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Statistical optimization of parameters affecting lipid productivity of microalga *Chlorella protothecoides* cultivated in photobioreactor under nitrogen starvation

Prakash Binnal[*], *P. Nirguna Babu*

Department of Chemical Engineering, Siddaganga Institute of Technology, Tumkur, 572103, Karnataka, India

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ABSTRACT

In the present work, a lab scale photobioreactor was used to evaluate lipid productivity and carbon dioxide fixation rate of microalgae *Chlorella protothecoides* under nitrogen deplete conditions. Effect of environmental conditions such as pH, temperature, light intensity, photoperiod (light to dark cycle ratio), CO_2 concentration in air and aeration rate on lipid productivity and carbon fixation rate of microalga was studied. Response surface methodology was adopted to optimize these conditions. All the parameters were found to be statistically significant. Best operating conditions were evaluated to be: pH-6.51, Temperature-28.63 °C, light intensity-5.31 klux, Photoperiod-15.36 h:8.64 h, CO_2 concentration in air-6.26% (v/v), Aeration rate −2.92 lpm. Lipid productivity under these conditions was found to be 274.15 mg/(L day) which was 3.94 times higher than the value obtained in N+ experiment (69.46 mg/(L day)). Carbon fixation rates under N+ and N− conditions were 286.12 and 273.66 mg/(L day) respectively.

1. Introduction

Lipid productivity (LP) is a critical variable for evaluating algal species for biodiesel production. It is calculated as the product of biomass productivity (BP) (g dry weight/L day) and lipid content (% dw) to give an indicator of oil produced on a basis of both volume and time (Griffiths and Harrison, 2009). Key environmental variables affecting LP of microalgae under phototrophic conditions include: light intensity (LI), temperature, pH, photoperiod (ratio of light to dark cycle), CO_2 concentration in air and aeration rate. Several reports are available in literature which illustrate the effect of these variables on LP under nitrogen replete (N+) conditions (Lee and Lee, 2001; Pal et al., 2011; Xin et al., 2011; Cabello et al., 2015; Razzak et al., 2015). But very few authors have reported the influence under nitrogen deplete (N−) conditions (Fernandez et al., 2012; Liu et al., 2012; Bruer et al., 2013; Toledo et al., 2013; Fakhry and Maghraby, 2015).

Moreover, the number of variables in these studies was restricted to a maximum of 3. However, LP of microalgae is significantly affected by all aforementioned environmental variables. Especially, under nitrogen deprivation, microalgal cells are more sensitive to environmental conditions. They modify their biosynthetic pathways to accumulate lipids during this phase. Thus, knowledge of the influence of environmental conditions on LP under N− conditions is very useful. It leads to development of useful kinetic expressions that could be applied for designing and modelling

* Corresponding author. Fax: +91 816 2214070.
E-mail addresses: prakashbinnal@yahoo.co.in (P. Binnal), paturi_nir@yahoo.com (P.N. Babu).
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photobioreactors to predict LP and optimize the operating conditions (Da Silva et al., 2006). In this regard, the present study aims at the statistical optimization of aforementioned environmental parameters influencing the LP and R_C of microalga *Chlorella protothecoides* in a lab scale externally illuminated photobioreactor (PBR) under N− conditions.

1.1. Design of experiments by response surface methodology

Response surface methodology (RSM) is a statistical technique which allows the simultaneous consideration of many variables at different levels and the interactions between those variables, using a smaller number of observations than conventional procedures. It is very useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM has important application in the design, development and formulation of new products, as well as in the improvement of existing product design. It defines the effect of the independent variables, alone or in combination, on the processes. In addition to analysing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical or biochemical processes (Anjum et al., 1997).

Several authors have described the use of RSM as an optimization tool (Singh et al., 2015; Amosa, 2016; Amosa and Majozi, 2016; Chiranjeevi and Mohan, 2016; Jami et al., 2016). Among RSM techniques, Box-Behnken design is considered as an efficient option and an ideal alternative to central composite designs. It combines a fractional factorial with incomplete block designs to avoid the extreme vertices and presents an approximately rotatable design with only three levels per factor. The number of experiments (N) required for the development of BBD is defined as $N = 2k(k-1) + C_0$, (where k is number of factors and C_0 is the number of central points). For, $k = 6$, Co = 6, N is 56 (Deming and Morgan, 1993). Considering all the linear terms, square terms and linear × linear interaction items, the quadratic response model can be described as:

$$Y = \beta_o + \sum_{i=1}^{i=k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i_{>j}}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where Y is the predicted response variable, x_i is the independent variable, β_o, β_i, β_{ii}, β_{ij} are the regression coefficients and ε is the random error. Other advantages of BBD are: i) It permits estimation of the parameters of the quadratic model (ii) building of sequential designs (iii) detection of lack of fit of the model and (iv) use of blocks (Ferreira et al., 2007).

2. Materials and methods

2.1. Construction of photobioreactor

The pictorial, schematic and PID diagrams of Photobioreactor (PBR) used in the present study are shown in Figs. 1–3 respectively. The set up consists of a 5 L borosilicate vessel connected to a panel of straight glass tubes through a diaphragm pump. The panel of straight glass tubes consists of 6 glass tubes (1 inch diameter, 80 cm long) connected to each other through bends, flexible couplings and O-rings. The media from 5 L vessel is circulated through straight glass tubes and returned to the vessel by diaphragm pump. The vessel is equipped with pH, temperature and light intensity controllers. pH electrode (Mettler Tolerdo) and Pt-100 sensors have been used to measure pH and temperature of media in reactor. LED panels are arranged around 5 L vessel and straight glass tubes. The light intensity (0–10 klux) was adjusted from control panel. Two rotameters are provided for controlling flow rate of Carbon dioxide and air (Range of rotameter for air: 1 LPM–5 LPM, range of rotameter for CO_2: 1 mLPM to 50 mLPM). The mixed CO_2 enriched air was bubbled through media in 5 L vessel using ring sparger.

2.2. Algae strain collection and culture condition

The original strain of *C. protothecoides* (SAG 211-10C) was obtained from Sammulung von Algenkulturen (SAG), Germany, and maintained on agar slants containing BG11 medium consisting of (g/L): $NaNO_3$-1.5 g, $K_2HPO_4 \cdot 3H_2O$-0.04, $KH_2PO_4 \cdot 3H_2O$-0.2, EDTA-0.0005, Fe ammonium citrate-0.005, citric acid-0.005, Na_2CO_3-0.02 and 1 ml of trace metal solution. The trace metal solution contains (g/L): H_3BO_3-2.85, $MnCl_2 \cdot 4H_2O$-1.8, $ZnSO_4 \cdot 7H_2O$-0.02, $CuSO_4 \cdot 5H_2O$-0.08, $CoCl_2 \cdot 6H_2O$-0.08 and $Na_2MoO_4 \cdot 2H_2O$-0.05. The pH of the medium was adjusted to 6.8. Stock cultures were inoculated into 100 ml of sterilized medium in 250 ml Erlenmayer flasks. The flasks were then incubated at 24 °C in a rotary shaker and agitated at 120 rpm. After seven days, the algal biomass was recovered by centrifugation (REMI c-24 bl) at 10,000 rpm for 10 min and used as inoculam to PBR containing 3 L BG11 medium with nitrogen (for N+ experiments) or BG11 medium without nitrogen (for N− experiments)and the

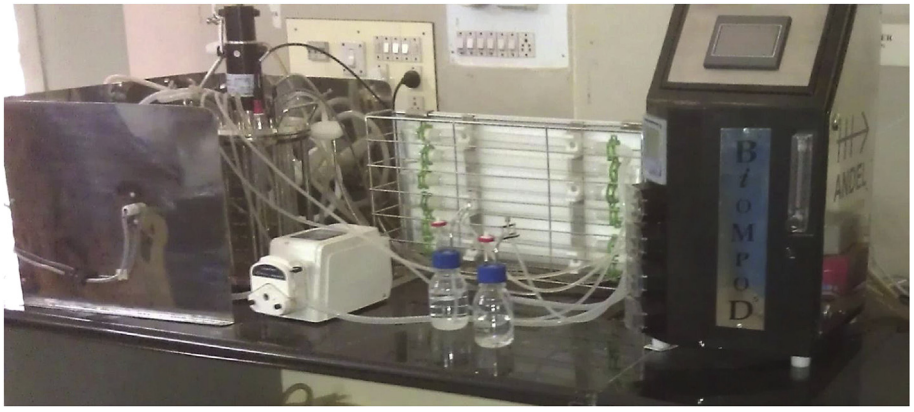

Fig. 1 – Pictorial view of photobioreactor.