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Transesterification synthesis of high-yield biodiesel from black soldier fly larvae by using the combination of Lipase Eversa Transform 2.0 and Lipase SMG1

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Abstract

The larvae of black soldier fly (BSFL) are rich in lipids, and in the current study, BSFL was used to produce biodiesel by enzymatic transesterification with methanol. Biodiesel obtained by free lipase-catalyzed transesterification is always with side-reactions. To address the limitation of free lipase, for the first time, Lipase SMG1 and Lipase Eversa Transform 2.0 were combined to use as catalysts at the same time. The effects of different reaction conditions on the yield of biodiesel were investigated, including the type of enzyme, reaction temperature, and molar ratio of BSFL to methanol. The molar ratio of BSFL to methanol 1:3, one-step addition of methanol at 25 °C were selected as optimal conditions. The fatty acid methyl ester content achieved to 98.45% after 8h reaction under the optimal conditions. Moreover, the main properties of the final biodiesel indicators met the EN14214 biodiesel standard.

Keywords: enzyme; black soldier fly larvae; biodiesel.

Practical Application: Lipase SMG1 and Lipase Eversa Transform 2.0 were combined to prepare biodiesel from black soldier fly larvae, which reduced the restrictions of free lipase.

1 Introduction

Considering the environmental threats associated with the excessive use of fossil fuels (Yin et al., 2020), it is crucial to explore alternative renewable energy sources. In recent years, biodiesel, produced from renewable sources such as vegetable oils, has attracted increasing attention as an alternative to traditional petroleum-based diesel. The main component of biodiesel is fatty acid methyl ester (FAME), which can be obtained from the transesterification of oils and alcohols (Bhatia et al., 2020; Costa et al., 2019). The commonly used feedstocks for biodiesel production include edible and non-edible oils which contain a large scale of triacylglycerols (TAG) (Knothe & Razon, 2017). However, using edible oils in biodiesel production is costly and may compete with food supply, thus rendering biodiesel less competitive in the fuel market (Nguyen et al., 2017). Using non-edible oils and waste cooking oils can largely reduce the cost in biodiesel production, but these oils usually require additional processing steps. Therefore, it is necessary to explore a new and cost-effective source of oils for biodiesel production to reduce the cost and simplify the process (Surendra et al., 2016).

The black soldier fly is a bi-winged insect based of the soldier fly family. Unlike other insects, the black soldier fly is not a pest and can survive in a variety of complex environments such as kitchen waste and poultry manure with a short growth cycle (Somroo et al., 2019). More importantly, it can inactivate pathogens and convert a large amount of low-value organic waste to high-value biomass, which makes them quite popular in many areas like waste-reducing (Singh & Kumari, 2019). The larvae of black soldier fly (BSFL) are rich of oils and proteins, containing 35-40% and 40-44% of lipids and crude proteins, respectively. The extracted lipids from BSFL are mainly composed of saturated fatty acids (SFFA) and have been used as in the production of biodiesel (Wang et al., 2017a; Cutrignelli et al., 2018; Ewald et al., 2020). Therefore, lipids extracted from BSFL represent a potential source for biodiesel production.

Compared with chemical catalysts, enzymes require a mild reaction environment and be able to display specific selectivity during transesterification and thus can produce biodiesel with limited by-products in an environmentally friendly way (Moazeni et al., 2019). The type of catalysts plays a crucial role in the yield of biodiesel (Martínez-Corona et al., 2020). There are two main types of enzymes used for transesterification: immobilized lipase and free lipase. The immobilized lipase has many advantages including reusability, stability, and high yield etc. (Wang et al., 2017b; Cardoso et al., 2021). For instance, Talukder et al. (2011) used Novozym 435 as catalyst for transesterification of palm oil and the yield of biodiesel reached up to 95-96% after 8 h of reaction. However, the commercial immobilized lipase usually costs a lot, while free lipase costs much lower than it. For instance, the price of immobilized lipase like Novozyme 435, is 2286.00 $/kg while the cost of Lipase Eversa Transform 2.0 is 150.00 $/kg only (Sun et al., 2021). But, the disadvantages of free lipase are also obvious including higher

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acid value, longer reaction time and higher content of unpleasant by-product. Thus, molecular distillation will be used to purify biodiesel, which means the cost will be more expensive and the process is complicated (Lv et al., 2021). Therefore, the catalysts and the process of biodiesel prepared still need to be improved with the reduction of cost and the increase of yield. Generally, monoacylglycerol (MAG) and diacylglycerol (DAG) lipases are used to remove partial glycerides from glyceride mixtures (Li et al., 2015). Many efforts had been done in the catalytic ability of Lipase SMG1, and it's the first time Lipase SMG1 was used in the production of biodiesel to obtain lower content of fatty acid (FFA), DAG, and MAG in the final system.

The aim of this study, therefore, is to develop a cost-effective and simple approach to produce a high yield of biodiesel using BSFL lipids and enzymes. The effect of enzyme types, molar ratio of BSFL lipids to methanol, and reaction temperature on biodiesel production were investigated. The properties of the final biodiesel products were further compared with literature and the EN14214 biodiesel standard.

2 Materials and methods

2.1 Materials

The BSFL lipids, a by-product from black soldier fly defatted meal factory, were kindly supplied by Guangzhou Fishtech Technology Co. Ltd. (Guangdong, PR China). Briefly, ground larval biomass was placed into a filter bag and soaked in petroleum ether for 48 h twice at room temperature. Lipase Eversa Transform 2.0, was purchased from Novozymes (Tianjin, China). Lipase SMG1 (free lipase) was produced according to the method of Xu et al. (2012). Lipase G50 (free lipase) was purchased from Amano Enzyme (Japan). Methanol (Analytically pure grade) was used as an acyl acceptor, other chemicals (n-hexane, iso-propanol et al.) were all HPLC grade. All chemicals were obtained from Sigma Aldrich (Shanghai, China).

2.2 Preparation of biodiesel by enzyme-catalyzed transesterification

Lipase Eversa Transform 2.0 alone or in combination with lipases SMG1 (or Lipase G50) were used as enzyme catalysts for transesterification of BSFL lipids and their efficiencies in the production of biodiesel were compared. The effects of molar ratio of BSFL lipids to methanol (1:2, 1:3, 1:4, 1:5) and reaction temperature (20, 25, 30, 35 °C) on the yield of biodiesel were investigated, respectively. Samples were collected periodically (every 2 h) till 10 h of reaction and were analyzed by high-performance liquid chromatography (HPLC).

2.3 Analysis of the composition of the reaction mixtures by HPLC

The analysis of the composition of the reaction mixtures was carried out using a normal-phase HPLC equipped with a refractive index detector and a Phenomenex Luna column (250 mM × 4.6 mM i.d., 5 μm particle size) according to the method previously described by Li et al. (2015) with a minor modification. The mobile phase consisted of n-hexane, iso-propanol and formic acid (21:1:0.003, by vol) with a flow rate of 1 mL/min. Peaks in HPLC were evaluated by comparison of their retention times with those known standards.

The yield of FAME was calculated follows the Equation 1:

$$FAME\ content\ (\%) = \frac{\mathrm{FAME}(\%)}{\mathrm{TAG}(\%)+\mathrm{DAG}(\%)+\mathrm{MAG}(\%)+\mathrm{FFA}(\%)+\mathrm{FAME}(\%)} \times 100\% \quad (1)$$

2.4 Analysis of fatty acid composition by GC

The final biodiesel was initially methylated to FAME according to the method described by Wang et al. (2010). Then, FFA composition of the final FAME product was determined using GC (Agilent 7890A) equipped with a capillary column CP-Sil 88 (60 mM × 0.25 mM,0.2 μm). The analysis was carried out according to the method described by Qin et al. (2011).

2.5 Analysis density, viscosity, flash point and acid value of biodiesel

The density, viscosity, flash point, and acid value of the final biodiesel product were all tested according to the methods of the American Society for Testing and Materials (ASTM).

2.6 Statistical analysis

All experiments were carried in triplicate. The results were presented as the means ± standard deviations (SD). The statistical significance of differences was measured by a one-way analysis of variance (ANOVA).

3 Results and discussion

3.1 The effect of enzyme type on the yield of biodiesel

Lipase Eversa Transform 2.0 alone or in combination with Lipase SMG1 (or Lipase G50) were used as catalysts for transesterification. As illustrated in Figure 1, the yield of FAME reached up to 66.74% when Lipase Eversa Transform 2.0 alone was used as a catalyst, and even didn't reach a reaction equilibrium after 8h. The low-quality of BSFL may make this phenomenon occurred, and the raw materials may have phospholipids and unpurified contents. Li et al. (2014) had reported that when the content of phospholipids exceeds 5%, the reaction rate will be decreased. However, the FAME yield increased dramatically when using the combination of Lipase Eversa Transform 2.0 and Lipase SMG1/LipaseG50 as the catalysts.

A yield of 98.45% was obtained in the presence of both Lipase Eversa Transform 2.0 and Lipase SMG1.Figure 2 illustrated that after the transesterification, the content of TAG (1.02%), DAG (0.26%), MAG (0.10%), and FFA (0.17%) occupied less than that of the raw materials, and there was a high yield of FAME (98.45%). Lipase Eversa Transform 2.0 is a TAG lipase that catalyzes TAG hydrolysis only (Remonatto et al., 2018) and Lipase SMG1 and Lipase G50 are partial glyceride lipases that have catalytic activity towards DAG and monoacylglycerol (MAG) according to Xu et al. (2012) and Zheng et al. (2014). Particularly, the Lipase SMG1 showed well catalyzed ability between FFA and methanol which has positive effect in increasing the extent of reaction and decreasing the acid value obviously

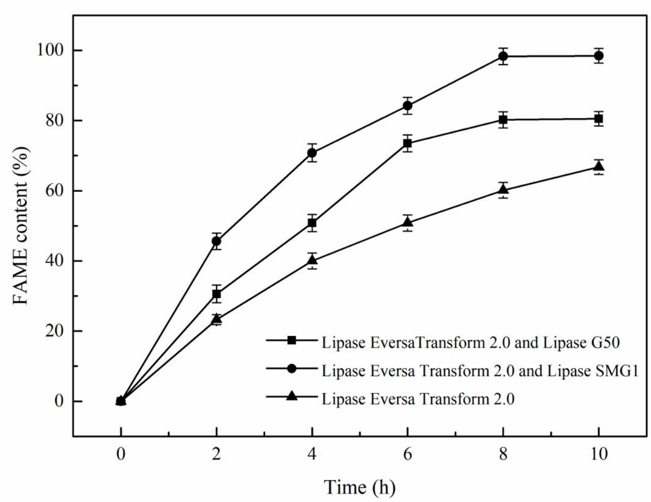

Figure 1. Effect of enzyme types on FAME content. (The transesterification was carried out at 25 °C with a molar ratio 1:3 between BSFL lipids and methanol, the lipase dosage was 50 U/g Lipase Eversa Transform 2.0 alone or 50 U/g Lipase Eversa Transform 2.0 combined with 50 U/g Lipase SMG1/ Lipase G50.)

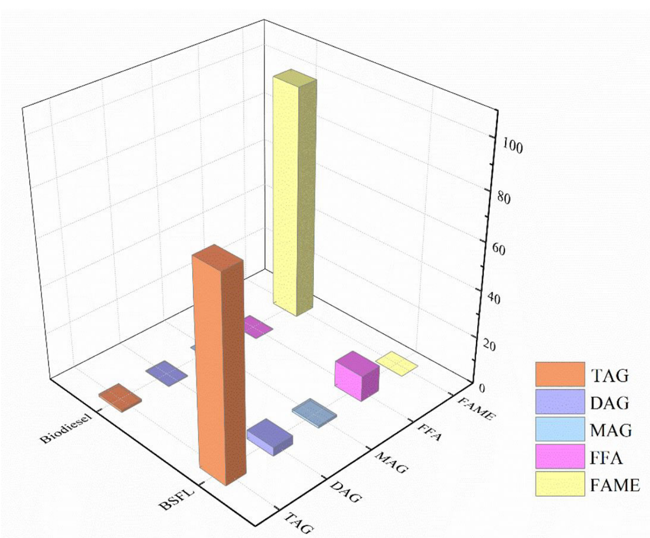

Figure 2. The composition of BSFL and biodiesel detected by HPLC.

(Li et al., 2016). In this study, after transesterification, the content of FFA dropped significantly from 11.35% to 0.17%, and the acid value was about 0.10 mg KOH/g. Therefore, the increase of FAME yield likely caused by the effective transesterification upon all types of glycerides, FFA, and even phospholipids which was consistent with the catalytic characteristics of Lipase Eversa Transform 2.0 and Lipase SMG1 mentioned before. Thus, the mixture of Lipase Eversa Transform 2.0 and Lipase SMG1 was used as catalysts in further study.

3.2 The effect of reaction temperature on the yield of biodiesel

Figure 3 showed the effect of reaction temperature on the yield of FAME. The yield of biodiesel increased from 73.90%

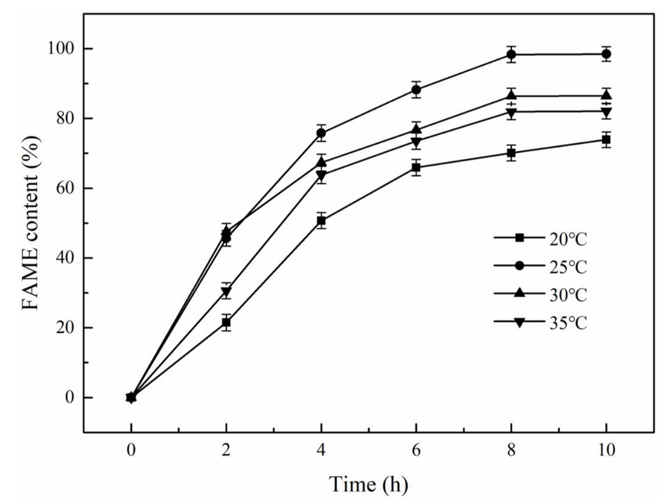

Figure 3. Effect of reaction temperature on the yield of FAME. (Lipase Eversa Transform 2.0 together with Lipase SMG1 were used as catalysts. Transesterification was performed with a molar ratio of BSFL lipids to methanol 1:3 and one-step addition methanol at varying temperature (20, 25, 30, and 35 °C).)

to 98.45% with the reaction temperature increased from 20 to 25 °C. When the temperature was further increased to 30 °C, although the initial yield in first 2 h was higher comparable to that at 25 °C but the final yield dropped to 86.43% after 10 h. The FAME production dropped markedly when the reaction temperature reached to 35 °C. This is likely because of a dual effect of temperature on enzyme catalytic activity, where a higher reaction rate and yield are obtained at a higher temperature, while increasing the temperature beyond certain point (30 °C) leads to enzyme inactivation. Therefore, the reaction temperature was set at 25 °C in the following study.

3.3 The effect of the substrate molar ratio on the yield of biodiesel

The effect of substrate molar ratio on FAME production was determined by setting the molar ratio of BSFL lipids to methanol at 1:2, 1:3, 1:4, and 1:5, respectively, in the transesterification. The results were showed in Figure 4. When the molar ratio of BSFL lipids to methanol increased from 1:2 to 1:3, the yield of FAME achieved up to 98.45%. A further increase of the molar ratio of BSFL lipids to methanol beyond 1:3 led to the yield of biodiesel decreased significantly. In the transesterification reaction, the ideal molar ratio of oil to methanol is 1:3. Increasing the molar ratio will make the reaction move forward and accelerate the reaction process. However, the short-chain alcohol has a toxic effect on the enzyme activity (Xu et al., 2012), resulting in a notable reduction on the final product content (Su'i et al., 2021). Therefore, the optimal condition for FAME production was established to be a molar ratio of BSFL lipids to methanol at 1:3, reaction time at 25 °C and a mixture of Lipase Eversa Transform 2.0 and Lipase SMG1 as catalysis. Under this condition, a maximum FAME yield of 98.45% was obtained after 8 h.

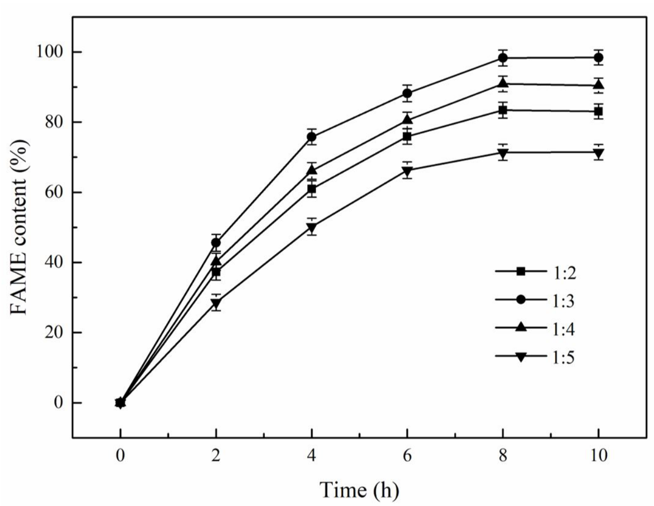

Figure 4. Effect of the molar ratio of BSFL to methanol on the yield of FAME. (Lipase Eversa Transform 2.0 together with Lipase SMG1 were used as catalysts. Transesterification was performed at 25°C, with a molar ratio of BSFL to methanol at 1:2, 1:3, 1:4, or 1:5.)

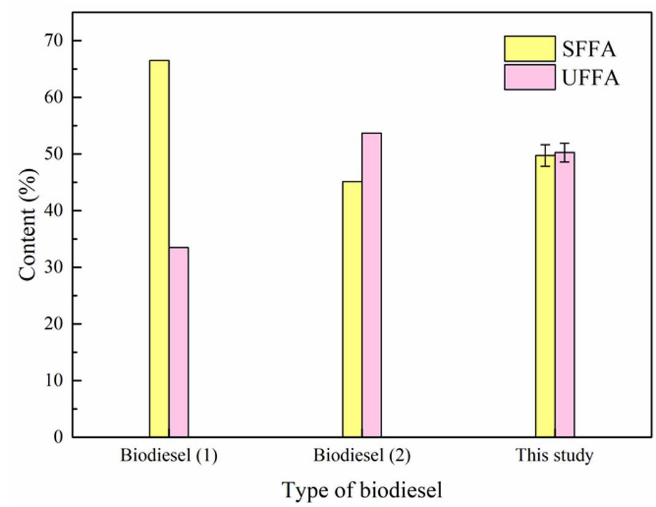

Figure 5. The comparison of UFFA and SFFA content among different type of biodiesel.

3.4 Properties of biodiesel

The cetane index, viscosity, ester content, acid value, and flash point are important properties of biodiesel. And the cetane index, viscosity, and flash point are related with carbon number, molar weight, and unsaturated carbon bonds number, etc. (Giakoumis & Sarakatsanis, 2018). Table 1 and Figure 5 compared the composition of FFA among the final biodiesel product obtained in this study, the biodiesel produced from BSFL using Novozym 435 as catalyst (Nguyen et al., 2018) and palm oil (Sajjadi et al., 2016) reported in the literatures before. And the main properties of the final biodiesel produced in this study and reported in the literatures were compared according to the standard EN14214 in Table2.

Although the raw materials of this study and the research reported by Nguyen et al. (2018) were both BSFL, the fatty acid composition of them had significant difference. Many studies have showed that the nutrient content of a BSFL product including lipids composition is highly related with the growing substrate of BSFL (Tschirner & Simon, 2015). And the BSFL biodiesel reported before had a lower unsaturated fatty acids (UFFA) content (33.50%) than that of this study (50.26%), which resulted a lower cetane index, higher viscosity, and flash point. This phenomenon was consistence with the relation between properties and FFA composition. The kinematic viscosity decreases while the cetane index increases as the total content of UFFA increases. (Giakoumis & Sarakatsanis, 2018; Kaisan et al. 2017). The flash point decreases with increasing of residual alcohol and other solvents with a low-boiling point (Černoch et al. 2010). Besides, higher flash point value means better for biodiesel handling, storage, and transportation (Alviso et al., 2020).

The biodiesel obtained in the current study has 49.74% SFFA and 50.26% UFFA totally, which had no significant difference from the biodiesel prepared by palm oil. Despite this, the cetane number, viscosity, and flash point between them still had difference, this may be related to the SFFA composition of them was different like BSFL biodiesel of this study had more lauric and myristic. What's more, the quality of raw material and processing conditions during the preparation of biodiesel also effects the properties of biodiesel. (Ramirez-Verduzco et al., 2012).

The final biodiesel obtained in this study meets the requirements of the EN14214 standard, and has similar properties to biodiesel produced from palm oil. Moreover, the acid value was 0.10 mg KOH/g which was lower than the limitation of EN 14214 (0.50 mg KOH/g), thus shows great potential in replacing diesel usage in industry.

Table 1. The fatty acid composition of the biodiesel produced from BSFL, palm oil and this study.

Fatty acid composition	Biodiesel (1) (%)	Biodiesel (2) (%)	This study (%)
Capric (C10:0)	2.80	ND*	ND*
Lauric (C12:0)	30.50	ND*	12.12 ± 0.02
Myristic (C14:0)	7.70	ND*	3.21 ± 0.01
Palmitic (C16:0)	21.40	39.83	24.42 ± 0.01
Palmitoleic (C16:1)	4.30	0.17	1.70 ± 0.03
Heptadecanoic (C17:0)	ND*	NR*	2.10 ± 0.05
Stearic (C18:0)	3.20	5.33	7.89 ± 0.02
Oleic (C18:1)	24.90	41.90	38.60 ± 0.02
Linoleic (C18:2)	4.30	11.46	9.60 ± 0.04
Linolenic (C18:3)	ND	0.15	0.36 ± 0.05
Nonadecanoic (C19:0)	0.90	NR*	ND*

NR*: Not be reported; ND*: Not be detected; (1): Biodiesel produced by BSFL (Nguyen et al.2018); (2): Biodiesel produced by palm oil (Sajjadi et al.2016).

Table 2. Main properties of different biodiesel types compared.

Biodiesel type	Cetane index	Viscosity (mm^2/s,40°C)	Ester content (%)	Acid value (mg KOH/g)	Flash point (°C)
Biodiesel (1)	50.00	5.20	98.30	0.80	121.00
Biodiesel (2)	60.21	4.53	NR*	0.20	176.70
This study	58.98 ± 1.21	4.57 ± 0.12	98.45 ± 0.24	0.10 ± 0.02	179.80 ± 1.01
EN14214	>51.00	3.50-5.00	>96.50	<0.50	>101.00

NR*: Not be reported; (1): Biodiesel reported from Nguyen et al. (2018); (2): Biodiesel reported from Sajjadi et al. (2016).

4 Conclusions

Here, we reported a cost-effective and simple method to produce biodiesel using BSFL lipids and enzyme catalysis. The results showed that using Lipase Eversa Transform 2.0 with Lipase SMG1 as catalyst was able to increase the yield of biodiesel and drop the acid value significantly. After optimization of the reaction condition, the highest FAME yield of 98.45% was achieved at 25 °C with a molar ratio of BSFL to methanol at 1:3. And the properties of the obtained biodiesel met the EN14214 standard. This study showed the combination of Lipase Eversa Transform 2.0 with Lipase SMG1 has great value in the production of biodiesel.

Conflict of interest

The authors declare that they have no conflict of interest.

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