ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 5, pp. 16903-16911, May, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

OPTIMIZATION OF BIOMASS PRODUCTIVITY AND CARBON DIOXIDE FIXATION ABILITY BY FRESHWATER MICROALGAE SCENEDESMUS BAJACALIFORNICUS BBKLP-07, A STEP TOWARDS SUSTAINABLE DEVELOPMENT

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0805.0242

ARTICLE INFO

Article History:

Received 15th February, 2017
Received in revised form 25th March, 2017
Accepted 28th April, 2017
Published online 28th May, 2017

Key Words:

CO_2 fixation · Biomass · *Scenedesmus bajacalifornicus* · Optimization · Response surface method

ABSTRACT

Microalgae carbon dioxide (CO_2) sequestration has been recognized as a promising technology in the arena of sustainable development to mitigate CO_2. The objective of the present study was to optimize the culture conditions for freshwater microalgae *Scenedesmus bajacalifornicus* BBKLP-07. Response surface methodology (RSM) was used to analyze biomass productivity (R1) and CO_2 fixation (R2) of microalgae *Scenedesmus bajacalifornicus* BBKLP-07 cultivated on media containing varying concentrations of CO_2, nitrate and phosphate at different pH conditions. The predicted second-order quadratic model for response variables was significant ($p < 0.01$). Additionally, predicted R-squared values 0.7111 (R1), 0.8616 (R2) of quadratic model indicated the satisfactory fit of the model. On the basis of statistical analysis of results, CO_2 concentration (15%), sodium nitrate (1.75 g/l/day), Di potassium hydrogen phosphate (0.06 g/l/day) and pH 7 i.e. $C_{15}N_{1.75}P_{0.06}H_7$, was found to be the best combination for maximum biomass productivity (0.93 g/l/day) and highest carbon dioxide fixation rate (0.13 g/l/day).

INTRODUCTION

Global warming has been reached to an alarming level due to the change in global environment. Industries related to natural gas processing, steel manufacturing, electricity generation, cement, iron and combustion of municipal solid waste are the chief contributors of atmospheric CO_2 because of their dependence on carbon sources like natural gas, coal, and oil (Inventory of U.S greenhouse gas emissions and sinks: 1990-2008). Increasing concentrations of gasses will increase the average surface temperature of the Earth by up to 6 ^oC during the 21st century (IPCC- Fourth assessment report 2007). In the year 2004 global electricity consumption was observed to be 131,000 GW h (EIA - International Energy Annual 2004); roughly around 86% of energy derived from fossil fuels which released approximately 29,000,000,000 tons of CO_2 to the environment (Raupach *et al*. 2007). Gigantic use of fossil fuels has increased the atmospheric CO_2 concentration to 385-395 ppm during the 2008 (NOAA. Earth system research laboratory; 2007); and even if CO_2 emissions are somehow instantaneously halved, CO_2 concentration would still ascent upto 540 ppm, approximately twice pre-industrial level, within next 30-40 years. Microalgae have been distinguished as one of the most potential sustainable biomass reserves due to their carbon neutrality toward natural environment and easy cultivation (Amaro *et al*. 2011). Microalgae dominate conventional crops in having higher carbon dioxide uptake rate, higher growth rate and lipid content and smaller land usage. However, the use of microalgae for biofuel production is still not economically feasible. This is principally attributed to the energy and cost constraints coupled with the cultivation and harvesting of microalgal cells (Barros. 2015). However, physicochemical surface properties of microalgal cells play a significant role in influencing both cultivation and harvesting of microalgae (Ozkan and Berberoglu 2013). Several species of microalgae have been examined under CO_2 concentrations of over 15%. For example, *Euglena gracilis* could grow under 5-45 % concentration of CO_2 and the optimum growth was observed with 5% CO_2 concentration (Nakano. 1996). *Chlorococcum littorale* has been studied under 60% CO_2 using the stepwise adaptation technique (Kodama. 1994). Another high CO_2 tolerant *Chlorella* sp. could grow successfully under

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10% CO_2 conditions (Hirata.1996a; 1996b) and it was also reported that the same species can be grown under 40% CO_2 conditions (Hanagata.1992). Furthermore, Maeda (1995) reported that a strain of *Chlorella* sp. T-1 can be grown under 100% CO_2, even though the highest growth rate was observed under a 10% concentration. *Scenedesmus* sp. could grow under 80% CO_2 conditions but 10-20% CO_2 concentration was optimum (Hanagata. 1992). *Cyanidium caldarium* (Seckbach. 1971) and some other species of *Cyanidium* can grow in pure CO_2 (Graham and Wilcox 2000).

In previous study, *Scenendesmus bajacaliforncus* BBKLP-07 strain had been isolated from freshwater ponds of Bagalkot district, which is a highly CO_2 tolerant (Patil and Kaliwal 2016). The present investigation was focused on optimizing the culture conditions such as CO_2 concentration, nitrate, phosphate concentrations and pH. For this purpose, a mathematical model response surface methodology (RSM) was adopted. Central Composite Design (CCD)/ RSM explores the relationships between several explanatory variables (CO_2, nitrate, phosphate and pH) and one or more response variables (Biomass productivity-R1 and CO_2 fixation rate-R2). RSM not only optimizes the process but also reduces cost and time required for experimentation by reducing the number of trials to be performed in laboratory. Additionally, RSM identifies optimal conditions of several variables in single set of experimental combination. Because of these advantages, RSM has been utilized in many ways for optimization of various parameters.

MATERIALS AND METHODS

Microorganism

Scenedesmus bajacalifornicus BBKLP-07 was isolated from freshwater ponds of Bagalkot District, Karnataka, India through repeated streak plate method on BG-11 medium at pH 7.1 (Patil and Kaliwal. 2016). The cells have a parietal chloroplast with a single pyrenoid, and walls are unornamented with knobby cell apices at the ends. Purity was checked through microscopic observation at regular intervals and pure cultures were maintained at 27 ± 3 ^oC temperature and 40 µmol/m^2/sec light intensity in culture room.

Table 1 Range and levels of experimental variables

Coded Variables	CO_2 (%)	$NaNO_3$ (g/l)	K_2HPO_4 (g/l)	pH
-1	5	0.5	0.02	5
0	15	1.75	0.06	7
1	25	3.0	0.1	9

Experimental design

Response Surface Methodology (RSM)

Optimization of CO_2, nitrate, phosphate and pH levels for different response variables, such as biomass productivity (R1), CO_2 fixation rate (R2) was done using statistical approach. For this, experiments were designed using Response Surface Methodology in Design expert version 10.0.4.0 (Statease Inc., Minneapolis, USA, trial version) and estimated the coefficients of a quadratic model using Box-Behnken design type. RSM was categorized in two models. First order model investigates linear relationship of response with its two independent variables and second order model, investigates a curvature on the response surface due to two or more than two variables (Kirana. 2016). An impressive feature of RSM is the designing of experiments with minimum number of combinations. The principle of response surface is based on the selection and identification of points having significant effect on responses. A full factorial approach is required to construct a model that can interact with and between 'n' number of variables and for analysis of all possible combinations. Factorial experiment is an approach in which combined effect of designed variables under various combinations can be studied by designing the variables simultaneously. Lower and upper limits of each of the variable were defined, as every variable is defined only at lower and upper boundary (i. e. two levels), then experimental design is known as 2 N full factorial. 2 N factorial design augmented with center(η_0), factorial (F) and axial points (star points, represented as A). Factorial points (F) represents a variance in optimal design for first order model whereas center point presents information concerning presence of curvature in the system. If curvature is present in the system, the axial points are included for the competent assessment of pure quadratic model and these points remain equidistant from each other providing rotatability to the model. In the present investigation, each factor was designed with 3 coded levels (1, 0, +1) as given in Table 1. The number of experiments in CCD can be calculated as:

$$n = F + A + \eta 0 \qquad \text{Equation (1)}$$

where, n = number of experiments, F = Factorial points, A = star points and η_0 = center point.

In the present study 29 experiments were performed as designed through Deign Expert version 10 (trial version) to study the effect of varying CO_2 concentration (%), nitrate ($NaNO_3$), phosphate (K_2HPO_4), pH levels and various combinations of input variables provided by software are designated with suitable codes as presented in Table 2. *Scenedesmus bajacalifornicus* BBKLP-07 was cultivated in 100 ml autoclaved BG-11 media with modified levels of nitrate and phosphate in 250 ml conical flasks and the initial inoculum cell concentration was maintained as 2 x 10^7 cells/ml. Cultures were cultivated in shaker at 100 rpm speed and 34 µmol/m^2/sec light intensity with 24hr photoperiod for 15 days.

The CO_2 optimization studies were carried out using the protocol given by Vidyashankar. (2013). For CO_2 optimization carbon dioxide from a pressurized cylinder was mixed with air pumped by a vacuum pump. Gases were mixed in a tee container, and then, the concentration of carbon dioxide in the introduced gas was calibrated using a Model 410i carbon dioxide gas analyzer Thermo Scientific (measurement range of 0 to 25 vol%). The gas was then introduced into the culture medium at a constant aeration rate of 0.26 vvm (volume gas per volume culture per min).

Determination of biomass productivity

Maximum biomass productivity (P_{max}, g/l/day) was estimated from Eq. (2), where X_t was the biomass concentration (g/l) at the termination of the cultivation period (t_x) and X_0 the initial biomass concentration (g/l) at t_0 (day) (Mariana. 2013).

$$P_{max} = (X_t - X_0) / (t_x - t_0) \qquad \text{Equation (2)}$$

Table 2 Central Composite Design matrix for four input variables and designated code used throughout the study.

		Factor 1	Factor 2	Factor 3	Factor 4	Coded variables
Std	Run	A: CO2	B: Nitrate Source	C: Phosphate source	D: pH	CNPH
		(%)	(g/l)	(g/l)		
5	1	15	1.75	0.02	5	$C_{15}N_{1.75}P_{0.02}H_5$
2	2	25	0.5	0.06	7	$C_{25}N_{0.5}P_{0.06}H_7$
22	3	15	3	0.06	5	$C_{15}N_{3.0}P_{0.06}H_5$
17	4	5	1.75	0.02	7	$C_5N_{1.75}P_{0.02}H_7$
15	5	15	0.5	0.1	7	$C_{15}N_{0.5}P_{0.1}H_7$
24	6	15	3	0.06	9	$C_{15}N_{3.0}P_{0.06}H_9$
4	7	25	3	0.06	7	$C_{25}N_{3.0}P_{0.06}H_7$
13	8	15	0.5	0.02	7	$C_{15}N_{0.05}P_{0.02}H_7$
27	9	15	1.75	0.06	7	$C_{15}N_{1.75}P_{0.06}H_7$
16	10	15	3	0.1	7	$C_{15}N_{3.0}P_{0.1}H_7$
1	11	5	0.5	0.06	7	$C_5N_{0.5}P_{0.06}H_7$
21	12	15	0.5	0.06	5	$C_{15}N_{0.5}P_{0.06}H_5$
25	13	15	1.75	0.06	7	$C_{15}N_{1.75}P_{0.06}H_7$
6	14	15	1.75	0.1	5	$C_{15}N_{1.75}P_{0.1}H_5$
12	15	25	1.75	0.06	9	$C_{25}N_{1.75}P_{0.06}H_9$
11	16	5	1.75	0.06	9	$C_5N_{1.75}P_{0.06}H_9$
18	17	25	1.75	0.02	7	$C_{25}N_{1.75}P_{0.02}H_7$
9	18	5	1.75	0.06	5	$C_5N_{1.75}P_{0.06}H_5$
8	19	15	1.75	0.1	9	$C_{15}N_{1.75}P_{0.1}H_9$
14	20	15	3	0.02	7	$C_{15}N_{3.0}P_{0.02}H_7$
20	21	25	1.75	0.1	7	$C_{25}N_{1.75}P_{0.1}H_7$
19	22	5	1.75	0.1	7	$C_5N_{1.75}P_{0.1}H_7$
7	23	15	1.75	0.02	9	$C_{15}N_{1.75}P_{0.02}H_9$
23	24	15	0.5	0.06	9	$C_{15}N_{0.5}P_{0.06}H_9$
29	25	15	1.75	0.06	7	$C_{15}N_{1.75}P_{0.06}H_7$
3	26	5	3	0.06	7	$C_5N_{3.0}P_{0.06}H_7$
28	27	15	1.75	0.06	7	$C_{15}N_{1.75}P_{0.06}H_7$
10	28	25	1.75	0.06	5	$C_{25}N_{1.75}P_{0.06}H_5$
26	29	15	1.75	0.06	7	$C_{15}N_{1.75}P_{0.06}H_7$

Determination of CO_2 fixation rate

Carbon dioxide fixation rate, R_{CO2} (g/l/day), was estimated from Eq. (3), as explained by (Tang. 2011).

$$R_{CO2} = C_C P_{max} (M_{CO2} / M_C) \quad \text{Equation (3)}$$

Where C_C was the carbon content of microalgal cells (% w/w), analysed by using a LECO CHNS-932 Elemental Analyser (USA), P_{max} was the maximum biomass productivity (g/l/day), M_{CO2} was the molar mass of CO_2 (g/mol) and M_C was the molar mass of carbon (g/mol).

Preparation of sample for Fourier transform infrared spectroscopy (FTIR)

50 ml of microalgal sample from optimized culture conditions was subjected to centrifugation at 3000 rpm for 10 min. The pellet was washed with double distilled water to eliminate any residue due to nutrient medium and was again pelletized by centrifugation (3000 rpm, 10 min). Samples was frozen overnight and freeze dried at 55°C. From the harvested and dried biomass, approximately 2-3 mg of sample was mixed with potassium bromide (KBr) and subjected to a pressure of about 8 × 106 Pa to obtain clear disc of 13 mm diameter, 1 mm thickness and examined using FTIR spectrometer (Bruker Optics TENSOR 27). Spectrum was developed with a DTGS detector over a wavelength of mid-IR region (4000 to 500 cm ^{1}). Empty ATR plate was practiced for background single beam spectra and the result was analyzed using OPUS control software.

Statistical analysis

All the experiments were carried out in accordance with set of conditions offered through Design Expert 10 (Table 2).

Noteworthy differences were determined by using examination of variance (ANOVA). Second order quadratic model was used to estimate the effect of CO_2 concentration, nitrate, phosphate levels and pH on response variables through following equation:

$$Ri=0+c_1A+c_2B+c_3C+c_4D+c_{12}AB+c_{13}AC+c_{14}AD+c_{23}BC+c_{24}BD +c_{34}CD+c_{11}A^2+c_{22}B^2+c_{33}C^2+c_{44}D^2 \quad \text{Equation (4)}$$

where Ri is Response variable; A, B, C and D are Independent variables i.e. CO_2, nitrate, phosphate and pH, respectively; c_0 is intercept term; c_1, c_2, c_3 and c_4 are linear terms; c_{11}, c_{22}, c_{33} and c_{44} are quadratic terms; c_{12} c_{13} c_{14} c_{23} c_{24} and c_{34} are interaction terms.

RESULTS

Central composite design / Response study

Observed and predicted values of CO_2 fixation rate and biomass productivity are presented in Table 3. To study the surface response for biomass productivity and CO_2 fixation rate by CCD, a quadratic model was studied with factors A, B, C, D, AB, BC, AC, AD, BD, CD, A^2, B^2, C^2, D^2 and intercept, which were analyzed as a function of the model (Table 4). Where A, B, C, and D represent CO_2 nitrate, phosphate and pH level, respectively. The quadratic model predicted for the response variable R1 (biomass productivity) and R2 (CO_2 fixation rate) were found statistically valid. ANOVA was utilized for assessment of factual noteworthiness of every quadratic model. Reaction factors were examined utilizing coefficients. p-Value was utilized to concentrate the coefficient relationship with its separate error. smaller p-value proposes the bigger estimation of coefficient when contrasted with error;

along these lines, guaranteeing the non-understanding of observed data and the null hypothesis.

Table 3 Observed and predicted values of biomass productivity (R1), and CO_2 fixation rate (R2).

Biomass productivity R1				
Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared
Linear	0.5349	< 0.0001	-0.0289	-0.1968
2FI	0.9507	< 0.0001	-0.2643	-0.8567
Quadratic	< 0.0001	< 0.0001	0.8996	0.7111
Cubic	0.0006	0.0034	0.9926	0.7841
CO_2 fixation rate R2				
Linear	0.8565	< 0.0001	-0.1062	-0.3511
2FI	0.9994	< 0.0001	-0.4515	-1.4852
Quadratic	< 0.0001	< 0.0001	0.952	0.8616
Cubic	0.948	0.0034	0.9187	-1.5093

The concluding quadratic equation for the response variable was established where positive sign before coefficient indicates a synergistic effect of a factor towards response and negative sign suggests an antagonistic effect. The quadratic model predicted for biomass productivity (R1) and CO_2 fixation rate (R2) using significant coefficients is given as:

R1 (Biomass productivity) = +0.93-(0.027)A +(5.000E-003)B+(1.667E-003)C+(0.088)D+(7.500E-003)AB+(7.500E-003)AC-(0.065)AD-(0.040)BC+(0.083)BD-(0.048)CD-(0.19)A^2-(0.034)B^2-(0.11)C^2-(0.28)D^2 Equation (5)

R2 (CO_2 fixation rate) = +0.13+(5.000E-003)A-(1.667E-003)B-(8.333E-004)C+(0.014)D-(2.500E-003)AB-(2.500E-003)AC+(1.000E-002)AD+(2.500E-003)BC+(5.000E-003)BD+(7.500E-003)CD-(0.038)A^2-(5.000E-003)B^2-(6.250E-003)C^2-(0.081)D^2

Equation (6)

Table 4 ANOVA for Response Surface Quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.76	14	0.054	8.64	0.0001	significant
A-CO2	0.023	1	0.023	3.58	0.0794	
B-Nitrate Source	0.036	1	0.036	5.76	0.0308	
C-Phosphate source	6.75E-04	1	6.75E-04	0.11	0.7482	
D-pH	0.099	1	0.099	15.72	0.0014	
AB	2.50E-03	1	2.50E-03	0.4	0.5388	
AC	2.25E-04	1	2.25E-04	0.036	0.8528	
AD	4.23E-03	1	4.23E-03	0.67	0.4265	
BC	6.40E-03	1	6.40E-03	1.02	0.3305	
BD	2.50E-03	1	2.50E-03	0.4	0.5388	
CD	1.60E-03	1	1.60E-03	0.25	0.6221	
A^2	0.27	1	0.27	42.16	< 0.0001	
B^2	0.036	1	0.036	5.77	0.0308	
C^2	0.048	1	0.048	7.63	0.0153	
D^2	0.43	1	0.43	67.54	< 0.0001	
Residual	0.088	14	6.30E-03			
Lack of Fit	0.088	10	8.79E-03	125.56	0.0001	significant
Pure Error	2.80E-04	4	7.00E-05			
Cor Total	0.85	28				

Factual importance was checked by Analysis of variance (ANOVA) and Analysis of variable factors (f test) for the recommended model and affirmed as significant with low p-values (P value <0.0001 for both biomass productivity and CO_2 fixation rate), showing the high certainty level. Importance of the model was affirmed by linearity between normal probability graphs i.e. the predicted value is in great concurrence with observed value (Fig 2 A and B). The predicted values of response demonstrated sensible concurrence with experimental values in this manner uncovering the centrality of model for every response variable. Three dimensional graphs mirror the impact of fluctuating concentrations of CO_2 and nitrate (A), CO_2 and phosphate (B), pH and CO_2 (C), and nitrate and phosphate (D) on biomass productivity (R1) and CO_2 fixation rate R2 (Fig 3 and 4) individually. Most elevated biomass productivity (0.93 g/l/day) and CO2 fixation rate (0.13 g/l/day) were found at $C_{15}N_{1.75}P_{0.06}H_7$. Hence, this condition (i.e. $C_{15}N_{1.75}P_{0.06}H_7$) is viewed as ideal for high biomass productivity and CO_2 fixation rate.

Effect of nutritional parameters on biomass productivity (R1) and CO_2 fixation rate (R2)

The present investigation reveals that the predicted values of response showed reasonable agreement with experimental values thus revealing the significance of model for each response variable. The effect of varying CO_2 concentration, nitrate, phosphate and pH levels on response variables i.e biomass productivity (R1) and CO_2 fixation rate (R2) is depicted in the perturbation graphs (Fig 1). The optimum response variable values for Biomass productivity as well as CO_2 fixation rate were 15% CO_2, 1.75g/l nitrate, 0.06 g/l phosphate and pH 7. The maximum biomass productivity (0.93 g/l/day) and CO_2 fixation rate (0.13 g/l/day) were obtained with the above-mentioned variables. The predicted and observed response at optimal condition ($C_{15}N_{1.75}P_{0.06}H_7$) were tabulated in table 5.

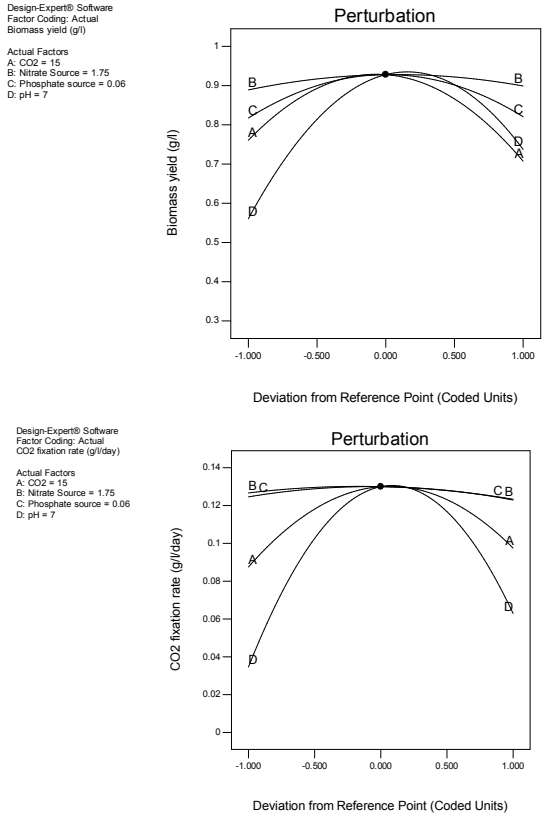

Fig 1 Perturbation graph showing the deviation of the optimized conditions for biomass productivity and CO_2 fixation rate of microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

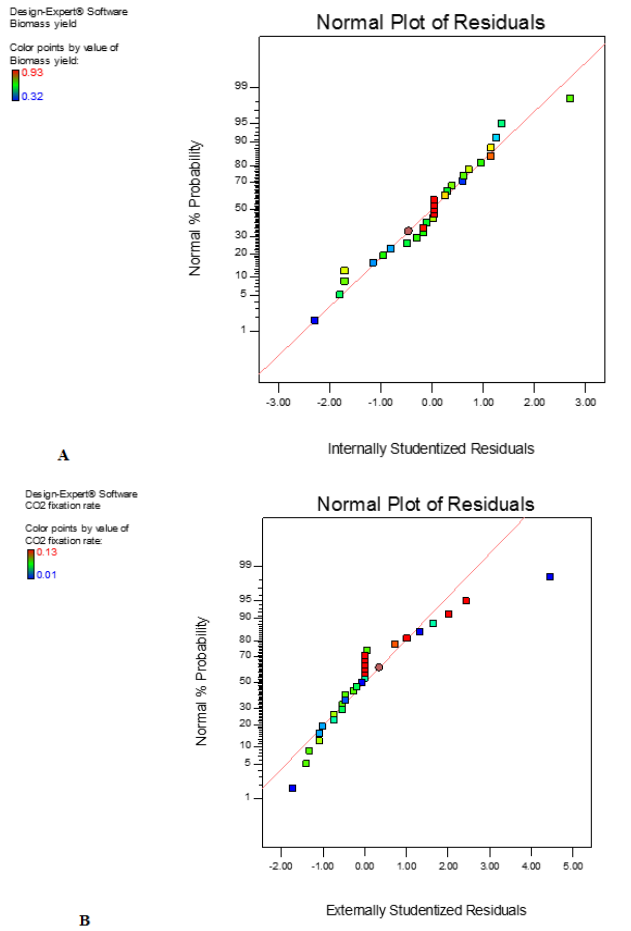

Fig 2 Normal (%) probability graphs for (A) Biomass productivity and (B) CO_2 fixation rate.

of microalgae *Scenedesmus bajacalifornicus* BBKLP-07. The highest biomass productivity and maximum CO_2 fixation rate were 0.93 g/l/day and 0.13 ± 0.002 g/l/day at 15% CO_2 concentration respectively at pH 7. The minimum biomass productivity and lowest CO_2 fixation rate were 0.35 g/l/day and 0.01 ± 0.002 g/l/day at 5% CO_2 concentration respectively at pH 5. As the concentration of CO_2 increases in the media, the biomass productivity as well as CO_2 fixation rate is elevated up to 15% CO_2 and further increase in the CO_2 concentration lead to decrease in biomass productivity as well as CO_2 fixation rate.

Nitrate and phosphate

Studies showed that the effect of nitrate and phosphate on biomass productivity and CO_2 fixation is insignificant. Even though the optimal conditions for the microalgal growth were $C_{15}N_{1.75}P_{0.06}H_7$, nitrate has very little effect on biomass productivity. Biomass productivity was found to be maximum at nitrate concentrate $N_{1.75}$ (0.93 g/l/day) at 15% CO_2,whereas the biomass productivity was found to be 0.6 g/l/day at $C_{15}N_{0.5}P_{0.06}H_5$ levels and 0.42 g/l/day at $C_{15}N_3P_{0.06}H_5$ levels. CO_2 fixation was not affected by nitrate concentration. Similarly, phosphate doesn't appear to have any effect on biomass productivity as well as CO_2 fixation.

pH

The pH has a very significant effect on biomass productivity and CO_2 fixation. At low pH 5, biomass productivity and CO_2 fixation rate were very minimum in all the studied experimental conditions whereas at high pH 9, biomass productivity and CO_2 fixation rate were moderately increased. Maximum biomass productivity and CO_2 fixation rate were found to be 0.93 g/l/day and 0.13 g/l/day at pH 7.

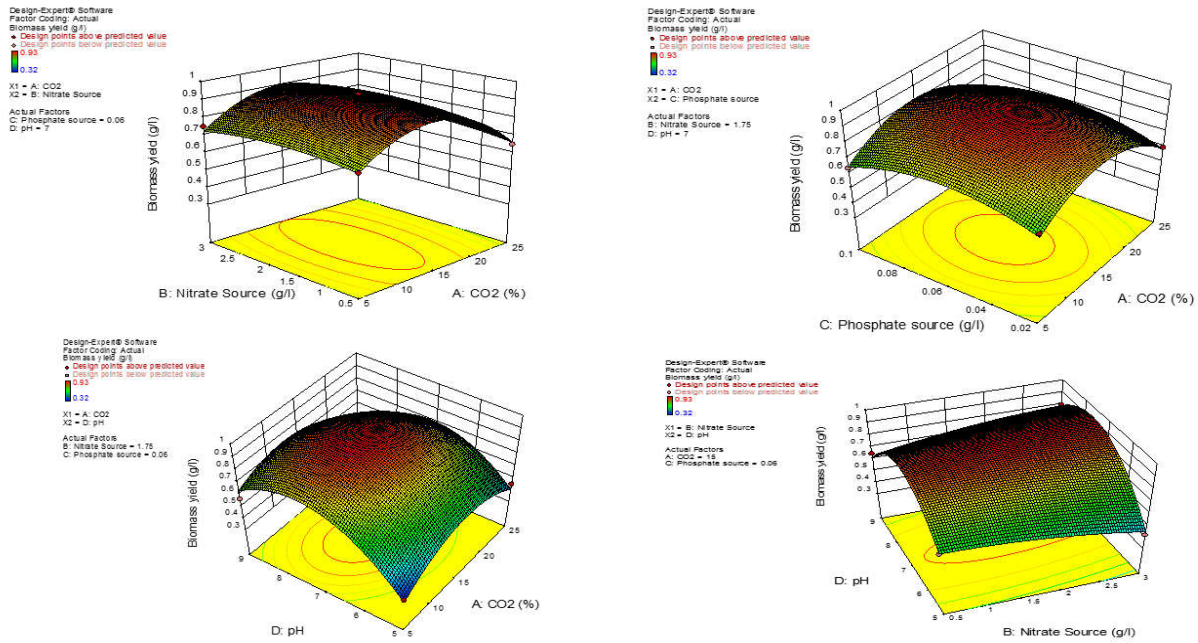

Fig 3 Effect of (A) CO_2, (B) Nitrate source, (C) Phosphate source, and (D) pH on biomass productivity of microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

CO_2 concentration

The CO_2 concentration played a very important role on growth parameters of microalgae isolates. It was observed that the 15% CO_2 concentration was found to be optimum for the culturing

The results indicate that pH 7 was optimum for the growth for microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

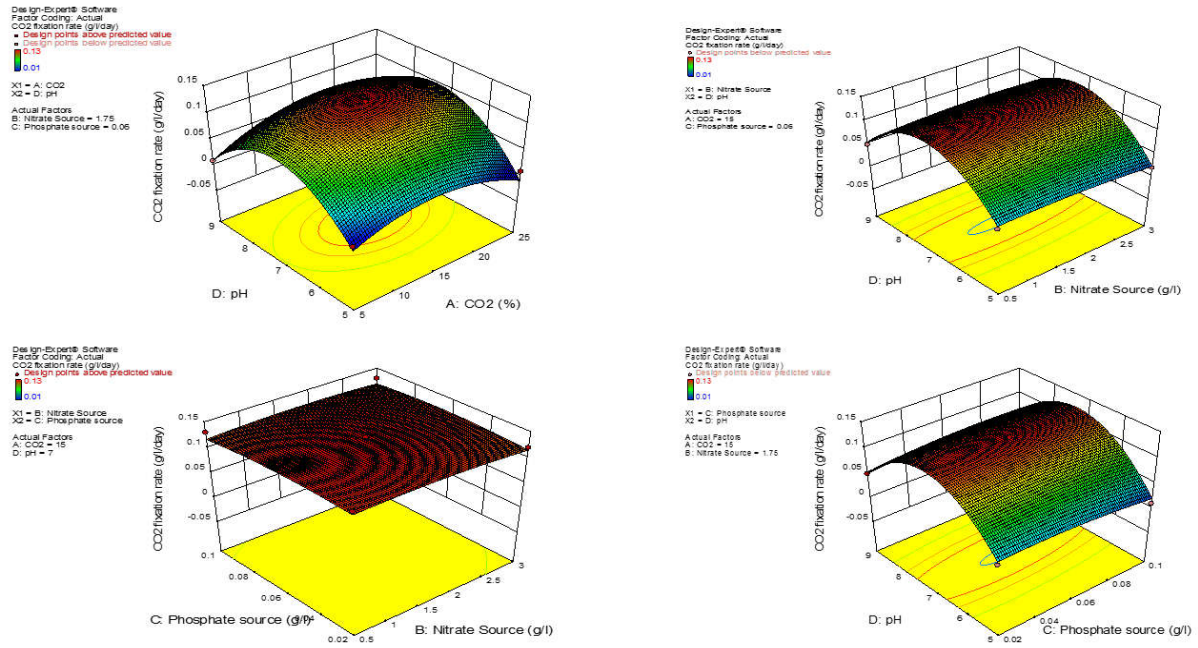

Fig 4 Effect of (A) CO_2, (B) Nitrate source, (C) Phosphate source, and (D) pH on CO_2 fixation rate of microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

Table 5 Predicted and observed response at optimal condition ($C_{15}N_{1.75}P_{0.06}H_7$).

Response	Predicted Mean	Observed Mean	Std Dev	SE Pred	95% PI low	95% PI high
Biomass productivity (g/l/day)	0.928	0.93	0.05579	0.061	0.8	1.06
CO2 fixation rate (g/l/day)	0.13	0.13	0.00954	0.01	0.11	0.15

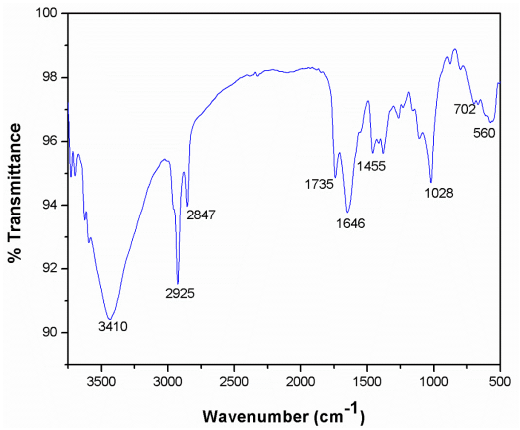

Fig 5 FTIR spectra at optimized CO_2, nitrate, phosphate and pH levels of microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

FTIR analysis

Recognizable proof depends on examination of the groups of the recorded FTIR spectra with those of a reference literature. The FTIR transmittance of the *Scenedesmus bajacalifornicus* BBKLP-07. algal species uncovers the proximity of -OH, -COOH, NH_2, and CO groups in the natural compound; aliphatic compounds: (500-800 cm^{-1}), phenols and alcoholic compounds (1000-1500 cm^{-1}), carboxyl compounds (1500-1700 cm^{-1}), hydroxyl compounds (3200-3,450 cm^{-1}). The band at 3410 cm^{-1} is expected to the O-H stretching vibration. The frail bands focused at 2925 and 2847 cm^{-1} are because of the nearness of asymmetric C-H stretching vibration.

Three unique groups were seen in the region of 1735, 1646, and 1455 cm^{-1}, which reveals the nearness of esters in the microalgae.

DISCUSSION

Central composite design / Response surface methodology

In statistics, RSM investigates the associations between quite a few illustrative variables and at least one response variables. The primary thought of RSM is to utilize a sequence of designed experiments to get an optimal response. RSM uses a second-degree polynomial model to perform the optimization. The experimental variables and responses were selected according to Box- Behnken design type (Box and Behnken 1960). Each independent variable is placed at one of three equally spaced values, usually coded as 1, 0, +1. The design is sufficient to fit a quadratic model, that is, one containing squared terms, products of two factors, linear terms and an intercept.

In the present four illustrative variables i.e CO_2 concentration (A), nitrate (B), phosphate (C) and pH (D) were used for the optimization of two responses i.e biomass productivity (R1) and CO_2 fixation rate (R2). The optimized conditions were found to be 15% (A), 1.75 g/l (B), 0.06 g/l (C) and 7 (D) and the maximum responses observed were 0.93 g/l/day (R1) and 0.13 g/l/day (R2). Similar studied have been performed by Kim . (2012) where they have optimized the culture conditions for biomass productivity of three different microalgae *Chlorella* sp., *Dunaliella salina* DCCBC2 and *Dunaliella* sp., and estimated the optimal growth conditions for *Chlorella* sp. (initial pH 7.2, ammonium 17 mM, phosphate 1.2 mM), *D. salina* DCCBC2 (initial pH 8.0, nitrate 3.3 mM, phosphate 0.0375 mM) and *Dunaliella* sp. (initial pH 8.0, nitrate 3.7 mM, phosphate 0.17 mM). The biomass productivities were 0.28, 0.54 and 0.30 g dry cell wt /l and the CO_2 fixation rates were 42.8, 90.9 and 45.5 mg/l/day respectively. RSM have been also

used for the optimization of lipid and biomass productivity of *Oocystis* sp. IM-04, where the variable parameters studied were temperature, nitrate and phosphate levels (Kirana. 2016; Satapute. 2012). The highest lipid productivity (7.0 mg/l/day) and biomass productivity (47.8 mg/l/day) were reported for the optimized culture conditions of sodium nitrate (750 mg/l), Di potassium hydrogen phosphate (0 mg/l) at 30 ^{o}C temperature. Statistical methods such as RSM has been used to standardize the production process of a special substance by optimization of operational factors with respect algal species (Berges. 2002).

CO_2 concentration

The CO_2 concentration assumed an imperative part on growth parameter of microalgae *S. bajacalifornicus* BBKLP-07. The microalgae detach *S. bajacalifornicus* BBKLP-07 demonstrated an extensive variety of CO_2 resilience capacity. It was examined that the 15% CO_2 conentration was observed to be ideal for the growth of microalgae *S. bajacalifornicus* BBKLP-07. The biomass productivity and CO_2 fixation were at peak at 15% CO_2 fixation. The greatest rates of biomass productivity and CO_2 fixation rate were 0.93 g/l/day and 0.13 g/l/day respectively. Fifteen percent CO_2 was basic for microalgal growth; Riebesell. (1993) has expressed that when CO_2 is underneath a critical concentration, algal growth gets to be distinctly constrained. This critical concentration not just relies on upon the rate of CO_2 supply and CO_2 affinity but also on cell size, growth rate, and conceivable nearness of extracellular carbonic anhydrase. Comparative outcomes were seen in case the of *Scenedesmus* obtusus, in which the microalgae demonstrated a maximum biomass productivity was observed at 15% CO_2 concentration and no noteworthy distinction in the biomass productivity was observed. However, critical concentrations of CO_2 for CO_2 fixation and biomass productivity were different for different microalgae species. The *Chlorella* sp. and *Scenedesmus* sp., isolated from a coalfired thermoelectric power plant exhibited maximum biomass productivity at 6 and 12% CO_2 respectively (Morais and Costa 2007); it is because of the fact that they require higher CO_2 concentration to fulfill their carbon demands (Burkhardt. 1999). Yang and Gao (2003) reported three species having contrasting cell shape and size i.e Chlormadinones *reinhardtii* and *Chlorella pyrenoidosa* with circular cell shape and *S. obliquus* with spindle shape having varying demands of CO_2 concentration to saturate the growth. The enhancement of growth rate with increased CO_2 is probably related to lower energy consumption. Lee. (1998) recommended hoisting the underlying cell density as an option way to deal with increment the tolerance against high levels of CO_2 and lessen the long adjustment time period.

Nitrate and phosphate

Nitrate and phosphate have a very insignificant effect on the biomass productivity as well as on CO_2 fixation rate of *Scenedesmus bajacalifornicus* BBKLP-07. However, phosphorous is known to have a significant role in cellular metabolic processes of microalgae. Several reports suggest that high concentration of phosphate and nitrate affects the biomass production in *Scenedesmus dimorphus* KMITL (Ruangsomboon. 2013, Mandal and Mallick 2009). Thus, the present study indicates that the effect of nitrate and phosphate on biomass productivity and on CO_2 fixation rate of microalgae *Scenedesmus bajacalifornicus* BBKLP-07 is insignificant.

pH

The role of pH on biomass productivity and CO_2 fixation is highly significant. In the present study pH 7 is observed to be optimum for the microalgal growth and CO_2 fixation, deviation in pH from 7 demonstrated adverse effect on *S. bajacalifornicus* BBKLP-07 biomass productivity and CO_2 fixation rate. The maximum biomass productivity and CO_2 fixation rate were 0.93 g/l/day and 0.13 g/l/day. However similar studies have been reported where *Chlorella* sp., have exhibited optimum growth conditions at pH 7.2, *Dunaliella salina* DCCBC2 and *Dunaliella* sp., have been exhibited maximum gowth at pH 8. The CO2 fixation rates of *Chlorella* sp., *D. salina* DCCBC2 and *Dunaliella* sp. were 42.8, 90.9 and 45.5 mg/l/day, respectively (Kim. 2012). The studies on *C. reinhardtii* showed that pH of 7.5 is optimum for microalgal growth however, excess CO_2 inhibited algae growth due to a significant decrease in pH (Kong. 2010). Contradictory reports suggest that biomass production by *Scenedesmus obliquus* and *Chlorella vulgaris* in laboratory cultures was significantly affected by the pH at which the cultures were preserved. Carbon fixation experiments exhibited that pH values in the range of 8 to 9 were important for influencing the free CO_2 concentrations in the medium (Azova 1982).

FTIR analysis

Fourier transform infrared (FTIR) spectroscopy is a novel method for monitoring carbon allocation in microalgae. This form of vibration spectroscopy can be used to collect mid-infrared absorbance spectra from air dried, intact microalgal samples. When applied to whole organisms the resulting spectrum reflects the biochemical complexity of the cells, with absorbance bands from lipids, nucleic acids, carbohydrates and proteins. The FTIR transmittance of the *Scenedesmus bajacalifornicus* BBKLP-07. algal species reveals the nearness of -OH, -COOH, NH_2, and CO groups in the microalgae; aliphatic compounds: (500-800 cm^{-1}), phenols and alcoholic compounds (1000-1500 cm^{-1}), carboxyl compounds (1500-1700 cm^{-1}), hydroxyl compounds (3200-3,450 cm^{-1}). The band at 3410 cm^{-1} is expected to the O-H stretching vibration. The frail bands focused at 2925 and 2847 cm^{-1} are because of the nearness of asymmetric C-H stretching vibration. Three unique groups were seen in the region of 1735, 1646, and 1455 cm^{-1}, which reveals the nearness of esters in the microalgae.

CONCLUSIONS

CO_2, nitrate, phosphate and pH have their individual and independent effect on biomass as well as on CO_2 fixation ability of *Scenedesmus bajacalifornicus* BBKLP-07. Utilization of RSM based CCD approach for determination of optimum growth levels proved to be an efficient and effective method. It is concluded that predicted values by quadratic model lies in close proximity with experimental values. Thus, for high CO_2 fixation and biomass productivity, process conditions optimized through CCD i.e. $C_{15}N_{1.75}P_{0.06}H_7$ for *Scenedesmus bajacalifornicus* BBKLP-07 are more suitable as compared normal BG-11 media. Since, optimal quantity of biomass was achieved at pH 7, therefore deflection in pH is not a prudent approach related to biomass production. Exploitation of such

optimized system for bioprocess engineering will not only result in high CO_2 sequestration but also will lead to high biomass in *Scenedesmus bajacalifornicus* BBKLP-07. Still further research is required to study and explore the impact of pilot scale studies in open/closed environment on the efficiency of this species in terms of CO_2 fixation and biomass productivity.

Compliance with Ethical Standards

Conflict of interest

Authors do not have any conflict of interest related to the manuscript.

Ethical approval

This article does not contain any studies related to animals and human participants.

Acknowledgments

The authors are thankful to the Department of Biotechnology, New Delhi for providing Bioinformatics lab facility, DBT-KUD-IPLS program (BT/PR14555//INF/22/126/2010) of Department of Biotechnology, New Delhi, University Grants Commission (UGC) for providing fellowship under University with Potential for Excellence (UPE) program. The authors are also thankful to the Department of Biotechnology and Microbiology, Karnatak University Dharwad for providing all the necessary facilities.

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How to cite this article:

Lakkanagouda Patil and Basappa Kaliwal.2017, Optimization of Biomass Productivity and Carbon Dioxide Fixation Ability By Freshwater Microalgae Scenedesmus Bajacalifornicus Bbklp-07, A Step Towards Sustainable Development. *Int J Recent Sci Res.* 8(5), pp. 16903-16911. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0805.0242

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