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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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 CO2 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 °C 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 CO2 to the environment (Raupach et al. 2007). Gigantic use of fossil fuels has increased the atmospheric CO2 concentration to 385-395 ppm during the 2008 (NOAA. Earth system research laboratory; 2007); and even if CO2 emissions are somehow instantaneously halved, CO2 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 CO2 concentrations of over 15%. For example, Euglena gracilis could grow under 5-45 % concentration of CO2 and the optimum growth was observed with 5% CO2 concentration (Nakano. 1996). Chlorella sp have been examined under 60% CO2 using the stepwise adaptation technique (Kodama. 1994). Another high CO2 tolerant Chlorella sp. could grow successfully under

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10% CO₂ conditions (Hirata, 1996a; 1996b) and it was also reported that the same species can be grown under 40% CO₂ conditions (Hanagata, 1992). Furthermore, Maeda (1995) reported that a strain of Chlorella sp. T-1 can be grown under 100% CO₂, even though the highest growth rate was observed under a 10% concentration. Scenedesmus sp. could grow under 80% CO₂ conditions but 10-20% CO₂ concentration was optimum (Hanagata, 1992). Cyanidium caldarium (Seckbach, 1971) and some other species of Cyanidium can grow in pure CO₂ (Graham and Wilcox, 2000).

In previous study, Scenedesmus bajacalifornicus BBKLP-07 strain had been isolated from freshwater ponds of Bagalkot district, which is a highly CO₂ tolerant (Patil and Kaliwal, 2016). The present investigation was focused on optimizing the culture conditions such as CO₂ 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 (CO₂, nitrate, phosphate and pH) and one or more response variables (Biomass productivity-R1 and CO₂ 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 °C temperature and 40 μmol/m²/sec light intensity in culture room.

**Table 1** Range and levels of experimental variables

| Coded Variables | CO₂ (%) | NaNO₃ (g/l) | K₂HPO₄ (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 CO₂, nitrate, phosphate and pH levels for different response variables, such as biomass productivity (R1), CO₂ 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(η₀), 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:

\[ n = F + A + η_0 \]  

**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 CO₂ concentration (%), nitrate (NaNO₃), phosphate (K₂HPO₄), pH levels and various combinations of input variables provided by software are designated with suitable codes as presented in Table 2.

**Table 2** Design of experiments

| Run | CO₂ (%) | NaNO₃ (g/l) | K₂HPO₄ (g/l) | pH |
|-----|---------|------------|--------------|----|
| 1   | 5       | 0.5        | 0.02         | 5  |
| 2   | 15      | 1.75       | 0.06         | 7  |
| 3   | 25      | 3.0        | 0.1          | 9  |

The CO₂ optimization studies were carried out using the protocol given by Vidyashankar. (2013). For CO₂ 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 0.26vvm (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 \( (t_t) \) and \( X_0 \) the initial biomass concentration (g/l) at \( t_0 \) (day) (Mariana, 2013).

\[ P_{max} = \frac{(X_t - X_0)}{(t_t - t_0)} \]  

**Equation (2)**
about 8 × 10^6 Pa to obtain clear disc of 13 mm diameter, 1 mm thick 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).

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

| Std | Run | A: CO2 (%) | B: Nitrate (g/l) | C: Phosphate (g/mol) | D: pH | CNPH |
|-----|-----|------------|------------------|----------------------|-------|------|
| 5   | 1   | 15         | 1.75             | 0.02                 | 5     | 6.5  |
| 2   | 2   | 25         | 0.5              | 0.06                 | 7     | 6.75 |
| 17  | 4   | 5          | 1.75             | 0.02                 | 7     | 6.75 |
| 15  | 5   | 15         | 0.5              | 0.1                  | 7     | 6.75 |
| 24  | 6   | 15         | 3                | 0.06                 | 9     | 6.75 |
| 4   | 7   | 25         | 3                | 0.06                 | 7     | 6.75 |
| 13  | 8   | 15         | 0.5              | 0.02                 | 7     | 6.75 |
| 27  | 9   | 15         | 1.75             | 0.06                 | 7     | 6.75 |
| 16  | 10  | 15         | 3                | 0.1                  | 7     | 6.75 |
| 1   | 11  | 5          | 0.5              | 0.06                 | 7     | 6.75 |
| 21  | 12  | 15         | 0.5              | 0.06                 | 5     | 6.75 |
| 25  | 13  | 15         | 1.75             | 0.06                 | 7     | 6.75 |
| 6   | 14  | 15         | 1.75             | 0.1                  | 9     | 6.75 |
| 12  | 15  | 25         | 1.75             | 0.06                 | 9     | 6.75 |
| 11  | 16  | 5          | 1.75             | 0.06                 | 9     | 6.75 |
| 18  | 17  | 25         | 1.75             | 0.02                 | 7     | 6.75 |
| 9   | 18  | 5          | 1.75             | 0.06                 | 5     | 6.75 |
| 8   | 19  | 15         | 1.75             | 0.1                  | 9     | 6.75 |
| 14  | 20  | 15         | 3                | 0.02                 | 7     | 6.75 |
| 17  | 21  | 25         | 1.75             | 0.06                 | 7     | 6.75 |
| 19  | 22  | 5          | 1.75             | 0.1                  | 7     | 6.75 |
| 6   | 23  | 15         | 1.75             | 0.02                 | 9     | 6.75 |
| 23  | 24  | 15         | 0.5              | 0.06                 | 9     | 6.75 |
| 29  | 25  | 15         | 1.75             | 0.06                 | 7     | 6.75 |
| 3   | 26  | 5          | 3                | 0.06                 | 7     | 6.75 |
| 28  | 27  | 15         | 1.75             | 0.06                 | 5     | 6.75 |
| 26  | 29  | 15         | 1.75             | 0.06                 | 7     | 6.75 |

Noteworthy differences were determined by using examination of variance (ANOVA). Second order quadratic model was used to estimate the effect of CO2 concentration, nitrate, phosphate levels and pH on response variables through following equation:

\[
R_i = c_0 + c_1 A + c_2 B + c_3 C + c_4 D + c_{12} A B + c_{13} A C + c_{14} A D + c_{23} B C + c_{24} B D + c_{34} C D + c_{13} A^2 + c_{14} A D + c_{24} B^2 + c_{34} C^2 + c_{23} D^2
\]  

Equation (4)

where R_i is Response variable; A, B, C and D are Independent variables i.e. CO2, nitrate, phosphate and pH, respectively; c_0 is intercept term; c_1, c_2, c_3 and c_4 are linear terms; c_{12}, c_{23} and c_{34} are quadratic terms; c_{13} c_{14} c_{24} and c_{14} are interaction terms.

### RESULTS

**Central composite design / Response study**

Observed and predicted values of CO2 fixation rate and biomass productivity are presented in Table 3. To study the surface response for biomass productivity and CO2 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 CO2, nitrate, phosphate and pH level, respectively. The quadratic model predicted for the response variable R1 (biomass productivity) and R2 (CO2 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.
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 CO2 fixation rate (R2) using significant coefficients is given as:

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

Equation (5)

\[
R2 \text{ (CO2 fixation rate) } = +0.13+(5.000E-003)A-(1.667E-003)B-(8.333E-004)C+(0.014)D-(2.500E-003)AB-(2.500E-003)AC+(1.000E-002)AD+(2.500E-003)BC+(5.000E-003)BD+(7.500E-003)CD-(0.038)A^2-(5.000E-003)B^2-(6.250E-003)C^2-(0.081)D^2
\]

Equation (6)

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 CO2 concentration, nitrate, phosphate and pH levels on response variables i.e biomass productivity (R1) and CO2 fixation rate (R2) is depicted in the perturbation graphs (Fig 1). The optimum response variable values for Biomass productivity as well as CO2 fixation rate were 15% CO2, 1.75g/l nitrate, 0.06 g/l phosphate and pH 7. The maximum biomass productivity (0.93 g/l/day) and CO2 fixation rate (0.13 g/l/day) were obtained with the above-mentioned variables. The predicted and observed response at optimal condition (C12N1.75P0.06H7) were tabulated in table 5.

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 CO2 fixation rate (R2).

| Source   | Sequential | Lack of Fit | Adjusted | Predicted |
|----------|------------|-------------|-----------|-----------|
| Linear   | 0.5349     | <0.0001     | -0.289    | -0.1968   |
| 2FI      | 0.9507     | <0.0001     | -0.2643   | -0.8567   |
| Quadratic| <0.0001    | <0.0001     | 0.8906    | 0.7111    |
| Cubic    | 0.0006     | 0.0034      | 0.9826    | 0.7841    |

### Table 4 ANOVA for Response Surface Quadratic model.

| Source          | Sum of Squares | df  | Mean Square | F Value | P-value | Prob > F |
|-----------------|----------------|-----|-------------|---------|---------|----------|
| Model           | 0.76           | 1   | 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     | 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  | 0.030       |         |         |           |
| Lack of Fit     | 0.088          | 10  | 0.079       | 125.56  | 0.0001  | significant |
| Pure Error      | 2.80E-04       | 4   | 7.00E-05    |         |         |           |
| Cor Total       | 0.85           | 28  |             |         |         |           |
The CO₂ concentration played a very important role on growth parameters of microalgae isolates. It was observed that the 15% CO₂ concentration was found to be optimum for the culturing of microalgae *Scenedesmus bajacalifornicus* BBKLP-07. The highest biomass productivity and maximum CO₂ fixation rate were 0.93 g/l/day and 0.13 ± 0.002 g/l/day at 15% CO₂ concentration respectively at pH 7. The minimum biomass productivity and lowest CO₂ fixation rate were 0.35 g/l/day and 0.01 ± 0.002 g/l/day at 5% CO₂ concentration respectively at pH 5. As the concentration of CO₂ increases in the media, the biomass productivity as well as CO₂ fixation rate is elevated up to 15% CO₂ and further increase in the CO₂ concentration lead to decrease in biomass productivity as well as CO₂ fixation rate.

**Nitrate and phosphate**

Studies showed that the effect of nitrate and phosphate on biomass productivity and CO₂ fixation is insignificant. Even though the optimal conditions for the microalgal growth were C₁₅N₁₇₅P₀.₀₆H₇, nitrate has very little effect on biomass productivity. Biomass productivity was found to be maximum at nitrate concentrate N₃ (0.93 g/l/day) at 15% CO₂ whereas the biomass productivity was found to be 0.6 g/l/day at C₁₅N₀.₅P₀.₀₆H₅ levels and 0.42 g/l/day at C₁₅N₃P₀.₀₆H₅ levels. CO₂ fixation was not affected by nitrate concentration. Similarly, phosphate doesn’t appear to have any effect on biomass productivity as well as CO₂ fixation.

**pH**

The pH has a very significant effect on biomass productivity and CO₂ fixation. At low pH 5, biomass productivity and CO₂ fixation rate were very minimum in all the studied experimental conditions whereas at high pH 9, biomass productivity and CO₂ fixation rate were moderately increased. Maximum biomass productivity and CO₂ fixation rate were found to be 0.93 g/l/day and 0.13 g/l/day at pH 7.

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

### 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 °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).

**CO₂ concentration**

The CO₂ 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 CO₂ resilience capacity. It was examined that the 15% CO₂ concentration was observed to be ideal for the growth of microalgae *S. bajacalifornicus* BBKLP-07. The biomass productivity and CO₂ fixation were at peak at 15% CO₂ fixation. The greatest rates of biomass productivity and CO₂ fixation rate were 0.93 g/l/day and 0.13 g/l/day respectively. Fifteen percent CO₂ was basic for microalgal growth; Riebesell. (1993) has expressed that when CO₂ is underneath a critical concentration, algal growth gets to be distinctly constrained. This critical concentration not just relies on upon the rate of CO₂ supply and CO₂ 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% CO₂ concentration and no noteworthy distinction in the biomass productivity was observed. However, critical concentrations of CO₂ for CO₂ 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% CO₂ respectively (Morais and Costa 2007); it is because of the fact that they require higher CO₂ concentration to fulfill their carbon demands (Burkhardt. 1999). Yang and Gao (2003) reported three species having contrasting cell shape and size i.e. Chloradimones reinhardtii and Chlorella pyrenoidosa with circular cell shape and *S. obliquus* with spindle shape having varying demands of CO₂ concentration to saturate the growth. The enhancement of growth rate with increased CO₂ 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 CO₂ 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 CO₂ 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 CO₂ fixation rate of microalgae *Scenedesmus bajacalifornicus* BBKLP-07 is insignificant.

**pH**

The role of pH on biomass productivity and CO₂ fixation is highly significant. In the present study pH 7 is observed to be optimum for the microalgal growth and CO₂ fixation, deviation in pH from 7 demonstrated adverse effect on *S. bajacalifornicus* BBKLP-07 biomass productivity and CO₂ fixation rate. The maximum biomass productivity and CO₂ 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, *Dunalialiella salina* DCCBC2 and *Dunalialiella sp.*, have been exhibited maximum growth at pH 8. The CO₂ fixation rates of *Chlorella* sp., *D. salina* DCCBC2 and *Dunalialiella 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 CO₂ 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 CO₂ 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, NH₂, 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 (3200-3450 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**

CO₂, nitrate, phosphate and pH have their individual and independent effect on biomass as well as on CO₂ 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 CO₂ fixation and biomass productivity, process conditions optimized through CCD i.e. C₁₀H₁₆N₂O₇: P₀₆H₄ 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 CO₂ sequestration but also will lead to high biomass in *Scenedesmus bajiocalifornicus* 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 CO₂ 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.

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