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Application of experimental design methodology for optimization of biofuel production from microalgae

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ABSTRACT

Optimization of biofuel productivity, in terms of lipid content, polysaccharide content, and calorific value, from microalgae was performed by varying four variables (temperature, light intensity, nitrogen content, and CO_2 addition) using a 2^4 full factorial design. A statistical analysis showing the influence of each variable and their interactions was conducted. The selected variables all influence biofuel productivity, but their importance varies according to the sequence: CO_2 addition > temperature > nitrogen content > light intensity. Interactive effects of temperature with light intensity and nitrogen with CO_2 addition for lipid and polysaccharide productivities were identified, respectively. For calorific value, interactive effects of CO_2 addition with light intensity and nitrogen content were observed. The highest biofuel productivity was obtained at the following conditions: temperature (>25 °C), light intensity (>60 μmol photons m^{-2} s^{-1}), nitrogen content (<50 mg L^{-1}), and CO_2 addition (>18 mL L^{-1} d^{-1}). 10 days was found to be the most favorable cultivation time for lipid production under the investigated conditions.

1. Introduction

Microalgae, which has higher biomass production and faster growth than those of other energy crops, has attracted a lot of attention as a potential source of biofuel. There are several types of biofuel, including biomethane produced by the anaerobic digestion of an algal biomass, photobiologically produced biohydrogen, and biodiesel derived from microalgal oil [1]. Microalgae are photosynthetic microorganisms that convert sunlight, water, and carbon dioxide into algal biomass. Microalgae can reduce land use and do not require agricultural land.

All microalgae contain proteins, carbohydrates, lipids, and nucleic acids in varying proportions. Many microalgae are exceedingly rich in lipid content, which can be converted into biofuel [2]. Carbohydrates and proteins can be used as carbon sources to produce ethanol under anaerobic conditions. The lipid and carbohydrate content of microalgae varies in accordance with culture conditions. Lipid content in microalgae can exceed 70% by weight of dry biomass [3]. The carbohydrate content in various strains of microalgae is also high, up to 50% of dry weight [2]. However, low biofuel productivity is often

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associated with high lipid and carbohydrate content of microalgae. Biofuel productivity is defined as the mass of lipids or carbohydrates produced per unit volume of culture per unit time; it depends on the algal growth rate and the lipid or carbohydrate content of the biomass.

Several factors influence the lipid and carbohydrate content of microalgae, including light (quality and quantity), temperature, nutrient concentration, O_2, CO_2, pH, salinity, and toxic chemicals. Few systematic studies have been conducted on the effects of these factors on the lipid and carbohydrate content and growth rate of microalgae. Light and temperature are major processing factors that affect the overall biomass productivity and biochemical composition of microalgae [4,5]. The effects of light and temperature are synergistic. Sandnes [6] observed that growth rates of *Nannochloropsis oceanica* increased with increasing light intensity at temperatures up to approximately 28 °C. At low light intensity, the growth rate is less affected by temperature. Renaud [7] studied the growth and nutritional content of four tropical Australian microalgal species and reported that the optimum temperature for growth was 25–27 °C for *Rhodomonas* sp. and 27–30 °C for *Prymnesiophyte* (NT19), *Cryptomonas* sp., *Chaetoceros* sp., and *Isochrysis* sp. *Chaetoceros* sp. has the highest percentage of lipids when cells are cultured at 25 °C, whereas *Rhodomonas* sp., *Cryptomonas* sp., *Prymnesiophyte* (NT19), and *Isochrysis* sp. have significantly higher amounts of lipids at temperatures in the range of 27–30 °C.

In addition, for a number of microalgae, CO_2 is the only carbon compound which can support growth. Increasing CO_2 content from 0.5% to 2% (v/v) increased carbohydrate content up to 20% and doubled the biomass for *Botryococcus braunii* [8]. Chrismadha [9] demonstrated that the addition of 5% CO_2 leads to enhanced carbohydrate accumulation for *Phaeodactylum tricornutum*. Although the growth rate increased with increasing CO_2 concentration, relatively low CO_2 concentration favored lipid accumulation. The highest lipid productivity was observed at low CO_2 concentration for *Chlorella vulgaris* [10]. Nitrogen limitation also improves the lipid accumulation of microalgae. Nitrogen limitation can result in a gradual change of lipid composition from free fatty acids to triacylglycerol. However, the biomass yield in nitrogen-limited cultures is lower than that in non-limited cultures. This difference is more significant under high light conditions [11].

Therefore, the optimum lipid and carbohydrate content and growth rate of microalgae depend on the species. The relationship between the factors of cell conditions is complicated and difficult to identify from simple experiments. Therefore, the optimization of these factors by statistical methods is required. However, few studies have been conducted on the optimization of cell conditions for lipid and carbohydrate content using a statistical analysis.

The present study was undertaken to examine the correlations among the factors (temperature, light intensity, nitrogen nutrient content, and carbon dioxide concentration) that affect the growth rate and lipid and carbohydrate content of biomass using a full factorial design method to optimize biofuel productivity. The results predicted by the multiple regression analysis method are compared with experimental results.

Table 1 – 2^4 Factorial experimental range and levels.

Variable	Symbol	Code levels	
		−1	+1
Temperature (°C)	X_T	20	30
Light intensity (μmol photons m^{-2} s^{-1})	X_L	40	80
Nitrogen content (mg N L^{-1})	X_N	35	70
CO_2 addition (mL L^{-1} d^{-1})	X_C	10	20

2. Methods and materials

2.1. Microalgal culture

One of the dominant green microalgal species, *Chodatella* sp., was isolated from local source water and cultured in a medium according to the method presented by Norris et al. [12]. Axenic cultures of *Chodatella* sp. were grown in batch mode in a 1-L modified serum bottle containing 800 mL of sterilized algal medium. The cultures were performed in an incubator according to the experimental design matrix. CO_2 was supplied to the cultures every day. Except for the growth phase stage test, cultures were harvested in the log growth phase after 7 days for experiments.

2.2. Biomass concentration and growth rate

The optical density of microalgal cells was determined daily by measuring absorbance at 684 nm (OD684) using an ultraviolet/visible spectrophotometer (Model U-2001, Hitachi, Japan). The dry weight of the microalgal biomass was determined gravimetrically. A known volume of microalgal culture was collected and dried at 90 °C for 3 h. The growth rate (μ) was calculated according to the equation, $\mu = (\ln A_1 - \ln A_0)/(T_1 - T_0)$, where A_1 and A_0 are the dry weights of the microalgal biomass at times T_1 and T_0, respectively.

2.3. Experimental design

A 2^4 full factorial design (FFD) was used to optimize the biofuel production [13]. The factorial design consists of four factors, namely temperature (X_T), light intensity (X_L), nitrogen content (X_N), and CO_2 addition (X_C) at two levels (low and high, coded as −1 and +1, respectively). Table 1 shows the real and coded values of the factors in the experimental designs. The FFD was used to determine the joint effects of several factors on a response. It was also used to determine the individual and cumulative effects of these variables and the mutual interactions between them. The complete design consisted of 16 runs, which were performed in duplicate to optimize the levels of selected variables. Data processing and calculations were carried out using a commercial statistical package, STATISTICA 9.0, to estimate the coefficients of the regression equation. The equations were validated using analysis of variance (ANOVA) to determine the significance of each term in the fitted equations and to estimate the goodness of fit in each case.

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