0.0391 g/L per day for the 30th day of growth and at a sodium bicarbonate concentration of 10 g/L, explicated in Fig. 3. The maximum optimized tolerable value of bicarbonate would be 8 g/L. Sodium bicarbonate supplied created the respiration environment for algae, justifying its incorporation in the growth medium. The high amount of carbon source ended with a high rate of carbon dioxide production, which increased the growth of algal culture.

Comparison of Experimental and Predicted Values by RSM

The predicted values of the growth rate of *Scenedesmus abundans* using the quadratic equation generated by RSM have been compared with the empirical values. The comparison showed that the model prediction satisfactorily matched with the investigational values within ± 2 %. An Analysis Of Variance (ANOVA) to determine the significant effects of process variables was conducted and the results are shown in Table 5. It can be noticed from the table, the F-values for the regressions were higher. The spacious F-value indicated that most of the variance in the response could be explained by the regression model equation. The associated p-value was used to calculate whether the F-statistics were large enough to indicate the statistical significance. The lower p-value (<0.0001) indicated that the model was statistically significant[32]. The model adequacy was checked by R^2 and R_{adj}^2. A higher value of R^2 (0.9535) showed that the model could predict the response successfully. The model adequacy has also been verified with R_{adj}^2 value. The ANOVA indicated that the second-order polynomial model was significant and adequate to represent the actual relationship between the response and the explanatory variables, with a small p-value (<0.0001) and a high value of R^2 (0.9535) for the growth rate of *Scenedesmus abundans.* Comparison of experimental and predicted data of growth rate is provided in Fig. 4.

Lipid Content Response on *Scenedesmus abundans*

The final quadratic equation (4) obtained for lipid content is given below:

Lipid Content (% dcw), $Y_2 = (0.327162 + (0.35542 \text{ x } A) + (0.09635\ B) + (0.26177\ C) + (0.015056\ AB) + (0.014616\ AC) - (0.022719\ BC) + (1.43232 \text{ x } 10^{-3} A^2) + (0.017466\ B^2) + (2.1981 \text{ x } 10^{-3} C^2))$ (4)

In order to improve the response model, a modification has been made in the design of the predicted model response equation. The results were found to be similar in both predicted and modified model responses to the lipid content. A summary of the actual, predicted, proposed model data and associated deviation is shown in Table 6. The absolute average deviation for predicting model was 1.3067 % and for the modified model was 0.6333 %. Root means square deviation for the predicted and as well as modified model has 13.3135 and 13.2546 % been depicted in Table 6.The customized model equation for lipid content is as follows.

Proposed model of Lipid content of S*cendesmus abundans* (% dcw), $Y_2 = (3.27162 + (0.3554\ A) + (0.09351\ B) + (0.26177\ C) + (0.015056 \text{ x } AB) + (0.014616 \text{ x } AC) - (0.022719\ BC) + (1.42324 \text{ x } 10^{-3} A^2) + (0.017466\ B^2))$ (5)

Effects of Culture time and Inoculum Concentration on Lipid Content

In this study, the maximum lipid content of 25.679 % dcw was obtained at the most favourable optimum conditions, which were

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along culture time of 30 days and a 10 % inoculum concentration. The maximum growth rate of *Scenedesmus abundans* was 0.0391 g/L/day, on the 30th day, and at inoculum concentrations of 2 and 10 %, as demonstrated in Fig. 5. Even every single biological organism requires a precise time interval to activate the cell multiplication mechanism and convert the growing culture environment into the adopted environment that in turn can be taken into account for further processes. Hence, culture time as well as inoculum concentration revealed an eminent role in influencing the lipid content.

Effects of Inoculum Concentration and Sodium Bicarbonate on Lipid Content

Expanding the inoculum concentration and sodium bicarbonate enhanced the lipid content of *Scenedesmus abundans*. Fig. 6 shows the maximum lipid content was 25.679 % of dcw that occurred owing to the high inoculum concentration (10 %) and sodium bicarbonate (10 g/L). The lipid content started to decline once the treatment with sodium bicarbonate began losing its vigor. This decrease in lipid productivity was largely due to the lack of biomass yield, thus correlating the biomass yield with the frequency of sodium bicarbonate treatment.

Effects of Culture time and Sodium Bicarbonate on Lipid Content

Fig. 7 explains that the maximum lipid content of 25.679 % dcw was achieved at two different optimal conditions such as a high culture time (30^{th} day) and sodium bicarbonate concentration (10 g/L). From the results, it was comprehended that the maximum lipid content reduced to 24.54 % dcw due to decrease of sodium bicarbonate up to 5 g/L. The present study confirms sodium bicarbonate as the most functional carbon source for *Scenedesmus abundans* growth, as the optimum bicarbonate treatment has been advantageous over maximum biomass production, and it also indicates that carbon source has a pronounced effect on microalgal growth and rapid production of biomass in tested strain leads to a higher lipid rate.

Comparison of Actual and Predicted Values by RSM

Fig. 8 shows the predicted values of lipid content using the quadratic equation generated by RSM, and have been compared with the investigated values. The comparison showed that the model prediction satisfactorily matched with the experimental values within ± 2%. An analysis of variance to determine the significant effects of process variables was conducted and the results are shown in Table 6. The ANOVA indicated that the second-order polynomial model was significant and adequate to represent the actual relationship between the response variable (lipid content) and the independent variables, with a small p-value (<0.0001) and a high value of R^2 (0.9586) for lipid content.

Study the Growth response of *Scenedesmus abundans* cultured in different levels of carbon source as substrate concentration in batch culture

In this study, a significant difference was shown by growing algae in the presence and absence of a carbon source. The optimum conditions arrived in this study were applied to a batch culture system containing M8 medium with supporting, controlling parameters such as 10% of inoculum, constant photosynthetic effect illuminated by artificial light in 14 photons and a time period of 30 days. The time profile corresponding to dry weight basis biomass is established in Fig. 9a; the effect was found to be steady and the maximum biomass growth was achieved in the 30^{th} day culture. The biomass concentrations in the culture growing both in the presence and absence of carbon source were 1.608 and 1.514 g/L, which occurred on the 30^{th} day. At the same time study species is subjected to several levels of carbon source as active substrate concentration in the batch culture system. The maximum biomass concentration occurs in zeroth and 10 g/L of carbon concentration. An increase of carbon concentration has significant results in the yield of biomass. The analyzed treatments have played a significant role in an effective result feature in experimental data and this data are valid by using kinetic models. The concept reflecting form of this study has been depicted in Fig. 9(a) and Fig. 9(b) which describes the increases in biomass concentration with respect to extending the carbon sources up to a certain level and equivalently the specific growth rate was decreased.

The effect of sodium bicarbonate was amplified with an increase in the pH of the medium due to the prevalence of a stress directed towards exponential production of the biomass via amplification of the photosynthetic activity. This study summarizes the effects of substrates which boost up the yield of lipids as well as biomass.

Determination of Statistical Error bars for predicted and proposed models

The accuracy of predicted and developed model for the growth rate and lipid recovery percentage has been estimated from absolute average deviation of the individual response. The average error percentage for predicted and proposed model for growth was 0.0006 and 1.2382 as well as predicted and modified model of lipid % response in 1.3067 and 0.6333. The error bars for different models has been shown in Figure 10. The average error percentage for Growth rate was less in predicted and for proposed this will become high it is due to optimum condition of microalgal culture was more sensitive. Lipid estimated from predicted will be high and proposed model have shown less

percentage of error. So the predicted model for growth rate and developed model for lipid % (dcw) were more reliable for batch study.

Kinetics Studies of Linear Models

The experimental data for substrate concentrations during the growth phase of *Scenedesmus abundans* in batch culture systems were used for the determination of kinetic parameters. The kinetic parameters were identified on the curve fitting models are shown in Table 7, i.e., Lineweaver-Burk, Langmuir Hanes, Eadie and Eddie Hofstee Plots [33-36]. These plotting methods determine the kinetic parameters from standard kinetic models. The kinetic parameters are $\mu_{max,}$ maximum specific growth rate and K_s half-saturation coefficient. Lineweaver-Burk plot method was used as a model which is linearized from the Monod equation. All the Methods used in this curve fitting procedure are based on the substrate concentration has been validated for the two kinetic parameters. The symbols used in the above mention models are μ is the specific growth rate (day^{-1}), μ_{max} is the maximum specific growth rate (day^{-1}), K_s is the half-saturation coefficients (g/L) and S is the substrate concentration (g/L). The obtained result from the model fitting methods with the experimental data had shown a relatively satisfactory relation between the regressions from the linear plots, i.e., 0.8259 for Lineweaver-Burk, 0.9888 for Langmuir Hanes and 0.885 in both Eadie and Eddie Hofstee plots are shown in Fig. 11. This experimental consistency could exhibit that sodium bicarbonate as limiting substrate in functional concentrations is not having any suppressing effect on the cell growth in a certain level of carbon source. The linearized model of Monod is Lineweaver-Burk plot from this method the maximum specific growth rate is 0.0598 day^{-1} and Monad constant is 0.6576 g/L respectively. As well as, for Langmuir Hanes, Eadie and Eddie Hofstee plots, the maximum specific growth rate and half-saturation coefficients are 0.0599, 0.0951, 0.0822 day^{-1} and 0.4918, 1.5770, 1.3958 g/L respectively.

Conclusion

This study concludes that the *Scenedesmus abundans* has ample potential for high biomass yield and lipid content, which were observed in the presence of the carbon source, a long culture time and both the base and lofty concentrations of the mother culture. The operating parameters such as culture time, inoculum concentration and sodium bicarbonate concentration were studied and optimized using Central Composite Design of Response Surface Methodology (CCD-RSM). The influences of individual and combined operating parameters on the growth rate and lipid content of *Scenedesmus abundans* were interpreted with a maximum growth rate of 0.0391 g/L per day and lipid content of 26.282 % dcw on the 30^{th} day, 10 % of inoculum concentration and 8 g/L of sodium bicarbonate. The studied batch processes of experimental data are also best fitted with Langmuir Hanes model than that of other Lineweaver-Burk, Eadie and Eddie Hofstee model. Hence, commercial production of biodiesel from the perennial algal source, *Scenedesmus abundans* is the future perspective of this work.

Notes and references

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Electronic Supplementary Information (ESI) available: [Native format of the figures is also uploaded].

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TABLES

Table 1 Composition of M8 medium

Components	*Quantity required (g/L)*
KNO_3	0.75
KH_2PO_4	0.185
$NaHPO_4$	0.065
$CaCl_2.2H_2O$	0.00325
$FeSO_4.7H_2O$	0.0325
$MgSO_4.7H_2O$	0.1
Na.EDTA.Fe	0.01

Table 2 Optimum levels of independent variables for growth study in *Scenedesmus abundans*:

Factors	*Unit*	*Range and levels*		
		-1	*0*	*+1*
Culture Time	Days	2	16	30
Inoculum Concentration	%	2	6	10
Sodium bicarbonate	g/L	0	5	10

Table 3 Design matrix and response data for *Scenedesmus abundans* growth study as determined by RSM:

Standard order	*Culture time (days)*	*Inoculum concentration (%)*	*Sodium bicarbonate (g/L)*	*Growth rate (g/L) per day*	*Lipid Content (% dcw)*
1	2	2	0	0.0377	5.810
2	30	2	0	0.0382	15.789
3	2	10	0	0.0378	6.245
4	30	10	0	0.0388	21.589
5	2	2	10	0.0379	7.625
6	30	2	10	0.0391	23.689
7	2	10	10	0.0378	8.235
8	30	10	10	0.0391	25.679
9	2	6	5	0.0378	4.358
10	30	6	5	0.0389	24.540
11	16	2	5	0.0385	10.259
12	16	10	5	0.0382	18.640
13	16	6	0	0.0381	12.590
14	16	6	10	0.0381	15.860
15	16	6	5	0.0381	13.569
16	16	6	5	0.0381	13.569
17	16	6	5	0.0381	13.569
18	16	6	5	0.0381	13.569
19	16	6	5	0.0381	13.569
20	16	6	5	0.0381	13.569

Table 4 Estimated data for Proposed Response Models as a Function of code operating variables

Standard order	*Growth Rate (g/L per day)*			*Lipid Content (% dcw)*		
	Experimental	*Predicted*	*Proposed Model*	*Experimental*	*Predicted*	*Proposed Model*
1	0.0377	0.0377	0.0377	5.8100	4.3053	4.3053
2	0.0382	0.0383	0.0376	15.789	16.375	16.375
3	0.0378	0.0379	0.0369	6.2450	6.9710	6.9710
4	0.0388	0.0388	0.0372	21.589	22.413	22.414
5	0.0379	0.0379	0.0384	7.6250	6.9808	6.7609
6	0.0391	0.0391	0.0389	23.689	23.143	22.924
7	0.0378	0.0377	0.0372	8.2350	7.8290	7.6091
8	0.0391	0.0391	0.0380	25.679	27.364	27.144
9	0.0378	0.0378	0.0376	4.3580	6.1871	6.1321
10	0.0389	0.0388	0.0380	24.540	21.990	21.935
11	0.0385	0.0383	0.0382	10.259	12.367	12.312
12	0.0382	0.0383	0.0373	18.640	15.811	15.756
13	0.0381	0.0379	0.0374	12.590	11.958	11.958
14	0.0381	0.0382	0.0382	15.860	15.771	15.551
15	0.0381	0.0381	0.0378	13.569	13.809	13.755
16	0.0381	0.0381	0.0378	13.569	13.809	13.755
17	0.0381	0.0381	0.0378	13.569	13.809	13.755
18	0.0381	0.0381	0.0378	13.569	13.809	13.755
19	0.0381	0.0381	0.0378	13.569	13.809	13.755
20	0.0381	0.0381	0.0378	13.569	13.809	13.755
AAD (%)	-	6×10^{-3}	1.2382	-	1.3067	0.6333
RMSD (%)	-	*0.*2366	1.7000	-	*13.3135*	*13.*2546

Note: AAD- Absolute average deviation; RMSD-Root mean square deviation

Table 5 Analysis of Variance of the 2^3 Factorial Designs for the Growth rate response of *Scenedesmus abundans*

Source	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F Value*	*P Value*
Regression	9	3.354×10^{-6}	3.726×10^{-7}	22.81	<0.0001
Residual error	10	1.634×10^{-7}	1.634×10^{-8}		(Significant)
Lack of fit	5	*1.631* $\times 10^{-7}$	*3.261* $\times 10^{-8}$		
Pure error	5	*3.072* $\times 10^{-10}$	*6.144* $\times 10^{-11}$		
Total	19	3.517×10^{-6}			

R^2=0. 9535, R_{adj}^2=0. 9117

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Table 6 ANOVA results of the quadratic models from lipid content of *Scenedesmus abundans*:

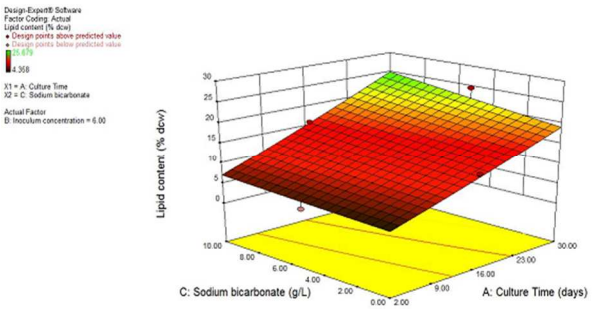
Source	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F Value*	*P Value*
Regression	9	707.45	78.61	25.70	< 0.0001 (Significant)
Residual error	10	30.59	3.06		
Lack of fit	5	*30.59*	*6.12*		
Pure error	5	*0.000*	0		
Total	19	738.04			

$R^2 = 0.9586$, $R_{adj}^2 = 0.9213$

Table 7 Linear plots

Curve Fitting Model	*Methods*
$\frac{1}{\mu} = \frac{Ks}{\mu max}\frac{1}{S} + \frac{1}{\mu max}$	Lineweaver-Burk plot
$\frac{S}{\mu} = \frac{Ks}{\mu max} + \frac{S}{\mu max}$	Langmuir Hanes plot
$\frac{\mu}{S} = \frac{\mu}{Ks} + \frac{\mu max}{Ks}$	Eadie Plot
$\mu = \mu \max - Ks\frac{\mu}{S}$	Eddie Hofstee Plots

GRAPHICAL ABSTRACT



FIGURES

Fig.1 Effects of different culture times and inoculum concentrations on growth rate of *Scenedesmus abundans*

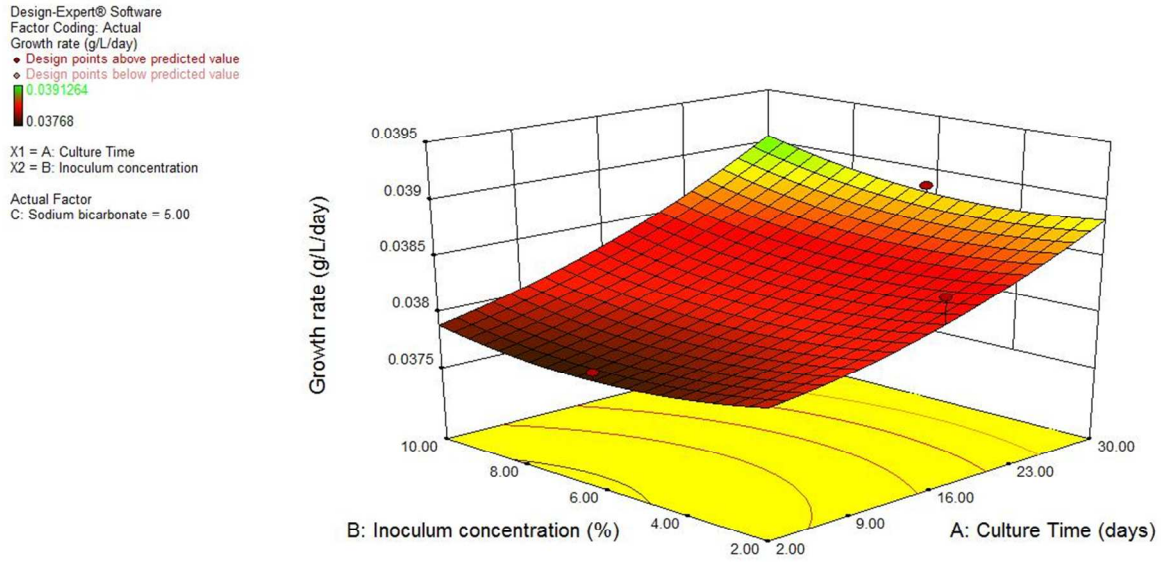


Fig. 2 Effects of inoculum concentration and sodium bicarbonate on the growth rate of *Scenedesmus abundans*

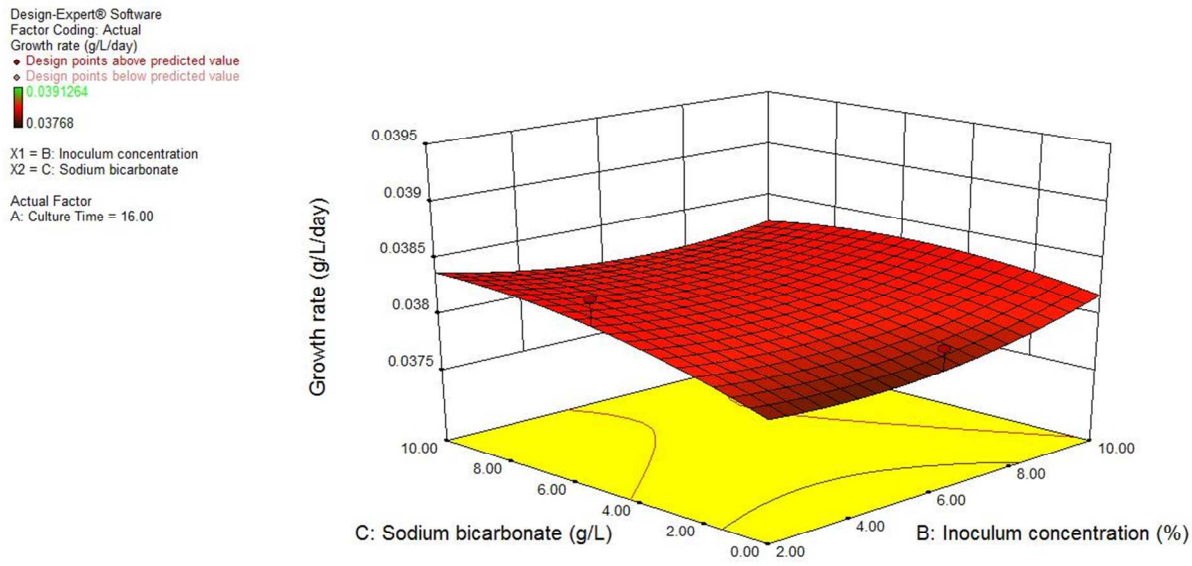


Fig. 3 Synergistic effect of culture time and sodium bicarbonate on growth rate of *Scenedesmus abundans*

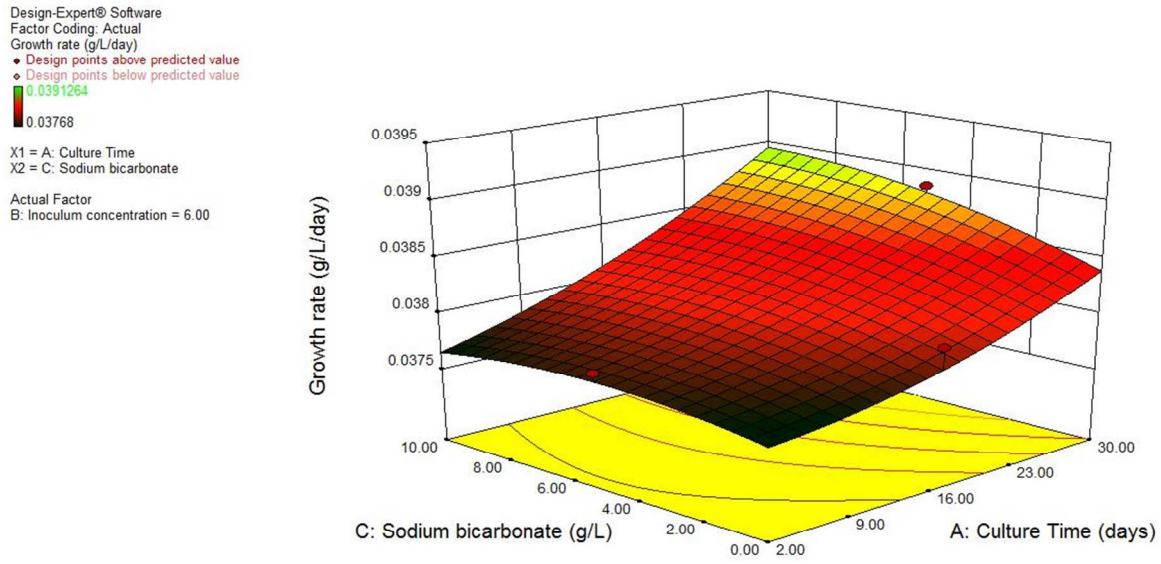


Fig.4 Comparison of predicted and actual (experimental) values of growth rate of *Scenedesmus abundans* by RSM methodology

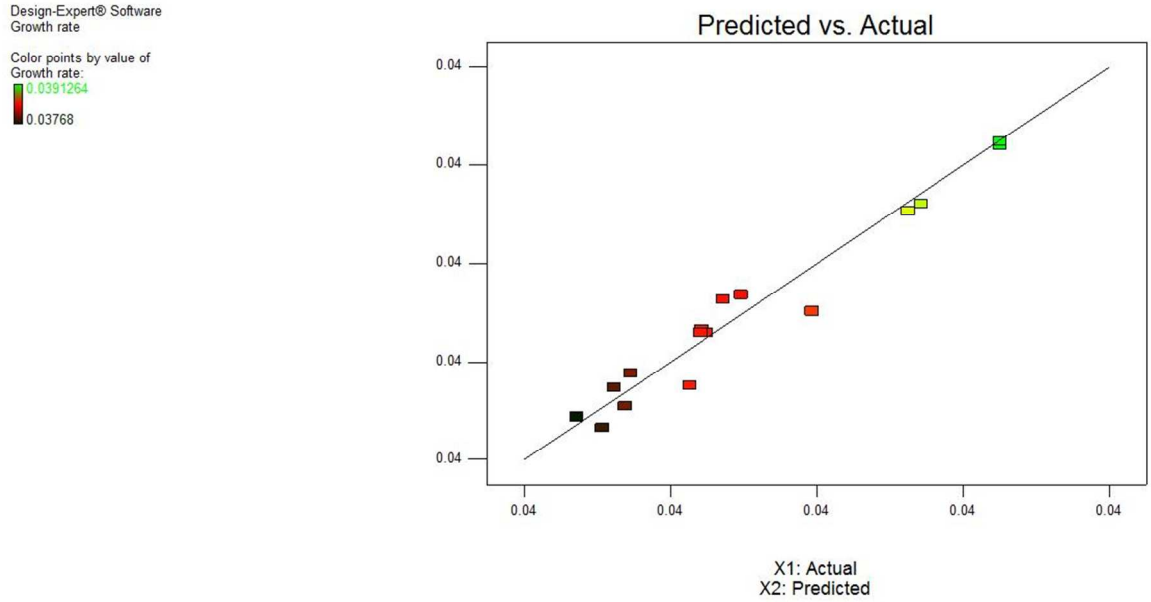


Fig. 5 Effects of culture time and inoculum concentration on lipid content of *Scenedesmus abundans*

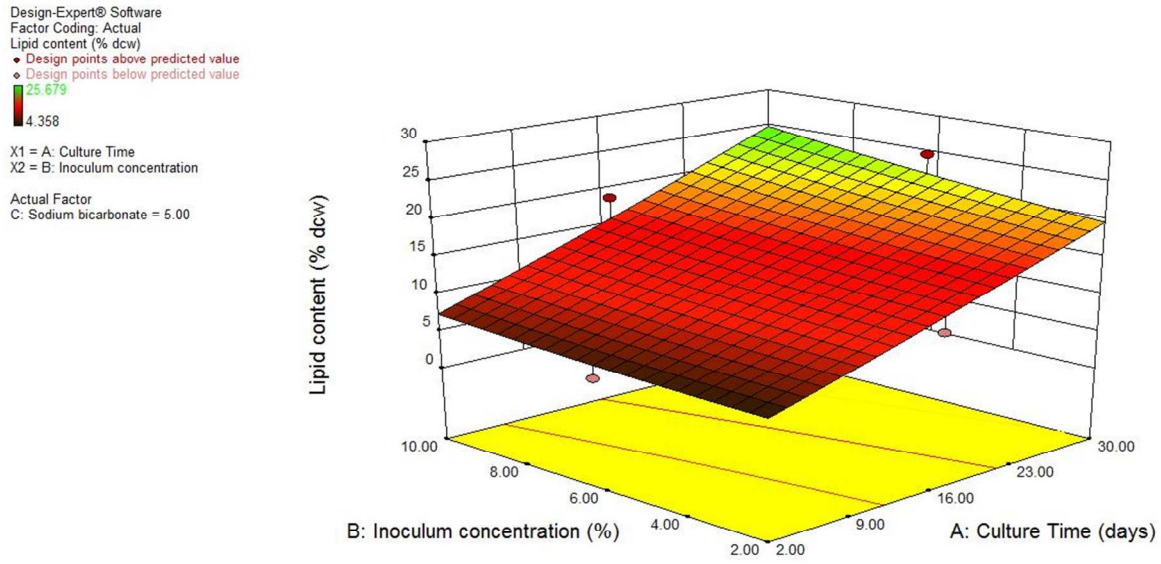


Fig. 6 Model for the combined effect of inoculum concentration and sodium bicarbonate on lipid content of *Scenedesmus abundans*

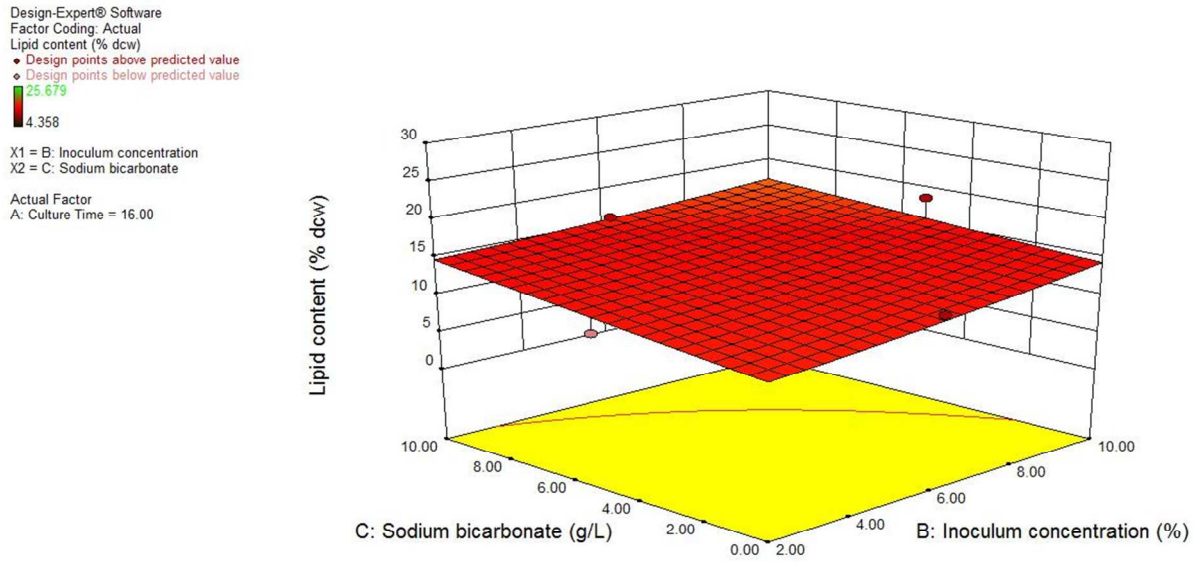


Fig. 7 Effects of culture time and sodium bicarbonate on lipid content in *Scenedesmus abundans*

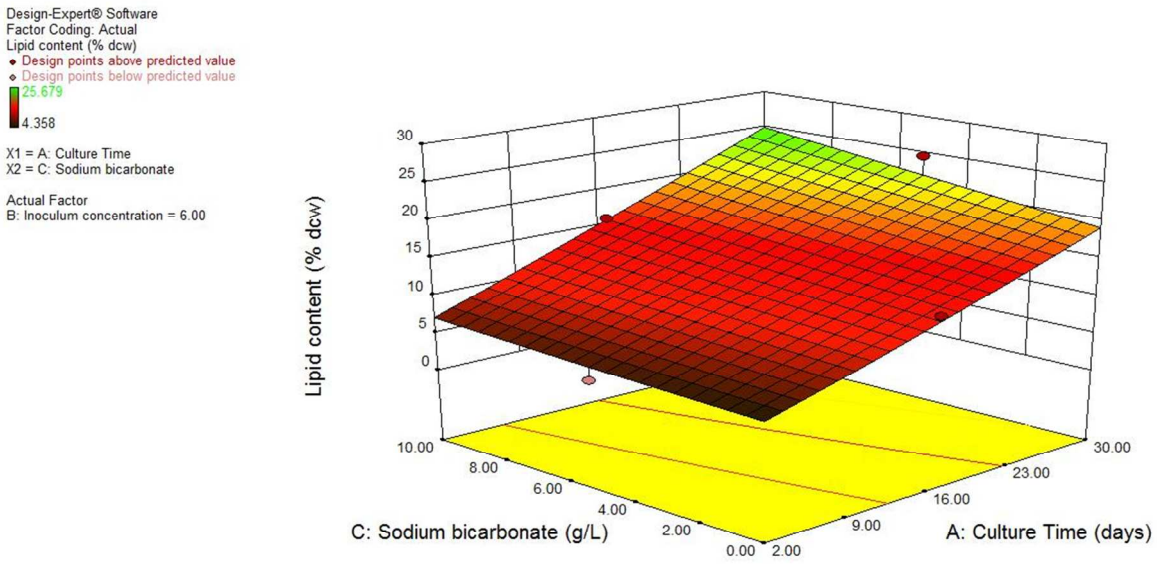


Fig. 8 Comparison of predicted and actual Values of lipid content of *Scenedesmus abundans originate from Design expert® 9*

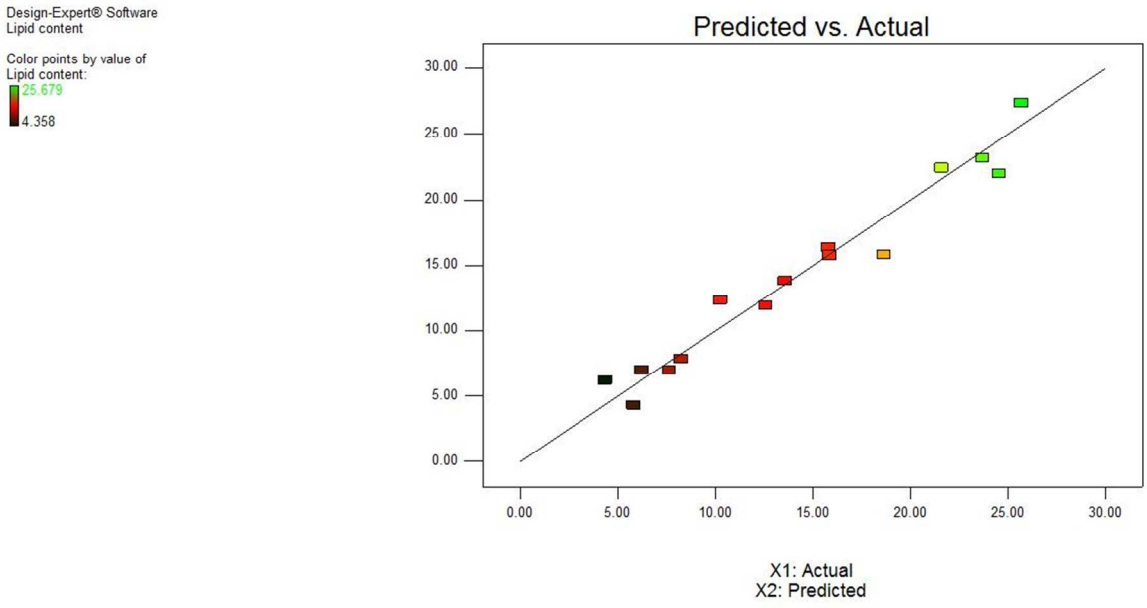


Fig. 9 (a) Illustration shows the individual growth response of *Scenedesmus abundans* subjected in different levels of substrate concentrations with respect to incubation time period and dry weight of biomass concentration

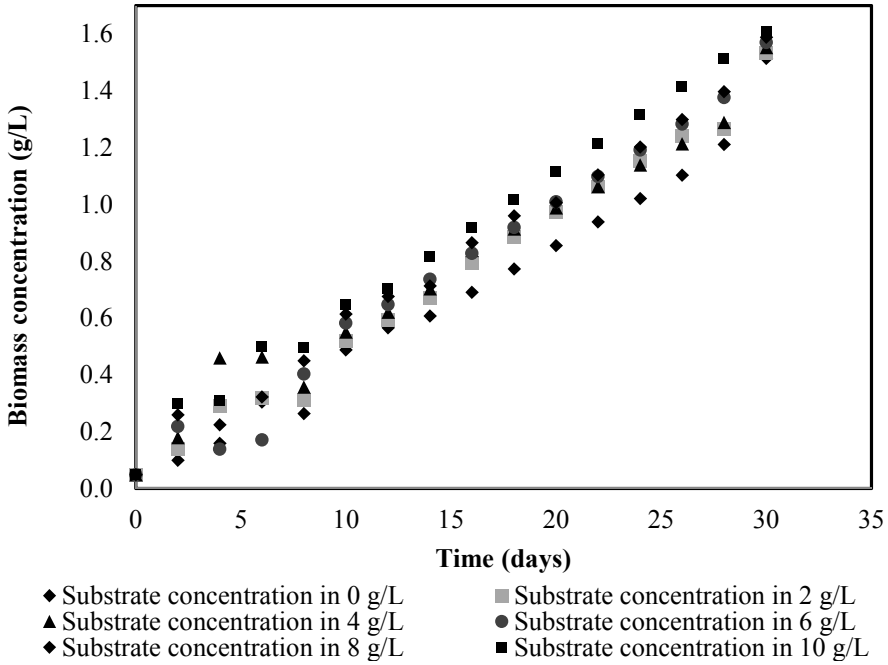


Fig.9 (b) Graph shows the effect of different substrate concentrations depicted in the form of time course of the specific growth rate of *Scenedesmus abundans.*

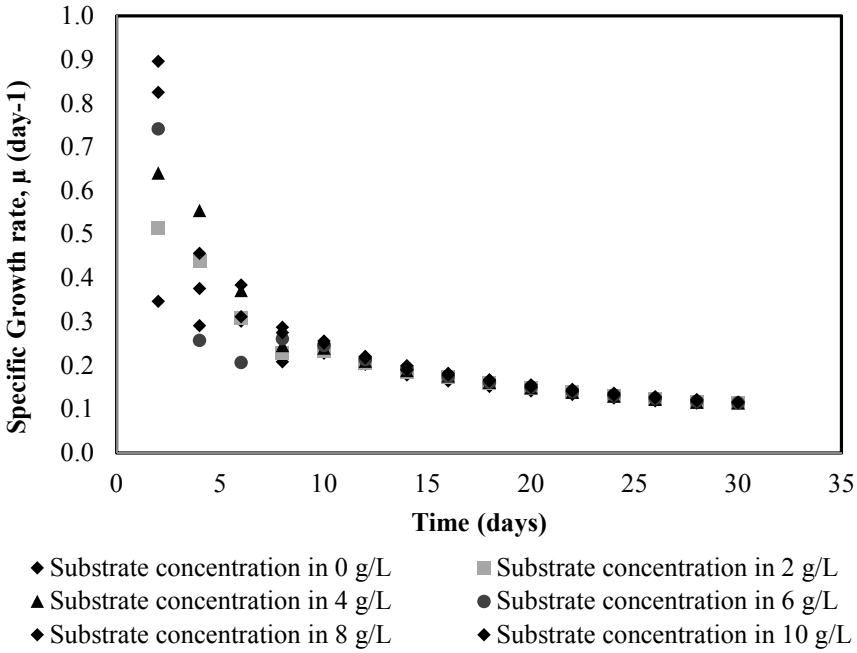


Fig.10 Error bar for predicted and proposed model of growth rate and recovery of lipid

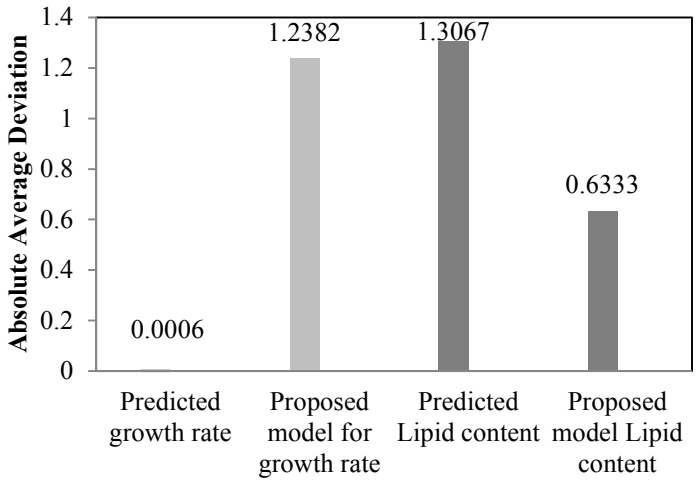
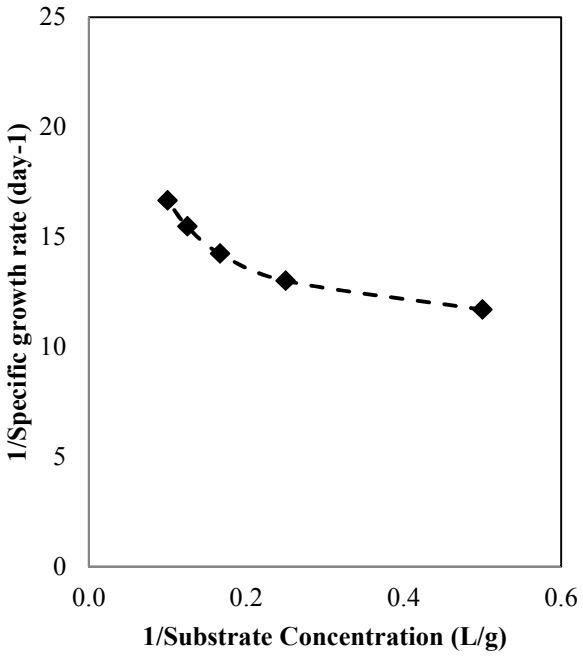
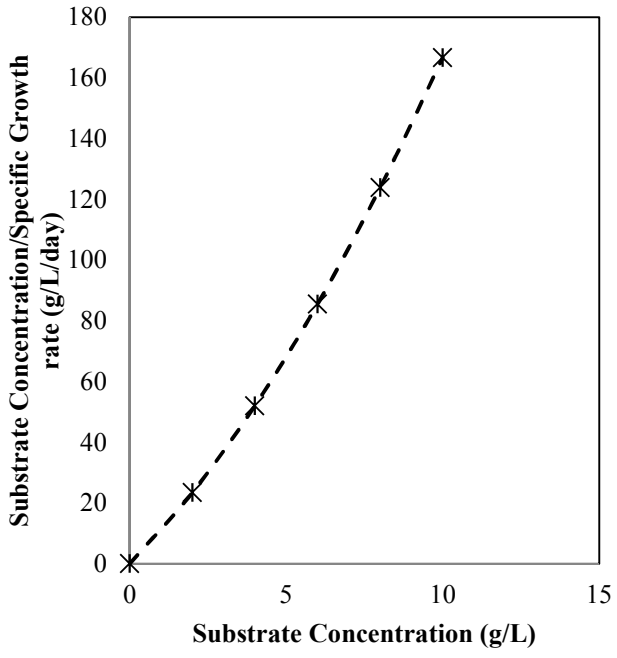


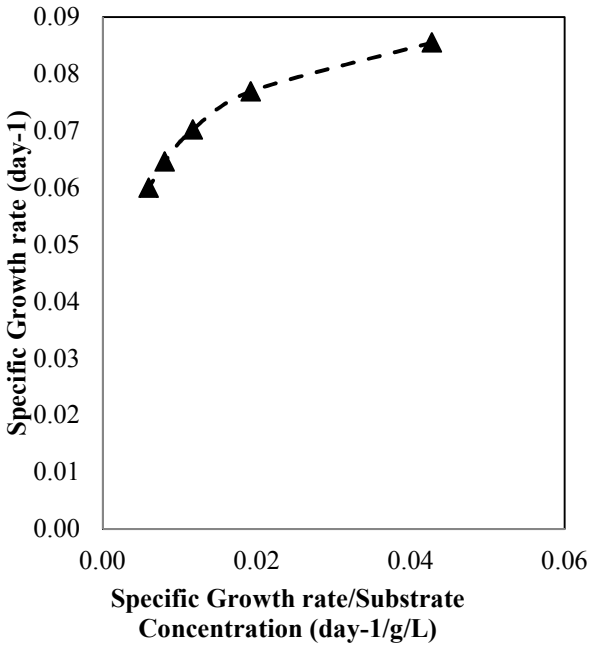
Fig.11 Fitting the explanatory data in the linear plots (a) Lineweaver-Burk plot for the inverse of substrate concentration versus specific growth rate, (b) Langmuir Hanes plot, (c) Eadie Plot and (d) Eddie Hofstee Plot



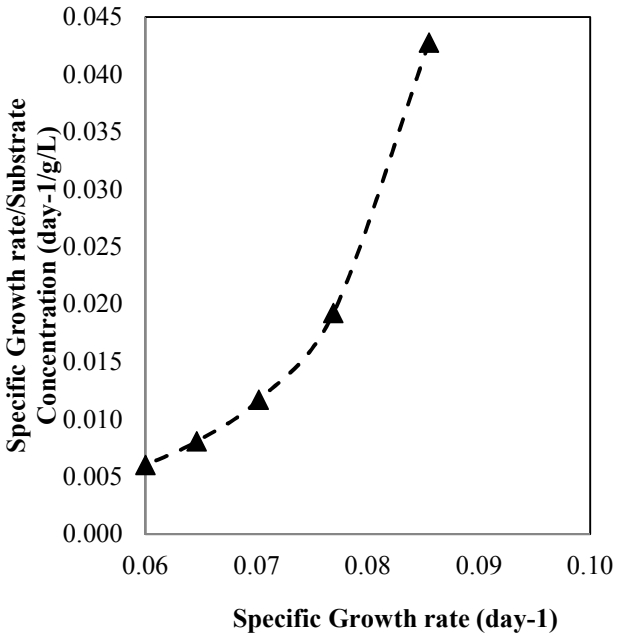
(a)



(b)


0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Specific Growth rate (day-1)
0.00
0.02
0.04
0.06
Specific Growth rate/Substrate Concentration (day-1/g/L)

(c)


0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Specific Growth rate/Substrate Concentration (day-1/g/L)
0.06
0.07
0.08
0.09
0.10
Specific Growth rate (day-1)

(d)