Fuel 197 (2017) 467–473

Contents lists available at ScienceDirect

Fuel

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

Full Length Article

Optimization of an effective method for the conversion of crude algal lipids into biodiesel

Sha Wu [a,b], Lirong Song [a], Milton Sommerfeld [c], Qiang Hu [a,c], Wei Chen [a,c,*]

[a] Key Laboratory of Algal Biology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei 430072, China
[b] University of Chinese Academy of Sciences, Beijing 100039, China
[c] Department of Applied Sciences and Mathematics, Arizona State University, 7001 E. Williams Field Road, Mesa, AZ 85212, USA

HIGHLIGHTS

- A novel transesterification method with microalgae lipids was developed and optimized.
- The conversion efficiency increased to 97% after adding co-solvent into the reaction system.
- Both algal polar lipids and neutral lipids could be converted into biodiesel.

ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 14 January 2017
Accepted 14 February 2017
Available online 25 February 2017

Keywords:
Biodiesel
Microalgae oils
Enzymatic transesterification
Lipase

ABSTRACT

Although microalgae represent a promising alternative feedstock for biofuels, the current solvent-based lipid conversion systems and processes are neither cost-effective nor energy-efficient due to the bad quality of algal crude lipids. In the present study, a novel lipase-catalyzed transesterification method with crude *Chlorella* lipids was systematically investigated and optimized. A highly efficient conversion protocol with 97% of fatty acid methyl esters (FAME) yield was obtained after the introduction of co-solvent. Then several crucial parameters including the reaction time, molar ratio of algae oils to methanol, ratio of algae oils to co-solvent, ratio of algae oils to lipase and reaction temperature and intensively studied and selected at 12 h, 1:12, 1:1 (m/v), 20:1 (m/m) and 40 °C, respectively. In addition, the reusability of the immobilized lipase was also investigated with crude algal lipids. The immobilized lipase could maintain 84% of its original activities even after five recycles. Polar lipid standards along with the polar lipids isolated from crude algal lipids were also tested and results indicated that they could also be converted into biodiesel very efficiently. This study provides an important insight into further decreasing the downstream processing costs in algae-based biofuel production.

1. Introduction

Continued use of fossil fuels is now increasingly recognized as unsustainable due to the depleting supplies and their significant contributions to the emission and accumulation of greenhouse gas carbon dioxide in the atmosphere and global climate changes [1–3]. Investigations concerning the exploration of renewable and environment-friendly energy resources have been widely carried out in the past decades [4]. In which, microalgal biomass derived biofuels have been recognized as one of the most attractive alternative biofuel sources due to its high-efficiency, low-poison, bio-degradable and renewable characters [5–8]. However, there is still a long way to scale the laboratory cultures up to the commercial production of biofuel feedstock due to the high cost from both up-stream biomass production, and the down-stream oil extraction/biofuel conversion processes as well [9].

At present, most studies associated with the development of algae-based biofuels were still focused on the up-stream processes, such as microalgal strain selection, algae cultivation, photobioreactor design and operation, prevention of contamination, biomass harvesting, etc [10–12]. Although there are some information about production of biodiesel from microalgae oil in recent years [13,14]. In some literatures, these processes were simply considered same as biodiesel production with the oil plant seeds as the feedstock, and algal oils could be easily extracted and converted into biodiesel by traditional chemical catalyzed transesterification protocols [14–16]. Also, there are several other articles reporting the conversion process by using the conventional hydrothermal

* Corresponding author at: Key Laboratory of Algal Biology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei 430072, China.
E-mail address: chenwei@ihb.ac.cn (W. Chen).

http://dx.doi.org/10.1016/j.fuel.2017.02.040
0016-2361/

treatment process derived from petrochemical industries [17,18]. However, the current downstream hydrothermal and solvent-based lipid extraction and conversion systems and processes are energy intensive [19]. The traditional protocol for biodiesel production is alkaline-catalyzed transesterification. It requires high-quality oils as the feedstock (usually refined oils with very low acid values and low-concentration of polar lipids) even having advantages of less reaction time and high biodiesel yields [20–22]. Unfortunately, crude microalgae oils contain high-concentration of free fatty acids and polar lipid contaminations up to 70–80% of total lipid content [6]. Prior to the alkaline catalyzed transesterification process, the complicated unknown refining protocols will be needed to obtain the high quality oil feedstock. This process will unavoidably lead to the significant enhancement of energy consumptions and total cost of algae-based biofuels [23,24]. In addition, the alkaline catalyzed transesterification reaction will also unavoidably lead to oxidation of high valued products, such as astaxanthin, lutein, polyunsaturated fatty acids, etc, and generation of large amount of alkaline wastewater containing both catalyst and glycerol [25–27]. Thus, it is essential to develop the conversation technologies for algae-based biofuel productions.

In recent studies, immobilized lipase have been employed as a potential efficient and low-cost method to convert higher plant oils to biodiesel due to its mild reaction conditions and the simple recycle protocols of its immobilized biocatalysts [28,29]. The purpose of this study was to test the possibility of using lipase catalyzed method in the conversion of bad-quality crude algae oils and optimization of reaction conditions, and finally establish a cost-effective protocol for converting algal oils into biodiesel.

2. Materials and methods

2.1. Organisms and culture conditions

The algae strain of *Chlorella vulgaris* was obtained from Freshwater Algae Culture Collection at the Institute of Hydrobiology, FACHB-collection. *Chlorella vulgaris* was maintained in BG-11 growth medium in a panel photo-bioreactor at 25 °C, and exposed to a continuous fluorescent lamp illumination at a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Culture mixing was provided by bubbling air containing 1% CO_2 (v/v).

2.2. Chemicals and reagents

Lipases, methyl ester standards and lipid standards were purchased from Sigma (USA). Silica gel plates used for thin layer chromatography (TLC) was obtained from Merck (Germany). Active silica gel for column chromatography was obtained from ICN Biomedicals GmbH (Germany). All other chemicals were of analytical grade and purchased from standard sources.

2.3. Oil extraction

The crude algal oils were extracted from dry *Chlorella vulgaris* biomass with two solvent systems containing methanol and DMSO, and hexane and ethyl ether, respectively, according to our previous publications [30]. The neutral lipids and polar lipids were isolated from crude algal oil with a column chromatography according to the method described by Chen et al. [31].

2.4. Chemical- and lipase- catalyzed transesterifications of crude algal lipids

For chemical-catalyzed transesterification, 50 mg crude algal oils and 5 mg heptadecanoic acid (as internal standard) were sampled into 8 ml glass vial with a PTFE septa, then 2 mL methanol containing 2% H_2SO_4 (m/m) was added into the vials and mixed thoroughly. The vials were then maintained at 80 °C for 2.5 h, oscillating the vial every fifteen minutes during the reaction. After transesterification reactions, 2 mL of saturated sodium chloride solution and 2 mL of hexane were added and mixed with a vortex mixer. The hexane layer which contained fatty acid methyl-esters (FAMEs) was carefully separated and then solvents were evaporated with N_2 stream [32]. Finally, FAMEs were re-dissolved in 1 mL hexane for further thin-layer chromatography (TLC) analysis. For GC–MS analysis, the above samples were diluted fifty time to obtain the proper concentration for mass detector.

The lipase catalyzed transesterification reactions were also carried out in 8 ml glass vial with PTFE septa in a thermostat shaking bed at a speed of 250 rpm. The reaction mixtures consisted of 50 mg crude algal oils and different dosage of lipases, solvents and methanol. After reaction, the samples were centrifuged and determined by TLC method [32]. Then 10 μL of the supernatant, 50 μL of methyl heptadecanoate standard (1.05 mg/mL) and 440 μL hexane were mixed thoroughly for further fatty acid analysis with GC–MS. The FAME conversion efficiency is calculated based on comparing the peak areas between lipase- and chemical-catalyzed transesterification. The FAME conversion efficiency can be calculated by the following equation:

$$\text{FAME conversion efficiency}(\%) = \text{Y(lipase)}/\text{Y(chemical)} \times 100$$

where Y (lipase) is the yield of FAME generated by lipase catalyzed transesterification, and Y(chemical) is the yield of FAME generated by chemical catalyzed transesterification with the same amount of microalgae oil.

For TLC analysis, samples and standards were loaded onto the silica gel and developed in a solvent mixture of hexane, diethyl-ether, and acetic acid (80:20:2, v/v/v). The developed TLC plate was then visualized by spraying a visualization reagents (a mixture containing phosphoric acid, 33% acetic acid, sulfuric acid and 0.5% copper sulfate acid (5:5:0.5:90, v/v/v/v)) and heated at 180 °C for 7 min [32].

For GC–MS analysis, a polar capillary column (HP-88, 60 m) was used. The oven temperature program consisted of an initial hold at 100 °C for 5 min, increasing to 240 °C at 3.5 $min^{-1,}$ and a final hold at 240 °C for 5 min. The injector temperature was kept at 260 °C, the flow rate of carrier gas (helium) was 1.5 $mL \cdot min^{-1}$ and the injection volume was 1 μL [32].

2.5. Optimization of different parameters of lipase-catalyzed transesterification

Biodiesel was produced from crude microalgae lipids through lipase-catalyzed transesterification. To obtain the optimal reaction condition, the following seven parameters were investigated systematically through single-factor experiment: (i) co-solvent, (ii) lipases, (iii) molar ratio of methanol to oil, (iv) ratio of co-solvent to oil, (v) ratio of lipase to oil, (vi) reaction temperature and (vii) reaction time. The effect of each factor was assessed by varying one of the above parameters while keeping the other constant. The co-solvent was screened from the following 11 kinds of solvents: hexane, acetone, ether, dichloromethane, 1-butanol, t-butanol, isopropanol, tetrahydrofuran, pyridine, ethyl acetate, MTBE. The lipase was selected from five kinds of immobilized listed as follows and their combinations cocktail: Lipase, immobilized on immobead 150 *Candida rugosa*; Lipase, immobilized on immobead 150 from *Pseudomonas cepacia*; Lipase acrylic resin from *Candida antarctica*; Lipase, immobilized on immobead 150 *Thermomyces lanuginosus*; Lipozyme, immobilized from *Mucor miehei*. The molar ratio of methanol to oil used were 3:1, 6:1, 9:1, 12:1, 18:1, 24:1, 30:1 (M/M). The ratio of co-solvent to oil tested were