Bioresource Technology 132 (2013) 197–201

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Chemo-enzymatic saccharification and bioethanol fermentation of lipid-extracted residual biomass of the microalga, *Dunaliella tertiolecta*

Ok Kyung Lee [a], A Leum Kim [a], Dong Ho Seong [b], Choul Gyun Lee [b], Yeon Tae Jung [c], Jin Won Lee [c], Eun Yeol Lee [a,*]

[a] *Department of Chemical Engineering, Kyung Hee University, Gyeonggi-do 446-701, Republic of Korea*
[b] *Marine Bioenergy Research Center, Department of Biological Engineering, Inha University, Incheon 402-751, Republic of Korea*
[c] *Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 121-742, Republic of Korea*

HIGHLIGHTS

- ▶ The residual biomass of *Dunaliella tertiolecta* after lipid extraction was saccharified and used for bioethanol fermentation.
- ▶ A pretreatment procedure was not required for enzymatic saccharification of the residual biomass.
- ▶ Saccharification yield based on the total amount of carbohydrates was 80.9% (w/w).
- ▶ Bioethanol was directly produced with 82% yield from the saccharification solution without additional pretreatment.
- ▶ The waste residual biomass generated during microalgal biodiesel production could be used for the production of bioethanol.

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form 29 December 2012
Accepted 4 January 2013
Available online 19 January 2013

Keywords:
Bioethanol fermentation
Dunaliella tertiolecta
Reducing sugar
Residual biomass
Saccharification

ABSTRACT

Chemo-enzymatic saccharification and bioethanol fermentation of the residual biomass of *Dunaliella tertiolecta* after lipid extraction for biodiesel production were investigated. HCl-catalyzed saccharification of the residual biomass at 121 °C for 15 min produced reducing sugars with a yield of 29.5% (w/w) based on the residual biomass dry weight. Various enzymes were evaluated for their ability to saccharify the residual biomass. Enzymatic saccharification using AMG 300L produced 21.0 mg/mL of reducing sugar with a yield of 42.0% (w/w) based on the residual biomass at pH 5.5 and 55 °C. Bioethanol was produced from the enzymatic saccharification products without additional pretreatment by *Saccharomyces cerevisiae* with yields of 0.14 g ethanol/g residual biomass and 0.44 g ethanol/g glucose produced from the residual biomass. The waste residual biomass generated during microalgal biodiesel production could be used for the production of bioethanol to improve the economic feasibility of microalgal biorefinery.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The depletion of fossil fuels has triggered the development of alternative renewable biofuels, such as bioethanol and biodiesel (Lin and Tanaka, 2006; Meher et al., 2006). Various types of biomass have been considered as feedstock for biofuel production (Demirbas, 2006). Starch and sugarcane have been used for commercial-scale production of bioethanol, i.e., first generation biomass. However, crop-based first generation bioethanol causes moral issues of using food for fuel production. Second generation bioethanol uses various cellulosic materials as feedstock. Despite intensive research on cellulosic bioethanol, commercial plants do not exist due to the difficulty and complexity of lignin pretreatment. One of the critical success factors of microbial bioethanol production from biomass is cost-competitiveness of the saccharification process. To address these technical challenges, much effort has been given to the development of cost-effective pretreatment and saccharification processes for cellulosic biomass (Nigam and Singh, 2011).

As the third generation biomass, microalgae and macroalgae have been considered as feedstock for biofuel production based on the expectation that large amounts of biomass will soon be available at an acceptable cost (John et al., 2011; Waltz, 2009). Biofuel production using algae has attracted much attention because it can be cultured using CO_2 and sunlight (Singh et al., 2011). Another advantage of microalgae and macroalgae is that they do not have lignin, allowing development of a cost-effective pretreatment process (Libessart et al., 1995; Miranda et al., 2012).

Microalgae is good feedstock for biodiesel production because it accumulates a high percentage of lipids on the basis of dry cell

* Corresponding author. Tel.: +82 31 201 3839; fax: +82 31 204 8114.
E-mail address: eunylee@khu.ac.kr (E.Y. Lee).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.007

mass (Nigam and Singh, 2011; Wahlen et al., 2011; Zou et al., 2009). Recently, we developed a large-scale cultivation method for the marine microalgae, *Dunaliella tertiolecta* LB999, with a photobioreactor using a semi-permeable membrane for the production of biodiesel (Kim and Lee, 2010).

During the production of biodiesel from *D. tertiolecta* LB999 biomass, a significant amount of residual biomass is generated as waste after lipid extraction. Biodiesel production from microalgae still suffers from high production cost. Most of the valuable cell components of microalgae need to be utilized to enhance the economic feasibility of microalgal biorefinery. The residual biomass can be used for the production of bioethanol because it has a large amount of polysaccharide. Many investigations on saccharification and bioethanol fermentation from microalgae and macroalgae biomass have been performed (Choi et al., 2010; Harun et al., 2010, 2011; Harun and Danquah, 2011; Kim et al., 2011; Lee et al., 2011; Wargacki et al., 2012). However, investigations on saccharification and bioethanol fermentation of the microalgal residual biomass after lipid extraction have not been reported. The main objective of this study is to produce bioethanol from the residual biomass of *D. tertiolecta* LP999 resulted from lipid extraction.

2. Methods

2.1. Cultivation of microalgae

The microalgae strain used in this work was *D. tertiolecta* LB999. The components of the culture medium were as follow: $NaNO_3$, 1.5 g/L; K_2HPO_4, 40 mg/L; $MgSO_4$, 75 mg/L; $CaCl_2$, 36 mg/L; $HOOC{\cdot}CH_2{\cdot}C(OH){\cdot}COOH{\cdot}CH_2{\cdot}COOH{\cdot}H_2O$, 6 mg/L; $C_6H_5O_7Fe_3H_2O$, 6 mg/L; EDTA, 1 mg/L; Na_2CO_3, 20 mg/L; $MnCl_2$, 1.8 mg/L; H_3BO_3, 2.8 mg/L; $ZnSO_4$, 2.2 mg/L; $Co(NO_3)_2$, 0.5 mg/L; $CuSO_4$, 0.8 mg/L; $Na_2MoO_4{\cdot}2H_2O$, 0.3 mg/L in artificial seawater (NaCl, 24.7 g/L; KCl, 0.66 g/L; $MgCl_2{\cdot}6H_2O$, 8.48 g/L; $CaCl_2{\cdot}2H_2O$, 1.9 g/L; $MgSO_4{\cdot}7H_2O$, 3.07 g/L; $NaHCO_3$, 0.18 g/L). *D. tertiolecta* LB999 was cultured in a 70 L plate type photobioreactor with fluorescent lighting (60 $\mu E/m^2$ s) at 20–25 °C for seven days. The culture was bubbled with air containing 2% (v/v) CO_2 (2 vvm; volume of air added to the liquid volume per minute). The initial biomass concentration was 0.3 g/L. The cells were harvested after seven-day culture. After centrifugation, we obtained the biomass concentration of 9.1 g fresh cell/L. The harvested cells were freeze-dried and used for lipid extraction.

2.2. Extraction of total lipids from microalgae biomass

The total lipids were extracted twice from the freeze-dried cells. Fifteen volumes of chloroform and methanol (1:2 (v/v)) were added to the freeze-dried biomass, and the lipid was extracted with magnetic stirring and reflux at 65 °C for 2 h or at room temperature for overnight. After lipid extraction, the residual biomass was dried at 42 °C for 2 h or at room temperature for overnight. The dried residual biomass was ground into powder using pestle and mortar. Approximately 80% of the residual biomass powder had diameter from 75 to 300 μm.

2.3. Chemicals and enzymes

Commercial cellulase (Celluclast 1.5L, Novoprime B957), amyloglucosidase (AMG 300L) and Viscozyme L were purchased from Novozymes (Denmark). Viscozyme L is a multienzyme complex containing arabanase, cellulase, β-glucanase, hemicellulase and xylanase. The enzymes activities of the Celluclast 1.5L, Novoprime B957, AMG 300L and Viscozyme L are 700 endoglucanase units (EGU)/g, 8000 high cellulase units (HCU)/g, 300 amyloglucosidase units (AGU)/mL and 100 fungal beta-glucanase units (FBG)/g, respectively.

All solvents used in this study were of analytical or reagent grade, and purchased from Sigma Co. (USA). All chemicals for making DNS reagent were purchased from Daejung Chemicals Co. (Korea). Glucose, galactose, and xylose were purchased from Sigma Co. (USA). Yeast extract, malt extract, peptone and dextrose were purchased from Merck (Germany).

2.4. Determination of the cellular composition of D. tertiolecta LB999

The lipid content of the dried biomass of *D. tertiolecta* LB999 at cell harvesting was determined using the Soxhlet method (AOAC, 2000; method 920.39). The carbohydrate content was determined using analytical methods of the National Renewable Energy Laboratory (NREL) (Sluiter, 2006). The protein content was analyzed using a micro-Kjedahl method (AOAC 2000, method 976.05). The ash content was determined by comparing the sample weight before and after heating in a furnace at 550 °C for 12 h (Kim et al. 2011).

2.5. Saccharification of residual microalgae biomass

For chemical saccharification, 5% (w/v) of the residual biomass was autoclaved at 121 °C for 15 min in the presence of HCl (0.1, 0.3, 0.5, 0.7, 1 M) or H_2SO_4 (0.05, 0.15, 0.25, 0.35, 0.5 M). Enzymatic saccharification was performed with 5% (w/v) of the residual biomass at various temperatures (35–55 °C) and pH (3.5–6.5). The reaction mixture was incubated with Celluclast 1.5L, Novoprime B957, AMG 300L, and/or Viscozyme L (Lee et al., 2011; Kim et al., 2011). Enzyme in the range of 0.1–1.0 mL/g was used based on the dry mass of the residual biomass. For the chemo-enzymatic saccharification, acid-generated hydrolysates were adjusted to a pH of 5.5 with 0.1 M sodium acetate buffer. Celluclast 1.5L, Viscozyme L, Novoprime B957, or AMG 300L was added for enzymatic saccharification. The samples were centrifuged at 7000 rpm and 4 °C for 10 min before analysis of the reducing sugars.

Batch saccharification reaction was performed in 1 L Erlenmeyer flask (at 55 °C, pH 5.5 and 0.4 mL enzyme/g residual biomass) on a rotary shaker (230 rpm). The reaction mixture of 1 mL was taken periodically and then the amount of reducing sugars was determined using DNS method for the analysis of saccharification reaction progress.

2.6. Ethanol production using enzymatic hydrolysates of the residual biomass

Saccharomyces cerevisiae YPH500 (ATCC 76626) was used for ethanol fermentation of the enzymatic saccharification products of the residual biomass (Boubekeur et al. 2001). For seed culture, *S. cerevisiae* YPH500 was cultured in 10 mL culture tubes containing 3 mL of culture medium at 30 °C and 200 rpm for 12 h. The composition of the culture medium was as follows: yeast extract, 3 g/L; malt extract, 3 g/L; peptone 5 g/L; dextrose 10 g/L (Lee et al., 2011). For a flask fermentation, 1 mL of the seed culture was inoculated to 200 mL of the enzymatic saccharification solution containing 17.5 g/L reducing sugars and 12 g/L yeast extract in a 500 mL culture flask at 30 °C and 200 rpm for 12 h. For a fermenter experiment, 10 mL of the seed culture was inoculated to the enzymatic saccharification solution (working volume: 1 L) in a 5 L fermenter with pH (6.5) and temperature (30 °C) control (Biotron, Korea). The glucose consumption and ethanol production were analyzed periodically. As a control, we conducted two independent fermentation experiments. Culture medium containing only 12 g/L yeast extract was used as the negative control experi-

Download English Version:

https://daneshyari.com/en/article/7084055

Download Persian Version:

https://daneshyari.com/article/7084055

Daneshyari.com