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Optimization and Production of *Botryococcus braunii* Biomass Using Commercial Nutrients by Response Surface Methodology

C. Kurinjimalar [1,*], G.Kavitha [1], S. Shamshath-Begum[2], G. Rajaram[1], S.Nagaraj[1], N. Senthilkumar[3] and R. Rengasamy[1]

[1] *Centre for Advanced Studies in Botany, University of Madras, Chennai, TN, India*
[2] *Department of Polymer Engineering, B.S. Abdur Rahman Crescent University, Vandalur, Chennai, TN, India*
[3] *Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, TN, India*

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A B S T R A C T

Biofuel production by a sustainable method using microalgae is entirely dependent on biomass production. However, commercialization at large scale using microalgae is a major obstacle using analytical grade growth nutrients, due to their cost effectiveness. Hence, development of a cost effective method is essential to reduce the production cost. Therefore, the present study envisaged the effect of low-cost commercial fertilizers such as urea, sodium bicarbonate, magnesium sulfate, potash and di-ammonium phosphate as growth nutrients for the production of biomass and total lipid of *Botryococcus braunii* were made. The biomass and total lipid production were optimized using Response Surface Methodology by 2^5 Central Composite Design. The result showed 225 mg L^{-1} of urea, 650 mg L^{-1} of sodium bicarbonate, 225 mg L^{-1} of magnesium sulfate, 150 mg L^{-1} of potash and 15 mg L^{-1} of di-ammonium phosphate supported the algal growth with a maximum biomass and total lipid of 0.792 gL^{-1} dry wt.. and 260 mg L^{-1} dry wt.., respectively. The biomass productivity of alga *B. brauniiat* the above condition recorded as 0.04 gL^{-1} day^{-1} with a generation time of 1.90 days.

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INTRODUCTION

Transportation and energy sectors have been major anthropogenic sources of greenhouse gas emission contributing to above 30 and 60%, respectively [1]. The continued use of fossil fuels and in turn involvement of these fuel to the accumulation of CO_2 in the environment is recognized as an environment damaging factors. Thus an efficient alternative source is a biofuel, of which microalgae is a promising feedstock for biofuel [2, 3]. The beneficiary effects of microalgae as an energy source over other energy producing terrestrial plants are high photosynthetic efficiency, fast growth rate and high biomass production rate with the ability to fix 50 times high carbon dioxide [4]. Also the characteristic features of biofuel such as non-toxic, biodegradable and renewable nature were considered as a prime candidate for biofuel production [5]. *Botryococcus braunii* a green, colonial hydrocarbon rich organism is widespread in freshwater bodies with an oil production of 29-75% [6, 7].

The production of microalgal primary metabolites such as protein, carbohydrate, and lipid mainly depends on media constituents. The major nutrients required for algal growth includes carbon, nitrogen, magnesium, potassium [8]. Production of algal metabolites can be abridged to a large extent if the main elements against growth factors were investigated. It is a time-consuming process to investigate effects of individual nutrients and also difficult to ascertain the interaction effects of all the different nutrients together. Therefore, Response Surface Methodology (RSM) was employed in present study in which experiments are followed accordingly [9]. The study explores the relation between the different combination of input variables taken and the output variable of the experimental model. Hence, with the changing energy demands it is needed to exploit the use of alga *B. braunii* using low-cost commercial nutrients. Thus, the study examines the production of biomass and

* Corresponding author: Chidambaram kurinjimalar
E-mail: cskurinjimalar@gmail.com

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total lipid optimized with RSM technique against urea, sodium bicarbonate, magnesium sulfate, potash and di-ammonium phosphate (DAP) as a source of nitrogen, carbon, magnesium, potassium, and phosphate for the growth of *B. braunii.*

MATERIAL AND METHODS

Microalga and growth conditions

In this study, a green microalga *B. braunii* experimented with the commercial inorganic nutrients namely urea, sodium bicarbonate, magnesium sulfate, potash and di-ammonium phosphate for growth. The stock culture was maintained in modified CHU-13 liquid medium [10].

The following experiments were carried out in 250 mL culture flask each containing 150 mL of respective media constituents. The optimally grown inoculum of 10% (v/v) was inoculated into the medium and allowed to grow for a period of 18 days. The chemicals used were commercial grade. The experiments were conducted at 25±1°C at 30 µmol m^{-2} s^{-1} light intensity of 12/12 (light/dark) photoperiod under laboratory conditions. Light from cool fluorescent white lamps was provided laterally.

Analytical estimation

Thirty milliliters of algal cultures were filtered using Whatman GF/C glass microfiber filters using a membrane filtration apparatus. Then the algal sample in a pre-weighed filter paper was dried at 80°C up to constant weight and its final dry weight was recorded. Dry weight was determined gravimetrically after subtracting the filter weight and recorded as g L^{-1} dry wt. [11]. The total lipid was extracted and estimated accordingly [12].

Biomass productivity ($gL^{-1}day^{-1}$) = $(X_2 - X_1)/(t_2 - t_1)$

where, X_1 and X_2 were the biomass (gL^{-1} dry wt.) on days t_1 (initial day) and t_2 (final day), respectively.

Experimental conditions and RSM analysis

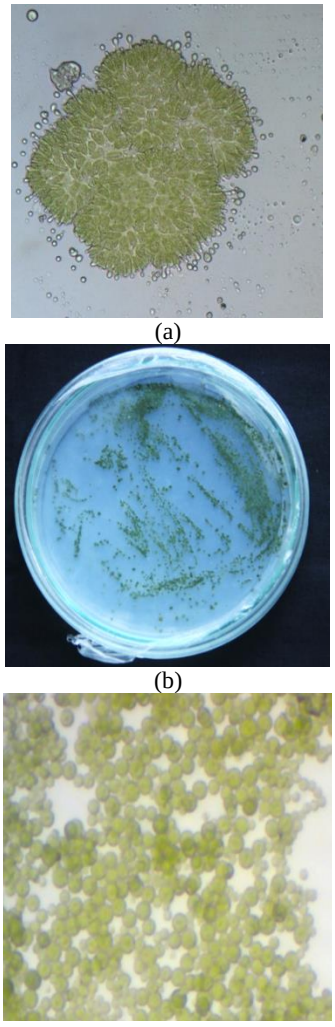
In RSM, Central Composite Design is one type was followed to find the optimum requirement of different commercial nutrients as a nutrient source taken in the present investigations. The five different commercial nutrients such as urea (A), sodium bicarbonate (B), magnesium sulfate (C), potash (D) and DAP (E) were analyzed with their varying levels been given in Table 1a. As given in Table 1b, 32 different factorial design were made and experiments were carried out and the response in terms of biomass and total lipid production were recorded. Also interpreted the linear, quadratic and interaction effects of variables along with main effect plot and response surface plot (3D) analysis. The experimental values were analyzed by ANOVA. The coefficient of determination (R^2) was used to represent experimental model fitness of the study.

Statistical Analysis

Data were analyzed statistically by Analysis of Variance (ANOVA) using Minitab 17, USA.

RESULTS AND DISCUSSION

The green colonial alga *Botryococcus braunii* was taken for the present investigation (Figs. 1a, 1b and 1c).



(a)

(b)

(c)

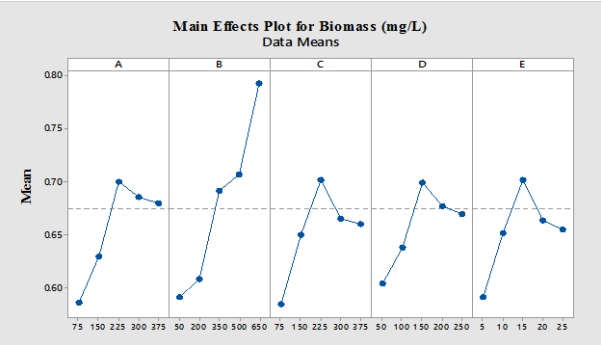
Figure 1. (a) Colony morphology of Botryococcus braunii Kütz. KMRR, (b) Botryococcus braunii colonies on CHU-13 agar medium on 30th day, (c) Single celled stage of *Botryococcus braunii* Kütz

In the present study RSM, a statistical experiment made by Central Composite Design method using commercial nutrients taken for algal growth under laboratory conditions. In this aspect, as per the statistical design, 32 different proportions were made and allowed the organism to grow for a period of 18 days (Table 1b).

The second order polynomial regression equations were used to fit the biomass and total lipid (dependent variables) to the selected variables such as urea (A), sodium bicarbonate (B), magnesium sulfate (C), potash (D) and DAP (E) (independent variables). Among the 32 different proportions, the organism amended with 225 mg L^{-1} of urea, 650 mg L^{-1} of sodium bicarbonate, 225 mg L^{-1}, of magnesium sulfate, 150 mg L^{-1} of potash and 15 mg L^{-1} of DAP gained with a maximum biomass of 0.792 g L^{-1} dry wt. and total lipid of 260 mg L^{-1} dry wt. (Table 1b). The study also revealed that the values recorded at 32 different conditions were found parallel to the predicted values in the RSM.

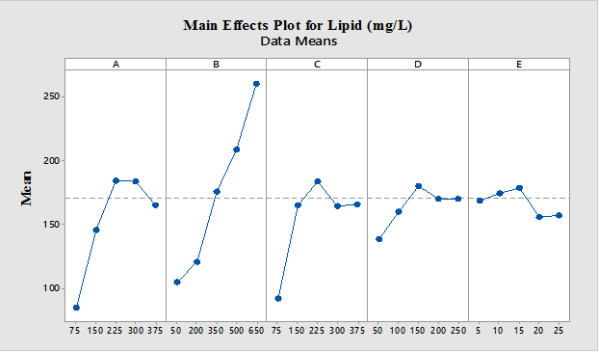
The individual effects of factors on algal growth response in terms of biomass and total lipid are represented in Main Effect Plots (MEPs) (Figs. 2a and 2b). With all the five different nutrients together showed that the concentrations above 225 mg L^{-1} of urea, 225 mg L^{-1} of magnesium sulfate, 150 mg L^{-1} of potash, 15 mg L^{-1} of DAP revealed less significance towards biomass and total lipid accumulation (Figs 2a and 2b). Whereas maximum sodium bicarbonate amendment with all other nutrients supported for maximum biomass at a given concentration of 650 mg L^{-1} (Fig. 2a-B). Also, the MEP's result showed that maximum biomass and total lipid were observed at urea, sodium bicarbonate, magnesium sulfate, potash and DAP of 225 mg L^{-1}, 650 mg L^{-1}, 225 mg L^{-1}, 150 mg L^{-1} and 15 mg L^{-1}, respectively. At the same condition, the alga showed maximum biomass productivity of 0.04 g L^{-1} day^{-1} with a maximum generation time of 1.90 days. It was observed that higher concentration of sodium bicarbonate (650 mg L^{-1}) resulted in maximum biomass level whereas lower concentration (50 mg L^{-1}) resulted in reduced growth in terms of biomass and also total lipid content (Figs. 2a, 2b and Table 1b). Thus, sodium bicarbonate is a significant factor to the growth of alga was observed in the present study. The higher the level of urea of 225 mg L^{-1} showed higher the production rate of biomolecules and furthermore increase in the level does not show much impact on the biomass production rate (Figs. 2a, 2b and Table 1b). The magnesium sulfate amendment of 225 mg L^{-1} as a source of magnesium is sufficient for growth of alga with a good accumulation of biomass. In addition, from the MEP's it was observed that lower the concentrations of potash and DAP with 50 mg L^{-1} and 5 mg L^{-1} resulted in lesser biomass accumulation and showed corresponding increase only at the optimal concentrations of 150 mg L^{-1} and 15 mg L^{-1}, respectively (Table 1b, Figs. 2a and 2b).

Analysis of regression coefficient values for the second order polynomial regression equation for the biomass and total lipid are given in Table 2. It was observed from the analysis (Table 2) that the coefficients for the linear effect of urea, sodium bicarbonate were highly significant (P =0.00) and linear effect for potash is not as highly as significant (P = 0.01) and for magnesium sulfate and DAP were found to be insignificant for biomass. Similarly, linear effects on total lipid showed urea, sodium bicarbonate and magnesium sulfate showed highly significant (P = 0.00) whereas potash and DAP showed insignificance. The quadratic effect of urea (A^2), magnesium sulphate (C^2), Potash (D^2) and DAP (E^2) were found to be highly significant (P =0.00) for biomass, similarly, the quadratic effects of urea (A^2), magnesium sulphate (C^2) were found



A- Urea (mg.L^{-1}) B- $NaHCO_3$ (mg.L^{-1}) C- $MgSO_4$ (mg.L^{-1}) D- Potash (mg.L^{-1}) E- DAP (mg.L^{-1}) Mean – Biomass (g.L^{-1} dry wt..)

(a)



A- Urea (mg.L^{-1})B- $NaHCO_3$ (mg.L^{-1}) C- $MgSO_4$ (mg.L^{-1}) D- Potash (mg.L^{-1}) E- DAP (mg.L^{-1}) Mean – Total lipid (mg.L^{-1} dry wt..)

(b)

Figure 2. (a) Main Effect Plot for biomass (g.L^{-1} dry wt..) of *Botryococcus braunii*,(b) Main Effect Plot for total lipid (mg.L^{-1} dry wt..) of *Botryococcus braunii*

TABLE 1a. Variable levels and their range for biomass and total lipid accumulation

Factors	Variables	Range of actual values for the coded variables				
		-α	-1	0	1	α
A	Urea (mg L^{-1})	75	150	225	300	375
B	Sodium bicarbonate (mg L^{-1})	50	200	350	500	650
C	Magnesium sulfate (mg L^{-1})	100	150	225	300	375
D	Potash (mg L^{-1})	50	100	150	200	250
E	Di-Ammonium phosphate (mg L^{-1})	5	10	15	20	25

TABLE 1b.

S. no.	A	B	C	D	E	Biomass Experimental (gL^{-1} dry wt.)	Biomass Predicted (gL^{-1} dry wt.)	Total lipid Experimental (mg L^{-1} dry wt.)	Total lipid Predicted (mg L^{-1} dry wt.)
1	225	350	225	150	15	0.775	0.764	220	214
2	225	50	225	150	15	0.592	0.605	105	115
3	150	500	150	100	10	0.587	0.562	192	172
4	150	200	150	100	20	0.595	0.585	89	76
5	375	350	225	150	15	0.680	0.716	165	180
6	300	200	150	100	10	0.613	0.594	119	103
7	75	350	225	150	15	0.587	0.611	85	103
8	300	500	150	100	20	0.778	0.757	241	225
9	225	650	225	150	15	0.792	0.814	260	284
10	225	350	225	150	5	0.592	0.635	169	194
11	300	200	300	200	10	0.623	0.610	168	166
12	300	200	300	100	20	0.617	0.612	81	82
13	150	200	300	100	10	0.592	0.584	128	124
14	225	350	225	150	15	0.775	0.764	220	214
15	225	350	225	250	15	0.670	0.705	170	183
16	150	500	150	200	20	0.643	0.623	158	143
17	225	350	375	150	15	0.660	0.675	166	157
18	300	500	300	100	10	0.668	0.648	236	229
19	150	500	300	100	20	0.655	0.644	192	188
20	225	350	225	150	25	0.655	0.672	157	165
21	225	350	75	150	15	0.585	0.630	92	134
22	225	350	225	150	15	0.775	0.764	220	214
23	300	200	150	200	20	0.628	0.613	157	147
24	225	350	225	50	15	0.605	0.631	139	159
25	225	350	225	150	15	0.775	0.764	220	214
26	225	350	225	150	15	0.775	0.764	220	214
27	300	500	150	200	10	0.775	0.745	233	215
28	150	200	300	200	20	0.615	0.611	92	94
29	150	200	150	200	10	0.585	0.567	133	118
30	225	350	225	150	15	0.775	0.764	220	214
31	300	500	300	200	20	0.780	0.764	235	234
32	150	500	300	200	10	0.770	0.751	184	178

to be highly significant (P = 0.00) for total lipid, whereas other quadratic effects showed little significance. Similarly, significance for the interaction effects was not found having P values >0.05. Equations 1 and 2 gives polynomial regression equation with coefficients for the biomass and total lipid, respectively. The significance of the predicted values was tested using ANOVA (Table 3). The reliability of the model can only be predicted using the coefficient of determination (R^2). The coefficient of determination (R^2) was found to be 0.93 and 0.92 for biomass and total lipid, respectively. The values show that the prediction obtained from the experimental data is quite satisfactory and Equations 1 and 2 can be used to predict the algal biomass and total lipid production from an amount of nutrients in the culture medium formulation. It was observed that the residual sum of squares (SS) and the lack of fit was also small with a zero value for pure error data. This shows that the experimental results elucidate the variables on biomass and total lipid production (dry wt.) in a more appropriate way.

The interaction between two variables and the response were represented in response surface plots (three-dimensional plane) by keeping the other independent variables as constant. With respect to the change in five operating parameters, the surface response plots were obtained for the biomass and total lipid are

Equation 1

Biomass (g L^{-1}) = -0.558 + 0.002551 A - 0.000023 B + 0.002857 C + 0.00318 D + 0.0434 E - 0.000005 A*A- 0.000001 B*B- 0.000005 C*C- 0.000 010 D*D- 0.001157 E*E+ 0.000001A*B- 0.000004 A*C 0.000001 A*D+ 0.000025 A*E+ 0.000000 B*C+ 0.000 002 B*D+ 0.000001 B*E + 0.000003 C*D - 0.000012 C*E - 0.000068 D*E

Equation 2

Total Lipid (mg L^{-1}) = -0.457+ 0.797 A+ 0.503 B+ 1.602 C+ 2.457 D+ 4.19 E - 0.002622 A*A- 0.000050 B*B- 0.002844 C*C- 0.004 05 D*D- 0.270 E*E- 0.000089 A*B+ 0.00109 A*C- 0.0 0003 A*D+ 0.0360 A*E- 0.000556 B*C- 0.000267 B* D- 0.00350 B*E- 0.00250 C*D + 0.0113 C*E - 0.0390 D*E

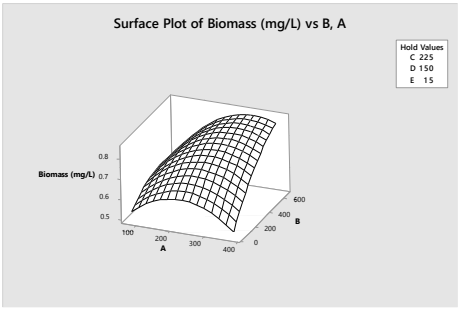
TABLE 2 Estimated regression coefficient for biomass and total lipid accumulation of *Botryococcus braunii*

Term	Biomass				Total lipid			
	Coefficient	SE coeff.	*T*	*P*	Coefficient	SE coeff.	*T*	*P*
Constant	0.766	0.013	58.29	0.000	214.30	9.77	21.94	0.000
A	0.026	0.006	3.88	0.003	19.25	5.00	3.85	0.003
B	0.049	0.006	7.36	0.000	42.25	5.00	8.45	0.000
C	0.011	0.006	1.65	0.128	5.92	5.00	1.18	0.261
D	0.018	0.006	2.75	0.019	6.00	5.00	1.20	0.255
E	0.009	0.006	1.39	0.193	-7.17	5.00	-1.43	0.179
A*A	-0.026	0.006	-4.34	0.001	-18.05	4.52	-3.99	0.002
B*B	-0.011	0.006	-1.94	0.079	-3.67	4.52	-0.81	0.434
C*C	-0.029	0.006	-4.79	0.001	-17.05	4.52	-3.77	0.003
D*D	-0.025	0.006	-4.18	0.002	-10.67	4.52	-2.36	0.038
E*E	-0.028	0.006	-4.75	0.001	-8.55	4.52	-1.89	0.085
A*B	0.015	0.008	1.91	0.082	8.50	6.12	1.39	0.192
A*C	-0.020	0.008	-2.49	0.030	-3.37	6.12	-0.55	0.592
A*D	-0.003	0.008	-0.41	0.690	9.38	6.12	1.53	0.154
A*E	0.009	0.008	1.14	0.279	4.00	6.12	0.65	0.527
B*C	0.004	0.008	0.49	0.637	3.25	6.12	0.53	0.606
B*D	0.015	0.008	1.87	0.089	-11.50	6.12	-1.88	0.087
B*E	0.000	0.008	0.11	0.917	6.88	6.12	1.12	0.285
C*D	0.012	0.008	1.50	0.161	0.12	6.12	0.02	0.984
C*E	-0.004	0.008	-0.53	0.606	-5.25	6.12	-0.86	0.409
D*E	-0.017	0.008	-2.06	0.063	-0.25	6.12	-0.04	0.968

TABLE 3. ANOVA results obtained from response surface optimization

source	Degree of freedom			biomass			Total lipid		
		Sum of square (SS)	Mean square (MS)	F	P	Sum of squares (SS)	Mean square (MS)	F	P
Regression	20	0.183	0.009	8.47	0.000	80562.4	4028.1	6.72	0.001
Linear	5	0.088	0.017	16.28	0.000	54671.8	10934.4	1.24	0.000
Square	5	0.071	0.014	13.23	0.000	19406.6	3881.3	6.47	0.005
Interaction	10	0.023	0.002	2.18	0.108	6484.0	648.4	1.08	0.447
Residual	11	0.011	0.001	-		6594.4	599.5	-	-
Lack-of-fit	6	0.011	0.001	-		6594.4	1099.1	-	-
Pure Error	5	0.000	0.000	-		0.000	0.000	-	-
Total	31	0.195	-	-		87156.9	-	-	-
	R2 = 0.9390; R = 0.8282					R2 = 0.9243; R = 0.7868			

given in Figs 3a and 3b, respectively. The response surface plot provides the clear indication about the maximal biomass and total lipid at a particular amendment conditions. Figures 4a and 4b shows the theoretically predicted and experimentally achieved values for biomass as well as total lipid accumulation of *B. braunii*. The graph displays a linear pattern and explains that the model is adequate enough to predict biomass and lipid accumulation within the CCD variable values. The values of coefficient of determination (R^2) for biomass (93.6%) and total lipid (92.2%) shows good agreement with the experimental model and experimental result obtained.



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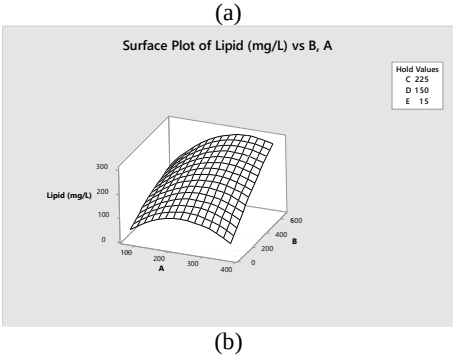
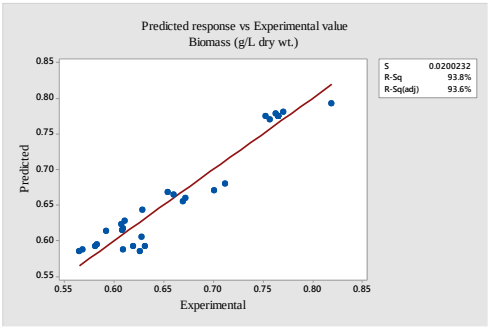


(b)

Figure 3. (a) Response surface plot for biomass (g.L^{-1} dry wt..) of *Botryococcus braunii*, (b) Response surface plot for total lipid (mg.L^{-1} dry wt..) of *Botryococcus braunii*

The obtained optimal conditions were checked by the validation and the validation results are shown in Table 4. Biomass and total lipid accumulation based on



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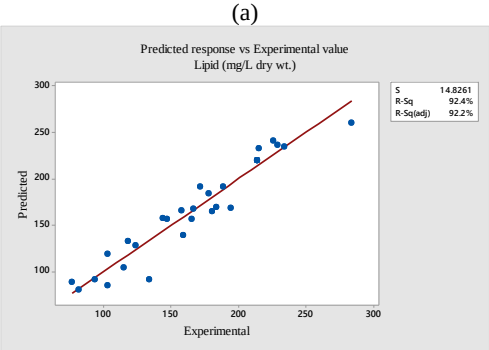

(b)

Figure 4. (a) Experimental and predicted values of biomass of *Botryococcus braunii*, (b) Experimental and predicted values of total lipid of *Botryococcus braunii*

validation experiments were found to be 0.814 g L^{-1} dry wt. and 284 mg L^{-1} dry wt., respectively. The values obtained from validation data were in good agreement with the experiments made showing the accuracy of the experiments. Finally, the comparison between the control (modified CHU 13 media) and optimized commercial media showed 15% and 18% increase in biomass and total lipid content, respectively (Table 5).

TABLE 4. Optimum conditions and validation results of *Botryococcus braunii*

Parameter (dry wt.)	A	B	C	D	E	Optimum values	Validation results
Biomass (g L-1)	225	650	225	150	15	0.792	0.814
Total lipid (mg L-1)	225	650	225	150	15	260	284

A= Urea, B= Sodium bicarbonate, C = Magnesium sulphate, D = Potash, E = Di-Ammonium phosphate

TABLE 5. Comparison of growth in optimized commercial media and Modified CHU-13

Parameter (dry wt.)	Optimized media	CHU-13 media
Biomass (g L-1)	0.792	0.685
Total lipid (mg L-1)	260	220

Discussion

Rapidly depleting fossil fuels with a concomitant increase in oil prices have made to look for alternative energy sources. Above all algal-based biofuel is an alternative to fossil fuels because microalgae feedstock production is more reliable [13]. *Botryococcus braunii* is one of the major source for biofuel production among many microalgae [14, 15]. It is already reported that quantity and quality of lipids within algal biomass may vary as a result of changes in growth media constituents. With this fact, the following experiment was carried out to study the effects of commercial nutrients as a source of nutrients for the growth of *B. braunii*. Growth medium with enough major nutrients such as carbon, nitrogen, phosphorus, sulfur has been reported for growth and lipid production of microalgae. The nitrogen source is responsible for the synthesis of the protein molecule, phosphate serve to enhance the algal growth, and magnesium constitute the major source for production of the chlorophyll molecule, and thereby altogether overall production rate was inclined to be increasing with the above nutrients altogether [16, 17]. There were no enough statistical optimization studies using commercial nutrients on *B. braunii*. The result of the present investigation revealed that major nutrients taken were proved to be positively promoting growth factors of *B. braunii*. It was observed that higher the levels of Sodium bicarbonate (650 mg L^{-1} dry wt.) resulted in maximum biomass level with concomitant decrease with the lower concentration (50 mg L^{-1} dry wt.). The results clearly show sodium bicarbonate as a potential carbon source to increase the growth of *B. braunii*.

The study pertained to show the positive effect of commercial fertilizers as a source of nutrients for total lipid production. The lipid estimation revealed that lipid content was increased to 18% in the optimized low cost media constituting 225 mg L^{-1} of urea, 650 mg L^{-1} of sodium bicarbonate, 225 mg L^{-1} of magnesium sulfate, 150 mg L^{-1} of Potash and 15 mg L^{-1} of di-ammonium phosphate compared to culture grown in modified CHU-13 media. Optimization studies on growth requirements of microalgae are time-consuming, whereas using RSM, a statistical method permits the analysis with fewer experiments [18-20]. The main variable that affects the growth has been identified as macronutrients as observed in the present study. Whereas there were no reports on interaction effects of commercial macronutrients.

Several studies have reported that medium with urea and sodium bicarbonate enhances lipid production of microalgae with increased multiplication rate [21]. The present study reveals that addition of 225 mg L^{-1} of urea, 650 mg L^{-1} of sodium bicarbonate, 225 mg L^{-1} of magnesium sulfate, 150 mg L^{-1} of potash and 15 mg L^{-1} of di-ammonium phosphate results in increased total lipid content of 21 mg L^{-1} dry wt. on a zeroth day to 260 mg L^{-1} dry wt. on the 18th day, respectively. Various studies revealed that nitrogen source was the major nutrient affecting algal growth [22, 23]. There was also evidence to suggest that nitrogen deficiency could promptly increase total lipid content . However in the present

investigation, the above phenomenon was not observed, instead, urea had a positive effect on lipid content (Table 1b) because urea did not reach the limiting level in the due course of experiments [24]. ANOVA (Analysis of Variance) was used to interpret the acceptability of statistical model. The model represented the coefficient of determination (R^2) values for biomass and total lipid was of 0.93 and 0.92, respectively. It clearly shows that a good correlation was obtained indicating good fitness of the model. The model could be acceptable with a $R^2 \geq 0.75$ [25].

CONCLUSION

Our study concludes that optimum concentration of 225 mg L^{-1} of urea, 650 mg L^{-1} of sodium bicarbonate, 225 mg L^{-1} of magnesium sulfate, 150 mg L^{-1} of potash and 15 mg L^{-1} of di-ammonium phosphate together enhanced biomass and total lipid production of *B. braunii*. Thus above all sodium bicarbonate contributes to influence the growth along with the level of urea, compared to other nutrients taken in the study. Thus, formulated and optimized medium for biomass and total lipid induction using RSM positively affecting the economic feasibility of mass scale production of biofuel using *B. braunii*.

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Persian Abstract

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چکیده

تولید سوخت زیستی با روش پایدار توسط میکروجلبک¬ها به تولید زیست توده بستگی دارد. اما استفاده از میکروجلبک و مصرف مواد مغذی رشد با گرید تحلیلی با توجه به مقرون به صرفه بودن آ¬ن¬ها با مانع تجاری سازی در مقیاس بزرگ روبه روست. بنابراین توسعه یک روش مقرون به صرفه برای کاهش هزینه¬های تولید حائز اهمیت است. مطالعه حاضر تاثیر تلقیح کننده¬های تجاری با قیمت پایین مانند اوره، سدیم بی¬کربنات، منیزیم سولفات، پتاس و دی¬آمونیم فسفات به عنوان مواد مغذی رشد برای تولید بیومس و مجموع چربی کل بتریوکوکسوس برانی ساخته شده را مورد بررسی قرار می¬دهد. تولید بیومس و چربی کل با استفاده از روش پاسخ سطح با طراحی مرکب مرکزی ۲۵ بهینه سازی شد. نتایج نشان دادند که ۲۲۵ میلی گرم بر لیتر اوره، ۶۵۰ میلی گرم بر لیتر سدیم بی کربنات، ۲۲۵ میلی گرم بر لیتر منیزیم سولفات، ۱۵۰ میلی گرم بر لیتر پتاس و ۱۵ میلی گرم بر لیتر دی آمونیوم فسفات رشد جلبک را پشتیبانی می¬کنند که مقدار بیشینه زیست توده و چربی کل به ترتیب ۰,۷۹۲ گرم بر لیتر وزن خشک و ۲۶۰ میلی گرم بر لیتر وزن خشک حاصل شد. بهره وری زیست توده جلبک در این شرایط ۰,۰۴ گرم بر لیتر در روز با زمان تولید ۱,۹ روز گزارش گشت.