Research Article

Effect of Critical Parameters on Biomass Yield from Botryococcus Braunii by Response Surface Methodology

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Abstract

The present investigation deals with the absorption of biomass from microalgae namely; Botryococcus Braunii. Effect of various parameters like light intensity, potassium nitrate dosage, pH and magnesium sulphate for the growth of Botryococcus Braunii (Bb) was evaluated by Response Surface Methodology and the optimum conditions for Botryococcus Braunii (Bb) growth and separation from biomass has been shown in the probability distribution curves. Botryococcus Braunii has been shown to grow best at a temperature of 23°C. The alga culture thus produced has the potentiality to produce hydrocarbons which can be chemically converted into fuels.

The present article provides a deep insight into exploring algal resources for bio fuel production to mitigate the Global Warming problem. The diversity of the results can be extended for other algal species development.

Keywords: Bio mass, Botryococcus Braunii, Microalgae, Response Surface Methodology

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Introduction

The traditional use of fossil fuels is now widely recognized as unsustainable because of diminishing supplies and the contribution of these fuels to the increased carbon dioxide concentration in the environment. Bio fuels are nature's alternative to fossil fuels [1]. They can be derived from plants sources and microalgae

Algae based bio fuel definitely has the potential to revolutionize the energy industry and play a leading role to fight against green house gas emissions and climate change [2]. Bio fuels produced from algae appears to be only feasible solution today for replacing petrol-diesel completely [3]. No other feedstock has the oil yield high enough for it to be in a position to produce such large volumes of oil. Considering global energy requirement, the use of cyano bacteria for sustainable energy production has gained importance because they utilize dissolved carbon dioxide hence carbon neutral production can be achieved [4 -7]. In addition to this, high energy compounds can be produced such as hydrogen, bio mass for biodiesel application and sugars for biomass fermentation or gasification. Neutral biomass mainly TAG are preferred to glycol-or phosphor biomass because of their high percentage of fatty acids and lack of phosphate and are involved with carbon and energy storage [8, 9]. It is known that nitrogen starvation triggers TAG accumulation to the detriment of cell division. Since maximizing biomass productivity is the goal it has been known that optimal culture conditions are different for cell biomass content and biomass productivity which becomes a concern for biomass productivity [10, 11].Compared with terrestrial crops which take a season to grow and only contain a maximum of about 5 percent dry weight of oil microalgae grow quickly and contain high oil content. The variation of oil content of different, micro alga is presented in **Table 1** [12].

Table 1 Oil content of microalgae

Micro alga	Oil content (% dry weight)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodiniumcohnii	20
Cylindrotheca sp.	16–37
Nitzschia sp.	45–47
Phaeodactylumtricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmissuecia	15–23

The oil production from microalgae is 30 times more than other sources. The biodiesel production is about 2-5 times higher than conventional diesel fuel.

Botryococcenes are unbranched isoprenoid tri terpenes having the formula C_nH_{2n-10}. The A race produces alkadienes and alkatrienes where n is an odd number 23 through 31. The B race produces botryococcenes where n is in the range 30 through 37, the bio fuels of choice for hydro cracking to gasoline-type hydrocarbons. The "L" strain makes oil not formed by other strains of Botryococcus braunii [13]. Within this major classification, various strains of Botryococcus will differ in the precise structure and concentrations of the constituent hydrocarbons oils [14].

The A-strain of Botryococcus would not function well as a feedstock for biomass based fuel production due to its slow growth (one doubling every 72 hours). The A-strain Botryococcus oil was found to be less than ideal, having most of its biomass as C_{29} to C_{34} aliphatic hydrocarbons, and less abundance of C_{18} fatty acids. This evaluation of Botryococcus Braunii oils was done in relation to their suitability for transesterification (i.e. creating biodiesel).

The practice of farming cultivating is known as algae culture. Botryococcus braunii has great potential for alga culture.It is reported that 86% of the dry weight of Botryococcus Braunii can be long chain hydrocarbons [15]. The vast majority of these hydrocarbons are Botryococcus oils: Botryococcenes, alkadienes and alkatrienes.

Botryococcus oils are oils of vegetable origin [16]. They are inedible and chemically very different, being triterpenes and lack the free oxygen atom needed for transesterification. Botryococcus oils can be used as feedstock for hydro cracking in an oil refinery to produce gasoline, kerosene. Botryococcenes are preferred over alkadienes and alkatrienes for hydro cracking as botryococcenes will likely be transformed into a fuel with a higher octane rating.

Strains of Botryococcus Braunii have been studied extensively as they grow photo-autotrophic ally and accumulate unusually high amounts of hydrocarbon molecules [17]. They can be cultured either in liquid cultures or on immobilized matrices. These features make Botryococcus Braunii an interesting candidate for the photosynthetic production of hydrocarbons thus providing a potential source of renewable and sustainable bio fuels. The L race yields a single C40 isoprenoid hydrocarbon derivative of lycopadiene.

Botryococcus Braunii races A and B are widely distributed in brackish and freshwater such as alpine, temperate and tropical lakes, those of race L are only observed in water samples collected in the tropics. Direct comparisons of productivity among different strains may not be valid. However, there is a need to directly compare yields and productivity of different Botryococcus Braunii strains so as to properly assess their potential for commercial exploitation [18].

Statistical optimization techniques are powerful tools for handling complex data [19-22].The experimental designs deal with the methods of analyzing variations in several factors simultaneously resulting in less number of experimental runs.

The experiment was carried out along with affecting parameters i.e. light intensity, concentration of potassium permanganate, concentration of magnesium sulphate, pH and their ranges tested to find the optimum condition for extraction of biomass from Botryococcus Braunii [23].

Materials and Methods

Culture and maintenance media

The species of standard Botyrococcus Braunii Kutzing NIES 2199 species is cultured in standard CHU 13 media as this is most suitable for the enhanced growth of the species [24].

Chu 13 media preparation

CHU 13 medium is a culture medium used in microbiology for the growth of certain algal species, first published by S.P. Chu in 1942 [25]. It is used as growth medium for the bio fuel candidate alga Botryococcus Braunii [26]. CHU 13 includes essential minerals and trace elements that are required by algae for growth, but does not include a carbon source and so is only appropriate for growth of prototroph. It can be prepared as either a liquid medium or as an agar medium. Hence it can be used to culture the combined species as well. The **Table 2** below illustrates the chemical nutrients in the CHU 13 culture media.

Figure 1 show the mother culture of the species of Botryococcus Braunii KUTZING NIES 2199 maintained in different 250 ml conical flasks containing 100 ml CHU 13 media under a 60 W tube light, at a distance of 20 cm and room temperature (28°C).

Growth Study

Growth studies were conducted for the species of standard Botyrococcus Braunii Kutzing NIES 2199 species. Dry weight method is employed to plot the growth curve. 100 ml of inoculated culture media from the mother culture at

pH range of 7.0 was taken in a poly propylene bag of dimensions 27 cm X 56 cm. The bag was autoclaved twice on alternate days. Clumps of the specimen were broken before inoculation and then incubated at room temperature. Similarly 6 more polypropylene bags of 100 ml each of inoculated culture media are prepared. These bags are subjected to light 60 W tube lights at a distance of 20 cm in air and grown in the Pollution control lab for 62 days. At days 0, 7, 11, 24, 33, 45, 62, one polypropylene bag is taken and drained of all water. The biomass is squeezed manually using a spatula. The weight of biomass is then noted which corresponds to the weight of the wet sample. The sample is then dried in a hot air oven at 50 °C for 48 hours. The dried sample is weighed which corresponds to dry weight of sample. At the end of 62 days, wet and dry weights of biomass are noted down. The growth curve is plotted by plotting dry weight in grams versus days. A tangent is drawn at each point and slope calculated to find specific growth rate reported in **Tables 3-4** and **Figure 2**.

Table 2 Preparation of Nutrient Media

COMPOUNDS	mg / Litre
KNO_3	400
K_2HPO_4	80
$CaCl_2$dihydrate	107
$MgSO_4$heptahydrate	200
Ferric Citrate	20
Citric acid	100
$CoCl_2$	0.02
H_3BO_3	5.72
$MnCl_2$tetrahydrate	3.62
$ZnSO_4$heptahydrate	0.44
$CuSO_4$pentahydrate	0.16
0.072 N H_2SO_4	1 drop

Figure 1 The mother culture of the species of Botryococcus Braunii

Table 3 Tabulation of Growth Kinetics

Days	Wet Weight In Grams	Dry Weight In Grams (X)
0	0.432	0.032
7	0.400	0.037
11	0.350	0.031
24	0.709	0.040
33	0.849	0.052
45	2.630	0.129
62	1.962	0.301

Table 4 Calculation of Specific Growth rate combined species of *Botryococcus Braunii* KUTZING NIES 2199 and Cladophora sp.

Days t	Dry weight X (grams)	Slope = dX/dt (g/day) *10^{-3}	1/X(g^{-1})	μ_g in day^{-1}
16	0.03	2.477	33.333	0.0826
33	0.052	9.042	19.231	0.1739
40	0.085	24.66	11.765	0.2901
62	0.301	17.073	3.322	0.0567
The average sp. Growth rate μ_g was found to be 0.150825day^{-1}				

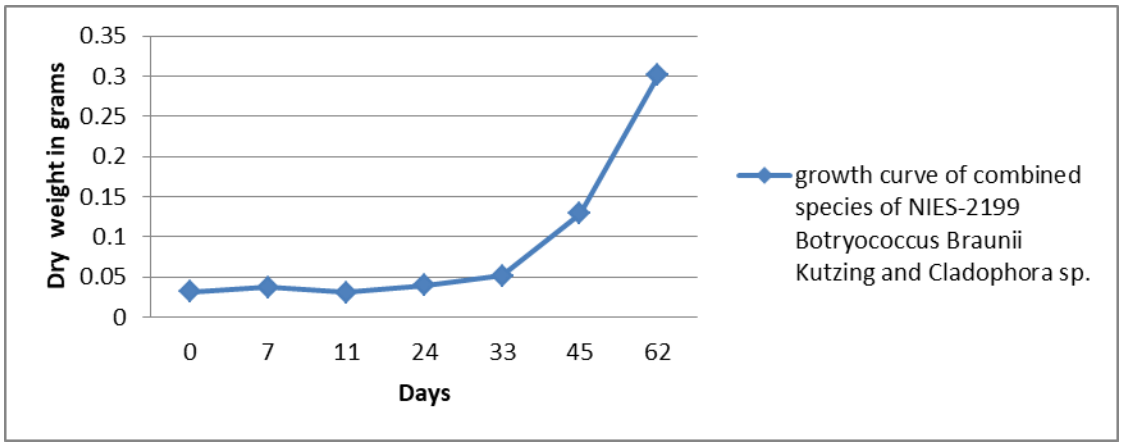

Figure 2 Growth curve of combined species of *Botryococcus Braunii* KUTZING NIES 2199 and Cladophora sp.

Scale up studies

Scale up studies was done to increase the biomass of the species. Initially 600 ml of combined species available from mother culture is cultured in CHU 13 media to give 6 litres of inoculated culture media. The inoculated culture media to scale up media ratio is taken as 1:10.

Preparation of 6 litres of inoculated culture media

100 ml of CHU 13 media which was inoculated with approximately 7g wet weight of species along with the required volume of water 1 litre at pH range of 7.0 was taken in a poly propylene bag of dimensions 27 cm X 56 cm. The culture chemicals were added to each bag in the same proportion and dilutions are carried out in the same way as mentioned before. The bags were autoclaved twice on alternate days. Clumps of the specimen were broken before inoculation. It is incubated at room temperature 28 °C. Similarly 5 more polypropylene bags of 1 litre each of inoculated culture media are prepared. These bags are subjected to 60 W tubes light in air and grown for 3 weeks in the Pollution control lab, CIFRI. **Figure 3** represents a view of inoculated culture media in six different packs.

Figure 3 pictorial views of inoculated culture media in six different packs

200 ml of CHU 13 is inoculated with approximately 14 g of species along with the required volume of water 2 litres at pH range of 7.0 was taken in a poly propylene bag of dimensions 27 cm X 56 cm. The chemicals of CHU 13 media were added in proportions of the table above by weight to each bag to meet the scaled up requirements. **Table 5** shows the preparation of above nutrient media.

The bags were autoclaved twice on alternate days. Clumps of the specimen were broken before inoculation. Incubation is done at room temperature 28^0C. Similarly 30 polypropylene bags of inoculated culture media of 2 litres each are prepared and arranged under 60W tube lights in air and further grown for 1 month at different intervals of time in the pollution control laboratory MSRIT, Bangalore as per **Figure 4**.

Table 5 Preparation of nutrient media

Compounds	Grams/ 1000ml of water
KNO_3	0.8
K_2HPO_4	0.16
$CaCl_2$ dihydrate	0.214
$MgSO_4$ heptahydrate	0.4
Ferric Citrate	0.04
Citric acid	0.2
$CoCl_2$	0.00004
H_3BO_3	0.01144
$MnCl_2$ tetrahydrate	0.00724
$ZnSO_4$ heptahydrate	0.00088
$CuSO_4$ pentahydrate	0.00032
0.072 N H_2SO_4	1 drop

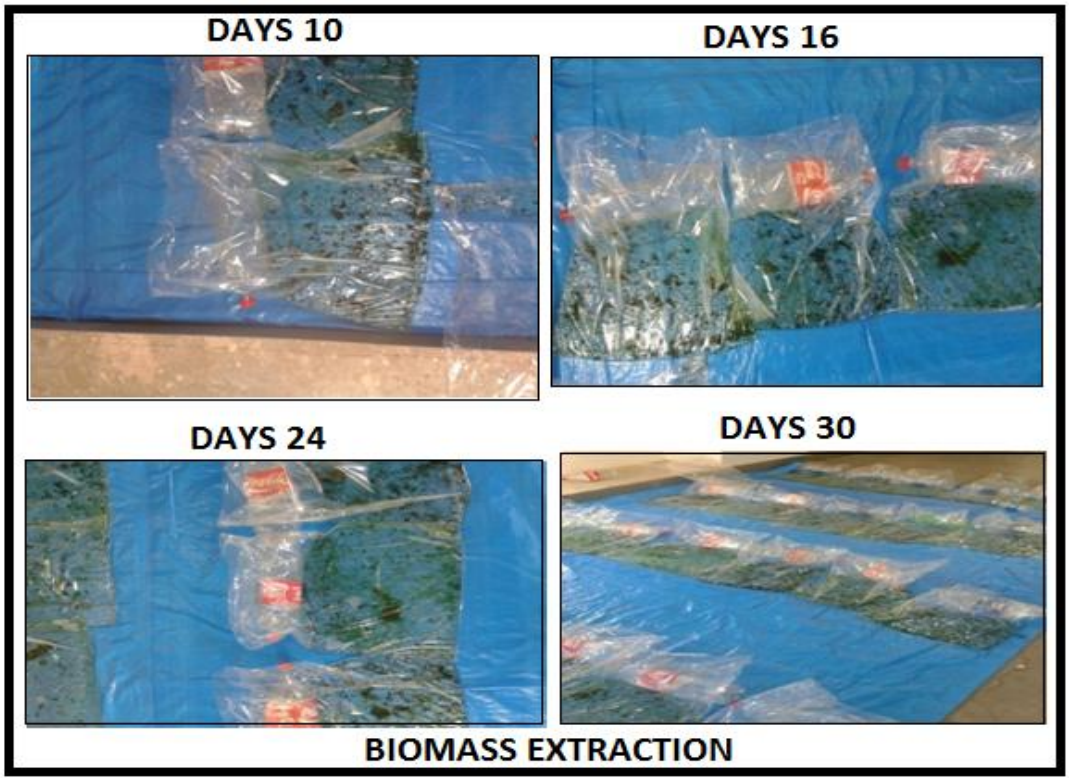

Figure 4 Polypropylene bags with inoculated culture media of combined species of *Botryococcus braunii*

Bligh and Dyer method for algal biomass extraction

Procedure

20 ml methanol and 20 ml chloroform were added to 1.797 grams dry weight of sample. The sample was homogenized for 10 min on the homogenizer (mortar and pestle). Additional 20 ml chloroform and 20 ml water was added. The sample was then filtered through black band filter paper (150 mm, Whatman, Dassel, Germany) in a glass funnel. The solvent was collected in a cylinder. The funnel was covered with a watch glass to reduce solvent

evaporation during the filtration. After the filtration, the solvent from the cylinder is allowed to settle in a separating funnel and after about 15 mins two layers are seen one the dark green biomass rich chloroform layer at the bottom and one clear methanol/ water layer on top. The bottom layer was transferred into the original flask from the separating funnel; 20ml chloroform was added into the flask. The sample was filtered again and the solvent was collected in the same cylinder. The methanol water layer is discarded.

Treatment of extract (chloroform phase)

The chloroform phase in the conical flask was transferred to a tray for evaporation using a volumetric pipette. The solvent was evaporated under an infrared lamp at 55- 60 °C. After the tray had cooled down, the flask with biomass was weighed. Initially empty weight of the conical flask was noted down. The difference in the two weights gives the weight of biomass extracted.

Treatment of water phase

Treatment of water phase was neglected due to negligible amount of biomass.

Newly Modified Bligh and Dyer method for algal Biomass Extraction

Procedure 40 ml methanol and 20 ml chloroform was added to 1.797 gram dry weight of sample and the sample was homogenized for 10 min on the homogenizer (mortar and pestle). Additional 20 ml chloroform and 20 ml water were added to it and homogenized using ultra sonicator for another 10 minutes. The sample was filtered through black band filter paper (150 mm, Whatman, Dassel, Germany) in a glass funnel. The solvent was collected in a cylinder. The funnel was covered with a watch glass to reduce solvent evaporation during the filtration. After the filtration, the solvent from the cylinder is allowed to settle in separating funnel and after about 15 minutes two layers are seen one the dark green biomass rich chloroform layer at the bottom and one clear methanol/ water layer on top. The bottom layer was transferred into the original flask from the separating funnel; 20ml chloroform was added into the flask. After homogenization for 5 min the sample was filtered again and the solvent extract mixture was collected in the same cylinder. The methanol water layer is discarded. **Figures 5-6** shows the preparation of Bligh and Dyer method for algal Biomass Extraction modified

Figure 5 Homogenization of algal sample using mortar and pestle

Treatment of extract (chloroform phase)

The chloroform phase in the conical flask was transferred to a tray for evaporation using a volumetric pipette. The solvent was evaporated under an infrared lamp at 55- 60 °C. After the tray had cooled down, the flask with biomass was weighed. Initially empty weight of the conical flask was noted down. The difference in the two weights gives the weight of biomass extracted.

Treatment of water phase

Treatment of water phase was neglected due to negligible amount of biomass.

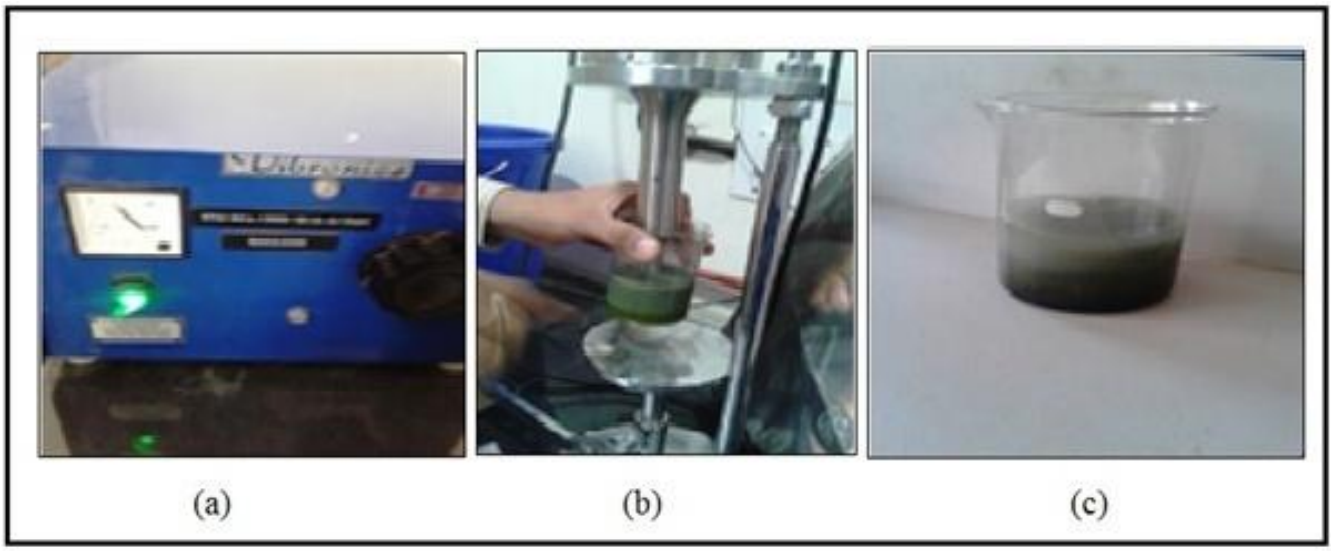

Figure 6 Ultrasonication: (a) Sonicator setup (b) Ultrasonication of algal sample taken in a beaker (c) The algal sample after ultra sonication

Central Composite Design Matrix

The conditions under which the experiment was carried out along with affecting parameters and their ranges tested to find the optimum condition for extraction of biomass is illustrated with Central Composite Design and analysis is performed to find fitting response surface using Response Surface Methodology.

Response surface methodology is a collection of mathematical and statistical techniques that are useful for modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The response is usually represented graphically by a surface [27, 28].

Central Composite Design Matrix

The first step in response surface methodology is to find a suitable approximation for the true functional relationship between Y and the set of independent variables. Usually, a low-order polynomial in some region of the independent variables is employed. If the response is well modelled by a linear function of the independent variables, then the approximating function is the first –order model as per equation 1.

$$\gamma = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots\ldots\ldots + \beta_K X_K + \in \qquad (1)$$

If there is curvature in the system, then a polynomial of higher degree must be used, such as the second-order model as represented by equation 2.

$$\gamma = \beta_0 + \in_{i=1}^{K} \beta_i X_i + \in_{i=1}^{K} \beta_{ii} X_i^2 + \in_{i \le j} \in \beta_{ij} X_i X_j + \in \qquad (2)$$

Almost all response surface methodology problems utilize one or both of these models. It is unlikely that a polynomial model will be a reasonable approximation of the true functional relationship over the entire space of the independent variables, but for a relatively small region they usually work quite well [29, 30].

The model parameters can be estimated most efficiently of proper experimental designs, which are used to collect the data. The selection of appropriate designs for fitting response surfaces will be discussed next. The independent variables and their levels studied are given in **Table 6**.

Table 6 Independent variables and their maximum and minimum levels

	-α	**-1**	**0**	**+1**
Light Intensity (Lux)	1000	1500	2000	2500
Potassium Nitrate (KNO_3)	300	350	400	450
Magnesium Sulphate ($MgSO_4$) mg/l	200	250	300	350
pH	8	8.5	9	9.5

Results and Discussions

Determination of optimum light intensity

Different light intensity of 1500 Lux, 2500 Lux, 1000 Lux, 3000 Lux, 2000 Lux were tried and tested to find the optimum range for yield. The highest yield was obtained at about 2000 lux light intensity.

Determination of optimum concentration of potassium nitrate

100 ml of inoculated culture media from the mother culture (pH 7.0) were taken in poly propylene bags of dimensions 27 cm X 56 cm. These bags are subjected to light 60 W tube lights at a distance of 20 cm, air and grown in the Pollution control lab for 62 days. Afterwards, biomass extraction was performed using Soxhlet Apparatus. The highest yield was noticed for about 400 mg/l of KNO_3.

Determination of optimum concentration of magnesium sulphate

The effect of $MgSO_4$ on the production of biomass was studied with its different concentration of while varying the other parameters like light intensity, KNO_3 concentration, pH etc. Then all experimental values are noted and hence observed that a concentration of about 300mg/l of magnesium sulphate favoured the highest yield of biomass.

Determination of optimum pH

pH is the most important factor compared to other parameters because Botrococcus Braunii prefers slightly basic and neutral pH conditions for growth. The optimum pH for the maximum growth was found to be 8.0.

A total of 26 experiments (runs) were carried out to find the optimum range for the four parameters that favours maximum biomass extraction from micro algal samples which is represented in **Table 7**.

Table 7 Comparison of Variation of Dry weight of biomass with respect to Light intensity, P^H, KNO_3 and $MgSO_4$ Concentration

Sl No.	Light Intensity (Lux)	Potassium Nitrate (KNO_3) mg/l	Magnesium Sulphate (MgSO4) mg/l	pH	Dry Weight of Biomass (gram/litre)	
					Experimental	Predicted
1	1500.000	350.0000	250.0000	8.50000	0.057	0.058542
2	1500.000	350.0000	250.0000	9.50000	0.052	0.054042
3	1500.000	350.0000	350.0000	8.50000	0.053	0.053208
4	1500.000	350.0000	350.0000	9.50000	0.056	0.049708
5	1500.000	450.0000	250.0000	8.50000	0.058	0.054208
6	1500.000	450.0000	250.0000	9.50000	0.055	0.052208
7	1500.000	450.0000	350.0000	8.50000	0.054	0.051375
8	1500.000	450.0000	350.0000	9.50000	0.051	0.050375
9	2500.000	350.0000	250.0000	8.50000	0.056	0.060208
10	2500.000	350.0000	250.0000	9.50000	0.058	0.063208
11	2500.000	350.0000	350.0000	8.50000	0.061	0.066375
12	2500.000	350.0000	350.0000	9.50000	0.063	0.070375
13	2500.000	450.0000	250.0000	8.50000	0.041	0.049875
14	2500.000	450.0000	250.0000	9.50000	0.052	0.055375
15	2500.000	450.0000	350.0000	8.50000	0.057	0.058542
16	2500.000	450.0000	350.0000	9.50000	0.064	0.065042
17	1000.000	400.0000	300.0000	9.00000	0.025	0.034250
18	3000.000	400.0000	300.0000	9.00000	0.066	0.050583
19	2000.000	300.0000	300.0000	9.00000	0.069	0.062250
20	2000.000	500.0000	300.0000	9.00000	0.052	0.052583
21	2000.000	400.0000	200.0000	9.00000	0.067	0.060750
22	2000.000	400.0000	400.0000	9.00000	0.065	0.065083
23	2000.000	400.0000	300.0000	8.00000	0.069	0.064417
24	2000.000	400.0000	300.0000	10.0000	0.068	0.066417
25	2000.000	400.0000	300.0000	9.00000	0.074	0.076000
26	2000.000	400.0000	300.0000	9.00000	0.076	0.076000

The experimental and predicted values from the above table are subjected to analysis of variance, ANOVA. In the preliminary step of optimization, the selected process variables were fitted to pseudo first order, pseudo second order and adsorption isotherms. The Box-Behnken experimental design is a very useful tool to determine the optimal levels and modelling of process parameters to a second order polynomial equation. The predicted response from the second order polynomial equation and the experimental data of Botryococcus Braunii were tabulated. Each experiment was repeated three times and the average experimental results were tabulated and repeatability of the experimental results was judged. The influence of solution pH, initial concentration of magnesium sulphate, dosage of potassium nitrate and light intensity present are studied at three levels. The regression equation obtained after application of analysis of variance was also tabulated in **Table 8** and **Figure 7** for the species.

Table 8 Analysis of variance

	SS	df	MS	F	P
Light Intensity (Lux)(L)	0.000400	1	0.000400	6.51487	0.025352
Light Intensity (Lux)(Q)	0.001504	1	0.001504	24.48223	0.000338
Potassium Nitrate (KNO3) mg/l(L)	0.000140	1	0.000140	2.28197	0.156762
Potassium Nitrate (KNO3) mg/l(Q)	0.000460	1	0.000460	7.49636	0.018000
Magnesium Sulphate (MgSO4) mg/l(L)	0.000028	1	0.000028	0.45856	0.511140
Magnesium Sulphate (MgSO4) mg/l(Q)	0.000228	1	0.000228	3.71570	0.077904
pH (L)	0.000006	1	0.000006	0.09768	0.759993
pH (Q)	0.000149	1	0.000149	2.43135	0.144901
1L by 2L	0.000036	1	0.000036	0.58609	0.458727
1L by 3L	0.000132	1	0.000132	2.15308	0.167999
1L by 4L	0.000056	1	0.000056	0.91577	0.357471
2L by 3L	0.000006	1	0.000006	0.10175	0.755221
2L by 4L	0.000006	1	0.000006	0.10175	0.755221
3L by 4L	0.000001	1	0.000001	0.01628	0.900583
Error	0.000737	12	0.000061		
Total SS	0.003145	26			

ANOVA; Var.: Dry Weight (gram/litre); R-sqr=.76566; Adj:.49227 (Spreadsheet1) 4 factors, 1 Blocks, 27 Runs; MS Residual=0.0000614 DV: Dry Weight (gram/litre)

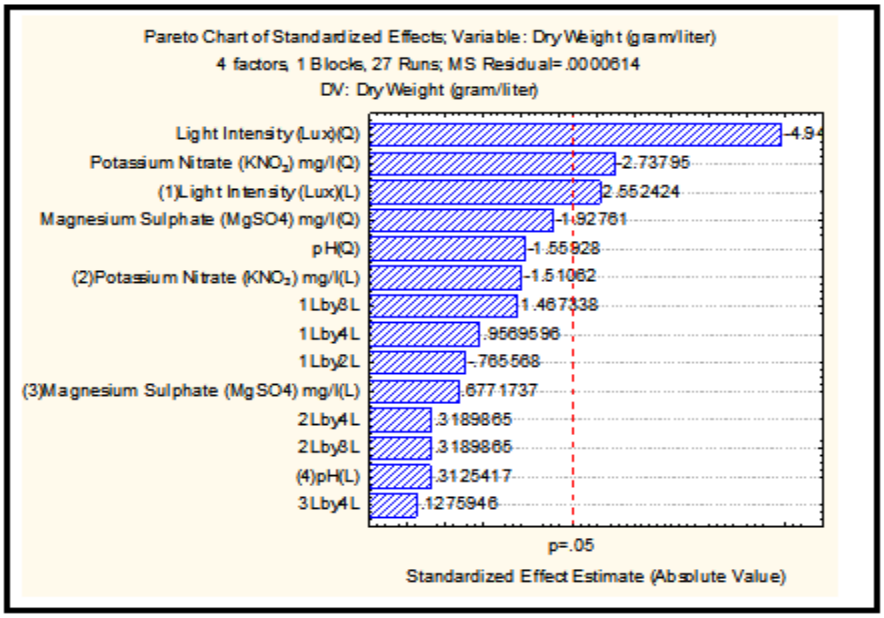

Figure 7 Standard Effect Estimate (Absolute Value)

The two dimensional contour plots (**Figure 8**) were drawn to determine the interactions of the light intensity, concentration of magnesium sulphate, concentration of potassium nitrate and pH on biomass obtained from **Table 9** for Botryococcus Braunii. Contour plot, shows the relative interaction of any two factors when the other two factors were maintained at constant level. pH and concentration of magnesium sulphate showed correct pattern by giving optimal values at pH 9 and magnesium sulphate concentration 300mg/l, the remaining graphs are considered to be

having saddle points i.e. they are having curves up in one direction and curves down in other direction and does not have optimal points. The circle represents optima minima or optima maxima and the trend towards reaching the circle, represents that the system is reaching towards the optimal biomass quantity. Here in the above graphs, optima maxima was achieved. The graphs between light intensity and potassium nitrate and magnesium sulphate and pH, the optimal light intensity is approximately near to 2000 lux and potassium nitrate concentration 400 mg/l and optimal magnesium sulphate concentration 300 mg/l and pH 9. These graphs will help in in-depth analysis of the variation of the independent variables.

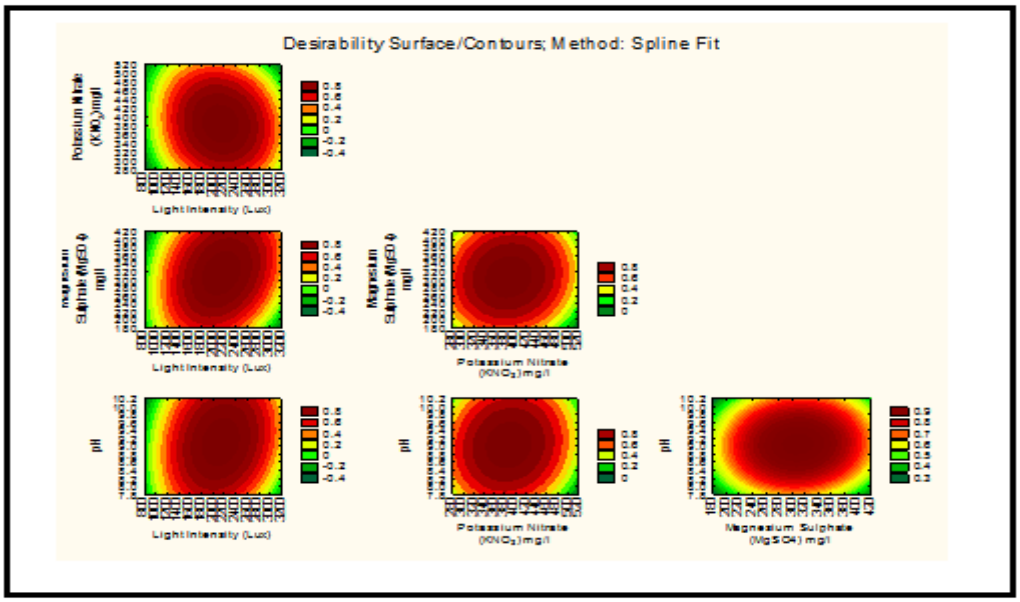

Figure 8 Contour plots showing behaviour among factors

Table 9 Critical values; Variable: Dry Weight (gram/litre) (Spread sheet1) Solution: maximum Predicted value at solution: 0.0772462

	Observed	Critical	Observed
Light Intensity (Lux)	1000.000	2170.465	3000.000
Potassium Nitrate (KNO_3) mg/l	300.000	385.898	500.000
Magnesium Sulphate (MgSO4) mg/l	200.000	314.799	400.000
pH	8.000	9.098	10.000

The p-values and F-values (Table 8) are used as tools to check the significance of each independent variable and also indicate the interaction between them. The smaller the p value and higher the F-ratio, the independent variable will become more significant. In the above ANOVA table, light intensity (lux) is found to be more significant when compared with other three independent variables for all three species and followed by potassium nitrate concentration. Magnesium sulphate concentration was found to be more significant for Botryococcus Braunii when compared to pH. For better understanding of the ANOVA table for the Botryococcus Braunii, profiles for predicted values and desirability graphs were plotted using Statistica V7.0 (Statsoft, USA).

A prediction profile for the concentration of magnesium sulphate (mg/l), concentration of potassium nitrate (mg/l), light intensity (lux) and pH consists of series of graphs of predicted percentage biomass extraction values for each independent variable and holding the levels of the other independent variables constant..

By applying the multiple regression analysis on the above factors, the final extraction values of the species are given in Table 7 and **Figure 9**. The difference between R^2 and adjusted R^2 was also tabulated for the above equations for better understanding of the above data. The value of the coefficient of determination (R^2 = 0.76556) for Botryococcus Braunii maxima indicates that only 0.23444 (1- R^2) percentage of the total variations in the independent variables were not explained by the model equation. The adjusted R^2 values explain the descriptive power of regression model equations. Every data point added to the model increases the R^2 value for better fit but adjusted R^2 compensates for the addition of variable and it increases and becomes close to the R^2 only if the experimental data points enhances the model and decreases when the data points enhances the model less. Liu et al. (2004) [31] explained about the adjusted R^2, that it corrects the R^2 value for the entire sample size and the number of terms in the model. If there are many terms in the model which does not fits to the equation and the sample size is not very large,

adjusted R^2 may be noticeably smaller than the R^2. The value of the adjusted coefficient of determination (Adj. R^2 = 0.49227) for Botryococcus Braunii maxima, are near to the R^2 confirming that that many terms in the model fits the equation. It is clear from the above values that the R^2 is greater than 0.76 showing that the experimental data fits to the predicted equation and the adjusted R^2 value is near to the R^2 for Botryococcus Braunii maxima and lot of variation was found for other two species. This signifies that most of the experimental data points were fitted significantly to the predicted equation for Botryococcus braunii maxima.

Each individual graph shows the pattern of variation of the percentage extraction of biomass at different levels. Using the above graphs, better understanding of the predicted responses and the most desirable response on the dependent variable can be found (Statistica V7.0, Stafsoft, USA).

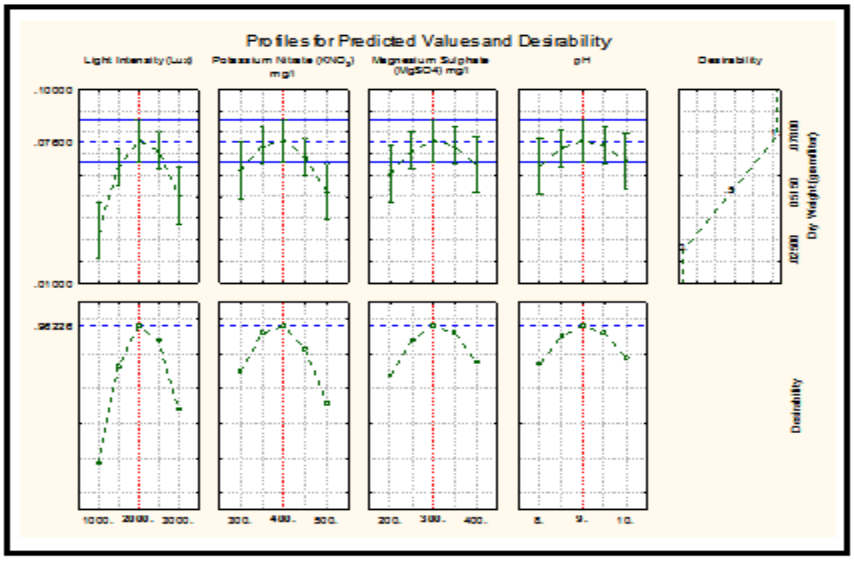

Figure 9 Profiles for predicted values and desirability showing the individual parametric effects on biomass quantity

Conclusion

The optimal values of light intensity, concentration of magnesium sulphate, concentration of potassium nitrate and pH were found to be 2000 lux, 300 mg/l, 400 mg/l and 9 respectively. These are well justified from optimal data points on contour plots and predictability-desirability chart showing maximum fit of the above data points and their interaction pattern.

Though the production of bio fuel from microalgae is yet not economical to compete with the cost of fossil fuels without additional support from the government and hence research is being done to make the process viable.

Recent researches such as metabolic engineering and genetic methods have been used to develop micro organisms for high productivity, having high energy value. Microalgae represent a group of organisms much simpler from plants, having no cell differentiation. Therefore, genetic manipulations can be done to increase their oil content. Microalgae having suitable amounts of fatty acids along with better oil extraction techniques can be used to decrease the overall cost of production.

After complete oil extraction, the resulting algae can further be processed into ethanol, methane, livestock feed and can also be used as an organic fertilizer with high nitrogen phosphorous ratio.

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