Energy Conversion and Management 158 (2018) 168–175

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Enzymatic production of biodiesel from insect fat using methyl acetate as an acyl acceptor: Optimization by using response surface methodology

Hoang Chinh Nguyen[a], Shih-Hsiang Liang[b], Shang-Sian Chen[c], Chia-Hung Su[c,*], Jhih-Huei Lin[c], Chien-Chung Chien[c]

[a] Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City 700000, Vietnam
[b] Hsinchu Branch, Livestock Research Institute, Council of Agriculture, Miaoli County 36841, Taiwan
[c] Graduate School of Biochemical Engineering, Ming Chi University of Technology, New Taipei City 24301, Taiwan

ARTICLE INFO

Keywords:
Insect fat
Interesterification
Lipase
Methyl acetate
Response surface methodology

ABSTRACT

Black soldier fly larvae (BSFL) are oleaginous insects that can assimilate organic waste for fat accumulation, and thus serve as an alternative feedstock for biodiesel production. In lipase-catalyzed transesterification, enzymes are deactivated by excess methanol. To address this obstacle, methyl acetate is suggested as an alternative acyl acceptor to methanol. In this study, methyl acetate was first used in the enzymatic production of biodiesel with BSFL as a triglyceride source. The interesterification of BSFL fat with methyl acetate was catalyzed using Novozym 435 as an efficient immobilized lipase. Response surface methodology was used to optimize the reaction and establish a reliable mathematical model for prediction. A maximum biodiesel yield of 96.97% was reached at a reaction time of 12 h, molar ratio of methyl acetate to fat of 14.64: 1, enzyme loading of 17.58%, and temperature of 39.5 °C. Under these optimal reaction conditions, Novozym 435 could be reused for up to 20 cycles without loss in enzyme activity. The properties of BSFL biodiesel were also investigated and all met the European standard EN 14214. This study indicates that the enzymatic interesterification of BSFL fat with methyl acetate is a promising and ecofriendly method for green fuel production.

1. Introduction

With rapidly increasing demands for energy and environmental protection, biodiesel has been increasingly developed worldwide as a green fuel to replace petroleum [1,2] because of its combustion efficiency, renewability, reduced environmental footprint, and compatibility with diesel engines without modification [3–5]. However, a major drawback of biodiesel compared with petrodiesel is its high price due to the high cost of feedstocks, which account for 75% of the production cost [6,7]. Therefore, developing a novel and inexpensive feedstock for biodiesel production is an urgent requirement.

Insects have attracted much attention as a feedstock source for producing biodiesel because of their high fat content, short life cycles, and high reproduction rates [8–10]. In particular, black soldier fly larvae (BSFL; *Hermetia illucens*) have been reported as a promising biodiesel feedstock [10,11]. These insects can degrade various organic wastes derived from animals and plants, such as animal manure [10,12], restaurant waste [13], and lignocellulosic biomass [14] to accumulate high fat content, which is subsequently used for biodiesel production [11,15]. The fuel properties of the resulting biodiesel were shown to meet the specifications of the European biodiesel standard EN 14214 [11]. In addition, the cell debris after fat extraction can be used as a high-protein feed for cultivating aquatic animals, poultry, and livestock [10,15]. Consequently, this insect has been increasingly used as feedstock for biodiesel production to reduce production costs and facilitate the green conversion of waste into energy.

In the conventional process of using BSFL for biodiesel production, larval fat is reacted with an acyl acceptor using sulfuric acid and sodium hydroxide as catalysts [12,15]. The most common acyl acceptors for biodiesel production are methanol and ethanol because of their availability and low price [12,16]. Although biodiesel can be successfully produced using a chemically catalyzed process, several associated problems exist, including corrosion damage to equipment and the complicated separation of saponified products and catalysts from biodiesel [11,17,18]. The use of supercritical alcohols in the transesterification of oil for biodiesel production addresses these problems; however, this process operates at extreme pressures (20–43 Mpa) and temperatures (350–400 °C), resulting in product degradation [19–21]. Lipase-catalyzed reactions were proven to be a promising method for biodiesel production to resolve the aforementioned limitations [11].

---

* Corresponding author.
E-mail addresses: nguyenhoangchinh@tdt.edu.vn (H.C. Nguyen), chsu@mail.mcut.edu.tw (C.-H. Su).

https://doi.org/10.1016/j.enconman.2017.12.068
Received 20 October 2017; Received in revised form 13 December 2017; Accepted 20 December 2017

Nguyen et al. [11] successfully produced biodiesel through the transesterification of BSFL fat with methanol using immobilized lipase under mild reaction conditions. The lipase-catalyzed process is energy saving and ecofriendly [22–24], and thus decreases environmental damage. Nevertheless, lipases are deactivated by high dosages of methanol or ethanol [11]. To overcome this obstacle, several solutions have been proposed, such as the use of solvents as diluents or the stepwise addition of alcohol [25,26]. However, these methods decrease the reaction rate because they maintain low concentrations of alcohol in the reaction mixture [27,28] and thus limit the industrial application of enzymatic processes for biodiesel production.

Methyl acetate, a novel acyl acceptor, has been developed for biodiesel production to overcome the problems associated with enzymatic transesterification using methanol [29,30]. The transesterification of triglyceride using methyl acetate is also known as interesterification or an ester exchange reaction, which involves three consecutive reversible reactions to convert triglycerides into biodiesel and triacetin [31,32]. High dosages of methyl acetate in the reaction have been shown to have no adverse effects on enzyme activity and stability, which are the key concerns in the enzymatic process, thus enhancing the reaction rate [33–35]. Du et al. [36] obtained a higher biodiesel yield by using methyl acetate compared with methanol, and lipase could be used continually for 100 cycles without loss of enzyme activity. In addition, triacetin, a byproduct of this process, is used as an additive in the tobacco, cosmetic, and pharmaceutical industries [31]. Studies have shown that triacetin can be added to the formulation of biodiesel at up to 10% to improve certain biodiesel properties [31,37,38]. Because of these merits, this method has been employed in biodiesel production using olive oil [38], soybean oil [36], sunflower oil [39], and waste cooking oil [35]. However, no study has reported the use of methyl acetate for biodiesel production from insect fat.

This study examined the lipase-catalyzed interesterification of BSFL fat using methyl acetate as an acyl acceptor for biodiesel production. Response surface methodology (RSM) was used to optimize the reaction conditions by analyzing the effects of reaction factors (reaction time, molar ratio of methyl acetate to fat, enzyme loading, and temperature) on the biodiesel yield. The reusability of the enzyme was also investigated under optimal reaction conditions. Finally, the properties of BSFL biodiesel were determined according to the American Society for Testing and Materials (ASTM) method.

2. Materials and methods

2.1. Materials

Novozym 435 (*Candida antarctica* lipase) was purchased from Novozymes A/S (Bagsvaerd, Denmark). Methanol (HPLC grade, Tedia, USA), n-hexane (HPLC grade, Tedia, USA), methyl acetate (HPLC grade), and other reagents were purchased from ECHO Chemical Co. Ltd. (Miaoli, Taiwan).

2.2. Insect species and growth conditions

BSFL (*Hermetia illucens*) were obtained from the Livestock Research Institute (Hsinchu Branch, Miaoli County, Taiwan) and maintained for more than 10 generations before being used in this study. To produce biomass, the larvae were inoculated into fermented wheat bran at a ratio of 1200 larvae per kilogram of substrate and incubated at 30 °C with 65% moisture. After 20 days, the larvae were harvested from residue and inactivated at 105 °C for 10 min. They were subsequently dried at 60 °C for 2 days and stored at 4 °C until use.

2.3. Fat extraction from BSFL

The BSFL were ground with a RT-02B micromill (Rong-Tsong Precision Technology Co., Taiwan) to produce BSFL powder. The BSFL powder was then immersed in n-hexane at a ratio of 1:5 (w/v) for 48 h at room temperature. The hexane phase containing crude fat was separated from cell debris through filtration. Finally, the crude fat was obtained by evaporating hexane using a rotary evaporator (N-1200, Eyela, Tokyo, Japan). The acid, iodine, and saponification values of crude fat were determined according to the standard method [40,41].

2.4. Effect of various acyl acceptors on biodiesel production

A comparative study was conducted on the enzyme-catalyzed transesterification of BSFL fat with methanol and interesterification with methyl acetate to investigate the effects of the acyl acceptor types (methanol and methyl acetate) on biodiesel yield. The reaction was initiated by adding 4% Novozym 435 into reaction mixtures containing the acyl acceptors and BSFL fat at various molar ratios (from 1:1 to 12:1). The reaction was then maintained at 40 °C with shaking for 12 h. After the reaction was completed, the sample was collected, washed 3 times with deionized water, and subsequently centrifuged to remove the aqueous layer [42]. The upper layer was collected and subjected to rotary evaporation (N-1200, Eyela, Tokyo, Japan) to recover the residual methyl acetate, after which biodiesel was obtained and used to determine the biodiesel yield.

2.5. Optimization of interesterification using RSM

A three-level and four-factorial Box–Behnken design was employed to investigate the effects of reaction factors on the biodiesel yield. Interesterification reactions with various reaction times (4–12 h), molar ratios of methyl acetate to fat (9:1–15:1), enzyme loadings (10%–20%), and reaction temperatures (30–50 °C) were performed in 150-mL Erlenmeyer flasks with vigorous shaking. The biodiesel yield was determined from the sample withdrawn from the reaction mixtures. The relationship between the determined biodiesel yield and reaction factors was established using the following quadratic equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \quad (1)$$

where Y is the biodiesel yield; X_1 is the reaction time; X_2 is the molar ratio of methyl acetate to fat; X_3 is the enzyme loading; X_4 is the reaction temperature; β_0 is the regression coefficient for the intercept term; β_1-β_4 are linear parameters; β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, and β_{34} are interaction parameters; and β_{11}, β_{22}, β_{33}, and β_{44} are quadratic parameters. The model parameters were determined using the least-squares method [43]. A mathematical model was then used to determine the optimal reaction conditions for obtaining the maximal biodiesel yield using a canonical method [43]. Minitab 16 (Minitab Inc., State College, PA, USA) was used to establish the empirical model, conduct an analysis of variance (ANOVA), and determine the optimal reaction conditions.

2.6. Reusability of the enzyme

Novozym 435 was reused in interesterification with methyl acetate and its stability was compared with that in the transesterification with methanol reported in our previous study [11]. The reaction was carried out under the optimal conditions determined using RSM. After the reaction was completed, Novozym 435 was removed from the reaction mixture through filtration. The recovered catalyst was then remixed with fresh reactants to initiate a new reaction. In each reaction cycle, the sample was withdrawn to determine the biodiesel yield.

2.7. Analysis

The biodiesel composition was determined using a gas