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Statistical optimization using Central Composite Design for biomass and lipid productivity of microalga: A step towards enhanced biodiesel production

Bala Kiran[a,b,*], Kratika Pathak[a], Ritunesh Kumar[a], Devendra Deshmukh[a]

[a] Department of Mechanical Engineering, Indian Institute of Technology, Indore 452017, M.P., India
[b] Centre for Biosciences & Biomedical Engineering, Indian Institute of Technology, Indore 452017, M.P., India

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ABSTRACT

Central Composite Design (CCD) was utilized to optimize the levels of nutrients (nitrate and phosphate) and temperature for high biomass and lipid productivity in *Oocystis* sp. IM-04 isolated from domestic wastewater. Effect of varying temperature, nitrate and phosphate levels and their interactions was studied for different response variables i.e. lipid productivity (R_1), biomass productivity (R_2) and lipid concentration (R_3). The predicted second-order quadratic model for response variables was significant ($p < 0.01$). Further R^2 values i.e. 0.8878 (R_1), 0.9944 (R^2), indicated satisfactory fit of the model. On the basis of statistical analysis of results, sodium nitrate (750 mg/l), Di potassium hydrogen phosphate (0 mg/l) at 30 °C temperature i.e. $N_{750}P_0T_{30}$, was found to be the best combination for highest lipid productivity (7.0 mg/l/d), biomass productivity (47.8 mg/l/d) and lipid concentration (109.5 mg/l).

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1. Introduction

Nitrogen and phosphorous are the two major elements which contribute to building blocks of bimolecular components such as DNA, proteins and lipids. The limited quantity of these two major elements is supposed to cause changes in cellular metabolism of microalgae as compared to normal conditions (Ruangsomboon et al., 2013). Few algal species are known to synthesize and accumulate significant quantity of lipids along with high biomass productivity. These species can proved to be potential strains for biofuel production (Dean et al., 2010; Kiran et al., 2014a, 2014b). The growth rate kinetics of microalgae depends upon various factors such as carbon dioxide aeration (Yue and Chen, 2005), nutrient composition (Dargone et al., 2011; Kiran et al., 2015), temperature (Converti et al., 2009), and salinity (Rao et al., 2007) etc. Nutrients concentration and temperature level at which microalgae flourishes are the fundamental factors affecting the growth and lipid accumulation within the cell.

Under nutrient limited/starved conditions, metabolic breakdown of carbohydrates release free carbon dioxide in cell matrix, which is utilized in constructive lipid metabolism resulting in increased lipid content per unit microalgal biomass. Studies also suggest that nitrogen and phosphorus starvation results in the induction of mono saturated fatty acid production with reduced quantity of PUFA and phospholipids (Pruvost et al., 2011; Rodolfi et al., 2009). Nutrient starvation also results in decomposition of thylakoid membrane which can be visualized through yellowing of cells. On the other hand, increased acyl hydrolysis leads to the induction of free Acyl-CoA within the cell enhancing lipid metabolism. Accumulation of lipid precursors (Acyl-CoA) in TAG synthesis has antagonistic relationship with the growth rate/biomass productivity (Pruvost et al., 2009). In addition to nutrients concentration, temperature also plays an important role. Studies suggest that temperature show an antagonistic relationship with nitrogen uptake (Rhee and Gotham, 1981; Whalen and Alexander, 1984) due to diminishing enzyme kinetics of nitrate reductase (NR) (nitrogen reducing enzyme) which is strongly affected under temperature variation (Berges et al., 2002). Thus it is very important to identify optimal conditions for enhanced lipids as well as biomass production.

Central Composite Design (CCD) is full-factorial two-level design which is a valuable mathematical approach and can be utilized in optimization of important process parameters. CCD not only optimizes the process but also reduces cost and time required for experimentation by decreasing the number of experiments to be performed in laboratory. Further the use of Response Surface Methodology (RSM)/Central Composite Design (CCD) identifies optimal conditions of several variables in single set of experimental

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* Corresponding author.
E-mail address: kiranb@iiti.ac.in (B. Kiran).

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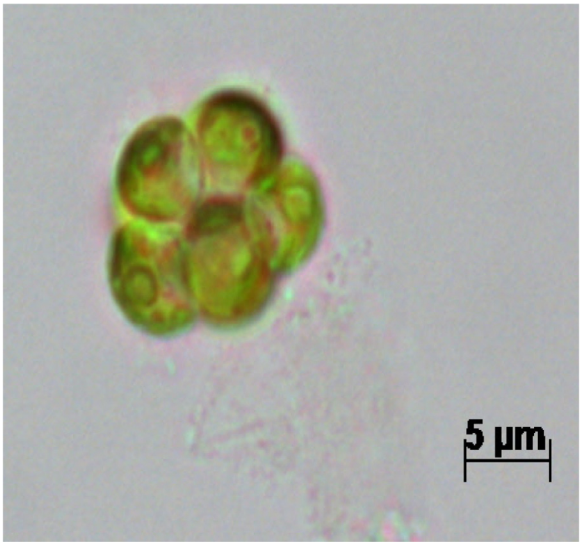

Fig. 1. *Oocystis* sp. IM-04 isolated from sewage wastewater.

combination. Due to above advantages, RSM/CCD has been utilized in many ways in previous studies such as, optimization of nutrient components like nitrate concentration, phosphate concentration, and pH for biomass production (Kim et al., 2012), optimization of process variables in catalyzed transesterification reaction (Renita et al., 2012), and the optimization of different conditions in lipid extraction process (Yang et al., 2014).

In the present study, the effect of three major factors i.e. nitrate, phosphate and temperature has been interpreted in terms of lipid and biomass productivity of *Oocystis* sp. IM-04. Indigenous species are supposed to perform better in open environmental conditions but initially cultivation conditions need to be optimized systematically under controlled conditions. Culture conditions for biomass and lipid productivity were optimized based on 2^n full factorial Face Centered Central Composite Design. Further the optimized conditions of lipid productivity from observed as well predicted values was analyzed and validated through FTIR spectroscopy which is an efficient and rapid method. Fourier Transform Infrared spectroscopy (FTIR) utilizes infrared absorption to study vibrational modes caused due to molecular groups present in the macromolecule of an organism. To the best of our comprehension, this is the first approach utilizing Response Surface Methodology for the optimization of nutrients in relation to biomass and lipid productivity in *Oocystis* sp.

2. Materials and methods

2.1. Microorganism

Oocystis sp. IM- 04 was isolated from wastewater collected from inlet of Kabirkhedi Sewage Treatment plant, Indore through repeated streak plate method on BG-11 medium at pH 8.5 (Stanier et al., 1971). The dominance of oval to ellipsoidal (size upto 10 µm) shaped colonies resembling morula (4–8 celled) stage present within a thin, hyaline mucilaginous envelope confirmed the monoculture conditions of *Oocystis* sp. IM-04 (Fig. 1). Purity was checked through slides viewed under light microscope (Unilab) at regular intervals and pure cultures are maintained at 27 ± 3 °C temperature and 40 µmol/m^2/sec light intensity in culture room.

Table 1
Range and levels of experimental variables where, α = 1 with 6 center point and 14 non center point.

Coded Variable	$NaNO_3$ (mg/l)	K_2HPO_4 (mg/l)	Temperature (°C)
−1	0	0	15
0	750	20	30
+1	1500	40	45

2.2. Experimental design

2.2.1. Response Surface Methodology (RSM)

Optimization of nitrate, phosphate and temperature levels for different response variables, such as lipid productivity (R_1), biomass productivity (R_2), and lipid concentration (R_3) was done using statistical approach. For this, experiments were designed using Response Surface Methodology in Design expert version 9 (Statease Inc., Minneapolis, USA, trial version) and estimated the coefficients of a quadratic model. RSM is categorized in two models. First order model studies linear relationship of response with its two independent variables and second order model, studies a curvature on the response surface due to two or more than two variables. An important feature of RSM is the designing of experiments with minimum number of combinations. Its principle is based on the selection and identification of points having significant effect on response surface. To construct a model that can interact with and between 'n' number of variables and for analysis of all possible combinations, a full factorial approach is required. Factorial experiment is an approach in which designed variables are studied together, i.e. combined effect of designed variables under various combinations can be studied simultaneously. Lower and upper limits of each of the variable should be defined and allowable range is further optimized at diverse levels (+α, +1, 0, −1, −α). When every variable is defined only at lower and upper boundary (i. e. two levels), then experimental design is known as 2 N full factorial. CCD is made up of two-level factorial design augmented with center (η_0), factorial (F) and axial points (star points, represented as A). Factorial points (F) symbolize a variance in optimal design for first order model whereas center point presents information regarding presence of curvature in the system. Axial points are added in the system if curvature is present for the competent assessment of pure quadratic model. These points remain equidistant from each other providing rotatability to the model.

In the present study, 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:

$$n = F + A + \eta 0 \tag{1}$$

where, n = number of experiments, F = Factorial points i.e. 2^k, A = star points i.e. 2k and η_0 = center point which is usually between 4 and 6 times to get a good estimate of experimental error.

Thus in the current study 20 experiments were performed as designed through Deign Expert version 9 (trial version) to study the effect of varying temperature (°C), nitrate ($NaNO_3$), and phosphate (K_2HPO_4) levels and various combinations of input variables provided by software are designated with suitable codes as presented in Table 2. *Oocystis* sp. IM-04 was cultivated in 100 ml autoclaved BG-11 media with modified levels of nitrate and phosphate in 250 ml conical flasks and inoculation of microalgal culture was done to set initial optical density at 0.1 (660 nm). Cultures were cultivated in shaker incubator at 100 rpm shaking speed and 34 µmol/m^2/sec light intensity with 24hr photoperiod for 15 days. In addition to replicates suggested by software, all of the experiments were performed in three replicates and average values along with standard deviations are reported here.

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