Bioresource Technology 163 (2014) 128–135

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Exploring the high lipid production potential of a thermotolerant microalga using statistical optimization and semi-continuous cultivation

Shih-Hsin Ho [a,b], Ching-Nen Nathan Chen [c,d], Yen-Ying Lai [a], Wei-Bin Lu [e], Jo-Shu Chang [a,f,g,*]

[a] Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[b] Organization of Advanced Science and Technology, Kobe University, Kobe, Japan
[c] Department of Oceanography, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
[d] Asia-Pacific Ocean Research Center, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
[e] Department of Cosmetic Science, Chung Hwa University of Medical Technology, Tainan 701, Taiwan
[f] University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan
[g] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan

HIGHLIGHTS

- High lipid productivity potential of a thermotolerant microalga was explored.
- Two-step response surface methodology was used to optimize batch culture conditions.
- Semi-continuous cultivation resulted in the highest lipid productivity.
- The 302 mg/L/d lipid productivity is the highest record in the literature.

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 4 April 2014
Accepted 8 April 2014
Available online 19 April 2014

Keywords:
Microalgae
Biodiesel
Response surface methodology
Fed-batch operation
Semi-continuous operation

ABSTRACT

A recently isolated thermotolerant microalga *Desmodesmus* sp. F2 has the traits of becoming potential biodiesel feedstock, such as high growth rate, high lipid content, and quick precipitation. Its overall lipid productivity was 113 mg/L/d when grown under non-optimal conditions using batch cultivation. A two-step response surface methodology was adopted to optimize its cultivation conditions. The overall lipid productivity was increased to 263 mg/L/d when the cells were grown under the optimized conditions of 6.6 mM initial nitrogen level and 6 days nitrogen depletion treatment in 700 μmol/m^2/s light intensity at 35 °C using batch cultivation. Fed-batch and semi-continuous cultivations were employed to further increase its lipid productivity to 213 and 302 mg/L/d, respectively. The 302 mg/L/d is the highest overall lipid productivity of microalgae ever reported in the literature. This study provides the information required for the design and operation of photobioreactors for large scale outdoor cultivation of this species.

1. Introduction

Microalgal biodiesel is recognized as the most promising alternative biofuel due to many reasons (Chisti, 2008; Jones and Mayfield, 2012; Mata et al., 2010). Unlike bioethanol production that goes through fermentation of sugars and distillation to concentrate ethanol, biodiesel production uses only a simple reaction of transesterification of lipids. The process of fermentation loses one third of carbon of the feedstock sugars in the form of CO_2 to the air, and distillation needs energy input. The two facts cripple the popularity of bioethanol. Compared to farming of other crops, cultivation of microalgae can use non-arable land and municipal wastewater or brackish water (Cho et al., 2011; Huang et al., 2012). Therefore, microalgal biodiesel production would not compromise food security and it can ameliorate water pollution. In addition to recycling residual nutrients in wastewater or brackish water, microalgae can utilize carbon dioxide in the flue gas emitted by industries, in the form of gas or bicarbonate, for photosynthesis (Chi et al., 2011). The lipid yield of microalgae per area is about 10 times or higher than those of other crops (Chisti, 2007). The high efficiency makes it possible to transform microalgal farms from aquaculture to industry.

* Corresponding author at: Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan. Tel.: +886 6 2757575x62651; fax: +886 6 2357146.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

http://dx.doi.org/10.1016/j.biortech.2014.04.028
0960-8524/

Similar to the circumstances in the dawn of Green Revolution, the current barriers to microalgal biodiesel development include the lack of elite microalgal species with high lipid productivity and a technology of low cost and high efficiency to extract lipids from microalgal cells. It has been reported that many species of microalgae can accumulate lipids to more than 50% of their dry biomass (Hu et al., 2008; Pan et al., 2011). However, only a handful of these species can be cultivated in the tropical and subtropical areas using photobioreactors that provide much better efficiency than the open pond systems (Ho et al., 2013b). Tropical and subtropical areas have more abundant solar energy than the temperate zone (Franz et al., 2012), but it also causes a high temperature problem in the photobioreactors. Thus, an applicable microalgal species must have the two traits of thermotolerance and high lipid content/productivity.

The quest for elite microalgal species leads to the discovery of many new thermotolerant species isolated from tropical Taiwan (Ho et al., 2013b). Among these species, *Desmodesmus* sp. F2 is among the best candidates for biodiesel production. These cells can live at 50 °C for 8 h. Their lipid content is higher than 50% after nitrogen starvation treatment, and 85% of the cells precipitate in about 3 h. The lipid productivity of this species grown in non-optimal conditions was 113 mg/L/d, which was already among the top performance in the literature. These traits demonstrate that this species has a great potential to serve as a feedstock for biodiesel production. Before this species is applied to large scale outdoor cultivation, it is essential to understand its full potential of lipid productivity in order to assist the photobioreactor design and operation.

Response surface methodology (RSM) is an effective way to optimize conditions for a variety of processes. RSM was adopted to determine the optimal light intensity and temperature to pursue the maximal growth rate of this species, and initial nitrogen level and duration of nitrogen depletion for its maximal lipid biosynthesis. The optimal conditions were then applied to fed-batch and semi-continuous cultivations to examine the maximal lipid productivity potential of *Desmodesmus* sp. F2. The result from the semi-continuous cultivation, 302 mg/L/d, is the highest microalgal lipid productivity ever reported to the best of our knowledge.

2. Methods

2.1. Microalga and growth conditions

The microalga *Desmodesmus* sp. F2 used in this study was isolated from tropical Taiwan (Kaohsiung City, 22°38′N, 120°16′E) (Ho et al., 2013b; Pan et al., 2011). The medium used in this research was based on a modified Bold 3 N medium which contained 4.4 mM $NaNO_3$, 0.17 mM $CaCl_2$, 0.3 mM $MgSO_4$, 0.22 mM K_2HPO_4, 0.65 mM KH_2PO_4, 0.43 mM NaCl, and the same levels of minerals and vitamins described in Berges et al. (2001). Specific light intensity, cultivation temperature and nitrogen level in each test are indicated in the results.

2.2. Batch operation of photobioreactor

The photobioreactor (PBR) was assembled using 1-L glass bottles (diameter 9.8 cm) and external light sources (T5 fluorescent lamps, 14 W, Philips Co., Taipei, Taiwan) mounted on the frame at both sides of the bottles. The microalgal stock for inoculation was maintained at mid-log phase. The inoculum size to each bottle equaled 60 mg dry cells per liter. The PBR was operated at initial pH 7.6, stir rate of 300 rpm, with CO_2 (2.5%) fed continuously at a rate of 0.2 vvm into the bottles.

2.3. Interaction of light intensity and temperature on the maximum biomass productivity

A modified on-face central composite design from Ho et al. (2012a) was applied to examine the interaction effects of light intensity and temperature on the biomass productivity. The ranges of light intensity and temperature were 300–1100 μmol/m^2/s (center point = 700 μmol/m^2/s) and 30–40 °C (center point = 35 °C). Thirteen batch experiments were carried out based on response surface methodology (RSM) design as shown in Table 1. Stepwise regression analysis was conducted by generating the following second-order polynomial equation (Eq. (1)):

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{12} X_1 X_2 \quad (1)$$

where Y is the expected response value predicted from RSM; α_i, α_j, and α_{ij} are the parameters estimated from the regression results. Biomass productivity (i.e., Y) was used to assess the interaction outcomes of light intensity (X_1) and temperature (X_2).

2.4. Interaction of initial nitrogen level and duration of nitrogen depletion on the overall lipid productivity

The experimental design in Section 2.3 was applied to investigate the interaction effects of initial nitrogen level and duration of nitrogen depletion on the lipid productivity of *Desmodesmus* sp. F2. Thirteen batch experiments were conducted in the optimal cultivation conditions (i.e., 700 μmol/m^2/s and 35 °C) as shown in Table 2. The ranges of initial nitrogen level and duration of nitrogen depletion were 4.4–8.8 mM and 4–8 days. Stepwise regression analysis was conducted by generating the following second-order polynomial equation (Eq. (2)):

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{12} X_1 X_2 \quad (2)$$

where Y is the expected response value predicted from RSM; α_i, α_j, and α_{ij} are the parameters estimated from the regression results. Lipid productivity (i.e., Y) was used to assess the interaction outcomes of initial nitrogen level (X_1) and duration of nitrogen depletion (X_2).

2.5. Fed-batch and semi-continuous operation of photobioreactor

The operation conditions for the fed-batch system were based on those for the batch cultivation except the initial nitrate level was increased to 6.6 mM. Concentrated medium stocks were fed into the cultures to replenish nitrate and other minerals (Section 2.1) at specified times indicated in the results. The semi-continuous cultivations were started with the batch mode, and a ratio of the cultures was replaced by fresh medium when the cell density reached 4 g/L dry biomass level. Six cycles of replacement were carried out in each test to investigate the stability of the semi-continuous operations.

2.6. Measurement of nitrate level

Nitrate level was determined according to an optical method (Collos et al., 1999; Ho et al., 2012b). Samples were filtered through a 0.22 μm pore size filter to remove cells and particles. The filtered medium was diluted with deionized water and its absorption at 220 nm wavelength was measured using a UV/VIS spectrophotometer (model U-2001, Hitachi, Tokyo, Japan). The nitrate level was determined by interpolating the OD_{220} reading into a standard curve.