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Critical parametric influence on microalgae cultivation towards maximizing biomass growth with simultaneous lipid productivity

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ABSTRACT

Enhancing microalgae biomass productivity through different abiotic and environmental factors optimization is crucial. Design of experimental (DOE) methodology using Taguchi orthogonal array (OA) was studied to evaluate the specific influence of eight important factors (light, pH, temperature, carbon concentration, nitrates, phosphates, magnesium ion concentration and carbon source) on the biomass production using three levels of factor ($2^1 \times 3^7$) variation with experimental matrix [L_{18}-18 experimental trails]. All the factors were assigned with three levels except light illumination (2^1). Substantial influence on biomass productivity is observed with carbon concentration contributing 16.8%, followed by nitrates 12.8% and light 9.3%. Experimental setup eight (Light, pH-8.5, Temperature 25°C, Carbon concentration 10 g/l, nitrates 1.5 g/l, phosphates 0 g/l, magnesium 150 mg/l, Carbon source (glucose)) showed maximum biomass growth (5.26 g/l) and good substrate degradation (63%, COD removal efficiency) contributing to carbohydrate production (257 mg/g biomass) which is further converted to lipids (20% Total lipid and 10% Neutral lipids). *Chlorophyll* (*a*, *b*), carbohydrates composition, FAME analysis for lipid percentage were monitored during process operation. Elemental analysis reveals that the carbon to hydrogen and oxygen ratio present in dried algal biomass can be hydrothermally liquefied (HTL) to produce biocrude.

1. Introduction

Microalgae is touted as an attractive alternative to traditional forms of biomass for biofuels production, due to high productivity per unit area, usage of non-arable lands for cultivation, utilization of wastewater as a substrate and CO_2 sequestration. Direct lipid extraction/Thermochemical conversion methods are widely being used to transform algal biomass into fuels along with high valued products [1]. Optimization of different factors such as nutrient stress, light, temperature, CO_2, salinity, etc. have been explored by several researchers to enhance lipid accumulation in microalgae rather than biomass [2]. The cell growth and microalgal biomass production will be influenced by sufficient supply of macro and micronutrients present in the medium and environmental factors (light, pH and temperature) [3]. Microalgae can survive in extreme environments as they can adapt their metabolism according to altering environmental conditions. Usually, microalgal biomass can be produced through autotrophic cultivation in open ponds or a photobioreactor using solar energy for fixing CO_2 [4,5]. Alternatively, they are also cultivated in heterotrophic mode of nutrition using organic compounds as energy and carbon sources [6–8]. Among the different modes of cultivation, mixotrophic operation offers several advantages like low-cost for biomass harvesting and substrate degradation [9]. Moreover, in mixotrophic operation, both cell growth and biosynthesis of products are significantly influenced by the nutrients present in the medium and by the environmental factors. The syntrophic association between microalgae and its existing microenvironment facilitates higher biomass production and lipid accumulation along with significant substrate degradation. Mixotrophic cultivation of microalgae provides a sustainable and viable route for the possible utilization/mitigation of CO_2 and organic waste present in wastewater for biofuel production [10].

Enhancing the biomass productivity with simultaneous lipid synthesis by optimizing influencial factors employing design of

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experiment (DOE) methodology has been followed in this communication. DOE methodology by Taguchi orthogonal array (OA) is a factorial based approach which merges statistical and engineering techniques [11–13]. Analysis of the experimental data using ANOVA (analysis of variance) provides information about statistically significant factors and their optimum levels for the design of experimental parameters. Taguchi method utilizes OAs to study a large number of variables with a small number of experiments, saving both time and cost [11–14]. The specific objective of this investigation is to study the methodological application of Taguchi orthogonal array (OA) design of experiment (DOE) to optimize various process parameters viz., Environmental factors (light, pH, temperature), abiotic factors (carbon concentration, nitrates, phosphates, magnesium ion concentration and carbon (glucose/acetate)) that influence the microalgae biomass and lipid productivity along with reducing sugars synthesis. The increased biomass can also be used in HTL for biocrude production.

2. Experimental methodology

2.1. Collection and cultivation of microalgae

Microalgal cultures collected from the outlet channel of Nacharam Lake (lentic water body), Hyderabad in pre-monsoon season was used as parent inoculum [8]. Prior to experimentation, the culture was washed twice with water and pelletized by centrifugation (3000 rpm; 10 min at 30°C) to remove associated debris. Microalgae was stored in rectangular plastic tubs (36 cm × 24 cm × 12 cm) exposed to sunlight. Domestic sewage (DS- pH, 7.8; COD, 220 mg/l; VFA, 165 mg/l; BOD, 120 mg/l; total alkalinity, 140 mg/l; chlorides, 175 mg/l; nitrates, 115 mg/l; color, 200 Hazen units) was used for algal cultivation as carbon and nutrient source. During initial period of culture maintenance, the biomass was mixed periodically to avoid settling and allow uniform distribution of sunlight to the cells. After consistent amount of biomass and cell density was achieved, this culture was used as inoculum for the experimental study.

2.2. Design of experimental (DOE) methodology

Taguchi's DOE methodology was employed. Selection/identification of important factors whose variation had a critical effect on the biomass growth and lipid production was primary step. Eight factors viz., light, pH, temperature, nutrient stress, salinity, nitrogen, phosphorous and trace metals were selected for optimization due to their significant role on photosynthetic machinery in context of biomass growth and synthesis of lipids (Table 1). Subsequently, an experimental matrix was designed considering three levels of factor orthogonal array (OA) layout, $2^1 \times 3^7$ with experimentation size by symbolic arrays of matrix [L_{18}-18 experimental trails] (Table 2). Except for nutrient stress operation (2^1), all other factors were assigned with three levels. In the designed OA, each column consisted of a number of conditions depending on the levels assigned to each factor and the diversity of factors can be studied by crossing OA of control factors.

2.3. Microalgal cultivation studies

Batch experiments for microalgal cultivation were conducted by employing 18 selected experimental variations (Table 2) in combination with seven factors at 3 levels and one factor at 2 levels (Table 1). According to the designed experiments, sterilized (autoclaved for 20 min at 121°C and 1.05 kg/cm steam pressure prior to the inoculation to avoid the bacterial contamination) 250 ml of modified growth medium (as per design, Table 1) was inoculated with microalgae inoculums (10% v/v; OD, 0.1). Growth phase (GP) was operated for a period of 8 days to accelerate algal biomass growth and to avoid bacterial contamination further antibiotic (Ampicillin: 0.2 g/l) was added to the each experimental setup for every alternate day of the operation. Total experimental setup was kept in a temperature controlled shaking incubator (120 rpm). During GP operation, biomass, cell density, pigment analysis (chlorophyll *a* and *b*) along with total cellular carbohydrate concentrations were estimated once in every alternate day. Lipid analysis was done at initial (before the GP) and end of GP. All the experiments were carried out in triplicates and the results presented here represent an average of three independent operations.

2.4. Lipid extraction and derivatization of FAME

After GP, the biomass was separated by centrifugation (5000 rpm; 5 min at 28°C) and the algal biomass pellet was subjected to solar drying followed by blending in to powder form. The blended powder was further disrupted using sonicator (20 kHz) for 30 min (Power Sonic 410) and the total lipids were extracted by modified Bligh and Dyer method using chloroform and methanol (2:1) as solvents and hexane was used for neutral lipid extraction [8]. Further followed by centrifugation were solvent: lipid layer was transferred into pre-weighed round bottom flask and the total and neutral lipids were determined gravimetrically in terms of percentage dry cell weight. Lipid productivity (%) was calculated based on the ratio of total lipid extracted to dry weight of algae as shown below

Lipid productivity = g of oil/dry weight of biomass

The resulted lipid was transesterified (using methanol-sulphuric acid mixture) to FAME and was analyzed using GC-FID. After conversion of fatty acids to methyl esters, the concentrated sample was used for the detection of FAME composition by GC with FID (Nucon-5765) through capillary column [Valcobond (VB) 30 mm (0.25 mm × 0.25 lm)] using nitrogen as carrier gas (1 ml/min). The temperature of the oven was initially maintained at 140°C (for 5 min), later increased to 240°C at a ramp of 4°C/min for

Table 1
Selected factors and assigned levels.

S.No	Factor	Level 1	Level 2	Level 3
1	Light (74 μmol m^{-2} s^{-1})	Yes	No	–
2	pH	6	7.5	8.5
3	Temperature (^{0}C)	20	25	30
4	Carbon Concentration (mg COD/l)	0	5000	10,000
5	Nitrates ($NaNO_3$) (g/l)	0	1.5	2.5
6	Phosphates (mg/l)	0	40	80
7	Magnesium (mg/l)	0	75	150
8	Carbon Source	Glucose (G)	Acetate (A)	Glucose + Acetate (G + A) (50% each)