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## Direct transesterification of black soldier fly larvae (*Hermetia illucens*) for biodiesel production

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### ABSTRACT

In this study, direct transesterification with a combination of methanol and a cosolvent was demonstrated to be promising for the production of biodiesel from black soldier fly larvae (BSFL) biomass. Of the solvents tested, n-hexane was identified as the most effective cosolvent for the reaction, resulting in a 14.5-fold increase in the biodiesel yield, compared with the reaction without a cosolvent. The direct transesterification using n-hexane as a cosolvent was then optimized to maximize the biodiesel yield. The highest biodiesel yield of 94.14% was achieved at an n-hexane:methanol volume ratio of 1:2 (v/v), a solvent dosage of 12 mL, a catalyst loading of 1.2 mL, a temperature of 120 °C, and a reaction time of 90 min. The properties of the BSFL biodiesel were also tested, and most—such as the biodiesel's density (875 kg/m<sup>3</sup>), water content (0.03 mg/kg), ester content (98.3%), acid value (<0.8 mg KOH/g), viscosity (5.2 mm<sup>2</sup>/s), flash point (121 °C), and cetane index (50)—met the specifications of the European standard EN 14214. This study suggested that direct transesterification using n-hexane as a cosolvent could be a promising method for biodiesel production from BSFL and decrease production costs.

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### 1. Introduction

Black soldier fly larvae (BSFL; *Hermetia illucens*) are increasingly considered a promising biodiesel feedstock because of their high fat content, rapid reproduction rate, and short life cycle [1–3]. These insects consume various waste materials such as animal manure [4], kitchen waste [5], and lignocellulosic biomass [1,6], and efficiently convert organic waste into biomass that is rich in protein and fat [7,8]. Fat can be extracted from the biomass and utilized for biodiesel production [1,6]; moreover, the remaining debris after fat extraction can be used as a protein source for aquatic animals, poultry, and livestock [3,4]. Because of these advantages, BSFL are economically viable for widespread application.

Conventional biodiesel production from BSFL comprises several steps: biomass drying, crushing, fat extraction, refinement, and transesterification [1,4,5]. However, the extraction of oil from oleaginous biomass and subsequent oil refinement are costly [9], consume high amounts of energy [10], and result in low biodiesel

yield [11], thereby limiting the competitiveness of the process [12]. To address these obstacles, direct transesterification has been proposed as a method for producing biodiesel in fewer steps [13–15]. In this method, methanol is simultaneously used as the solvent to extract oil from the cell and as the reactant to transesterify the extracted oil into fatty acid methyl esters [16–18]. To enhance the efficiency of oil extraction, high volumes of methanol have been used to overcome the mass transfer difficulties [17,19,20]. In accordance with Ehimen et al. [21], various molar ratios of methanol to oil between 105:1 and 524:1 have been used for the direct transesterification of *Chlorella microalgae*. However, excess methanol weakens the catalyst, which serves as both a catalyst for the reaction and the cell wall disruptor agent, thereby reducing the biodiesel yield [22,23].

Recently, the use of cosolvents such as n-hexane, n-pentane, chloroform, acetone, and petroleum ether mixed with methanol for direct transesterification has been developed to reduce methanol usage [24–27]. These solvents have exhibited efficiency for extracting oil because of their capability for dissolving long-chain triglycerides [24]. Several studies have revealed that this process produces higher biodiesel yields, requires less solvent consumption and time than do conventional methods, and prevents oil loss during the extraction process [28–30], thereby likely reducing

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biodiesel production costs. This method has been successfully employed for several oil-bearing materials such as microalgae [24,30], fungus [29], and oilseeds from plants [31–33]. However, direct transesterification has not yet been used to produce biodiesel from insects.

In this study, the direct transesterification of BSFL biomass through the use of a cosolvent was investigated with the objective of reducing the energy consumption of the fat extraction process and improving the biodiesel conversion yields. To prevent the saponification reaction caused by an alkali catalyst and enhance the biodiesel yield, sulfuric acid was used as a catalyst for the reaction because it converts triglycerides and free fatty acids into biodiesel [16,21,34]. The effects of the reaction factors (*i.e.*, solvent-methanol volume ratio, solvent dosage, catalyst loading, temperature, and reaction time) on the biodiesel yield were investigated and the fuel properties of the synthesized biodiesel were determined using the methods of the American Society for Testing and Materials (ASTM).

## 2. Materials and methods

### 2.1. Materials

Methanol, n-hexane, chloroform, petroleum ether, acetone, sulfuric acid, and other reagents were obtained from ECHO Chemical Co. Ltd. (Miaoli, Taiwan). All chemicals were analytical grade ( $\geq 99.0\%$  purity).

### 2.2. Insect species and growth conditions

BSFL obtained from the Livestock Research Institute (Hsinchu Branch, Miaoli County, Taiwan) were maintained for more than 10 generations before being used in this study. To produce BSFL biomass, the larvae were inoculated into fermented wheat bran (1200 larvae per kg of substrate) and incubated at 30 °C and 65% relative humidity. After 20 days, the BSFL were harvested from the residue and washed using distilled water. These larvae were then inactivated at 105 °C for 10 min, dried at 60 °C for 2 days, and stored at 4 °C until used in experiments. To investigate the fat content of BSFL, the dried BSFL was extracted using n-hexane according to the method reported by Nguyen et al. [1]. The fat yield extracted from BSFL was 30.2%, indicating a high fat content in BSFL.

### 2.3. Direct transesterification of BSFL biomass

BSFL were ground using an RT-02B blender (Rong-Tsong Precision Technology Co., Taiwan) to reduce the average particle size to less than 0.25 mm. Direct transesterification was initiated by mixing methanol, cosolvent, and sulfuric acid with the fine BSFL powder in a sealed reactor. The dosage of BSFL powder was constant (2 g) to avoid variation in the stirring power per unit quality [35]. To investigate the effect of solvent type (acetone, chloroform, petroleum ether, or n-hexane) on the biodiesel yield, direct transesterification was performed by incubating 2 g of BSFL powder with 1.2 mL of sulfuric acid and 14.8 mL of mixed solvent (solvent:methanol volume ratio of 1.17:1) at 90 °C for 60 min with stirring. After the reaction, the reactor was cooled to room temperature. Subsequently, 6 mL of n-hexane and 6 mL of deionized water were added to the reaction mixture for phase separation. The residue of BSFL and sulfuric acid was located in the water phase, whereas the upper layer (n-hexane phase) contained fatty acid methyl ester, which was collected for determination of the biodiesel yield.

After the optimal cosolvent was determined, a series of experiments using different cosolvent:methanol volume ratios (1:3 to 3:1, v/v), solvent dosages (8–16 mL), catalyst loadings (0.4–2.0 mL),

temperatures (90–130 °C), and reaction times (30–120 min) were performed to study the effect of the reaction conditions on the biodiesel production. After a reaction was completed, the sample was prepared as described for biodiesel yield determination. All experiments were conducted in triplicate to ensure the reproducibility of results.

### 2.4. Analysis

A gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector and a capillary column (Stabilwax, Restek, USA) were used to determine the fatty acid composition and biodiesel yield. The carrier gas used was nitrogen at 29 mL/min. The injector and detector were maintained at 220 and 250 °C, respectively. The column temperature was set at 140 °C for 5 min, then programmed to heat to 240 °C at a rate of 4 °C/min, after which it was maintained at 240 °C for 15 min. Methyl pentadecanate was used as the internal standard to quantify the amount of biodiesel. Fatty acid composition of BSFL biodiesel was identified from reference standards (Supelco 37, Sigma-Aldrich, USA). The BSFL biodiesel yield is defined as the percentage of biodiesel actually produced versus the maximum biodiesel that could be produced from the BSFL fat. The maximum biodiesel yield was determined using the procedure previously reported [5].

The synthesized biodiesel was purified according to the method reported by Uliana et al. [36] to remove all unreacted reactants before determining the biodiesel properties. Biodiesel properties such as density, water content, sulfur content, ester content, acid value, viscosity, flash point, and cetane index were determined according to the ASTM standard methods.

## 3. Results and discussion

### 3.1. Effect of cosolvent on biodiesel yield

Various solvents—chloroform, n-hexane, acetone, and petroleum ether—were individually mixed with methanol at a volume ratio of 1.17:1 (v/v) and their effects on the direct transesterification of BSFL biomass were evaluated. As illustrated in Fig. 1, the use of no cosolvent resulted in an extremely low biodiesel yield (4.73%). This can be attributed to low oil solubility in methanol, thus limiting the mass transfer that could occur during the reaction [32]. Several studies have suggested that the use of a cosolvent can improve the biodiesel yield because the cosolvent is not only capable of dissolving oil but is also miscible with short-chain alcohols, thus resulting in homogeneous catalysis [35]. Among the solvents tested, n-hexane resulted in the highest yield of 63.37%, followed by acetone (54.83%), chloroform (48.50%), and petroleum ether (35.67%). Therefore, n-hexane was discovered to be the optimal cosolvent for the extraction of fat and production of biodiesel from BSFL biomass. Additionally, n-hexane is eco-friendly and relatively cheap compared with other solvents [24]; therefore, it was selected for further study and reaction condition optimization.

### 3.2. Effects of reaction factors on biodiesel yield

The effects of the n-hexane:methanol volume ratio (v/v), solvent dosage, catalyst loading, temperature, and reaction time on biodiesel yield were investigated (Table 1). A higher biodiesel yield was obtained when lower volume ratios of n-hexane to methanol were used. Lower n-hexane:methanol volume ratios had a positive effect on the reaction because the higher methanol content in the reaction solution leads to a higher molar ratio of methanol to fat, thus producing a higher reaction yield [5,24,37]. In this study, the n-hexane:methanol volume ratios of 1:2 (v/v) was selected and kept constant for further study to evaluate the effects of the mixed

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