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From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible

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ABSTRACT

Biodiesel is a renewable and environmentally friendly liquid fuel. However, the feedstock, predominantly crop oil, is a limited and expensive food resource which prevents large scale application of biodiesel. Development of non-food feedstocks are therefore, needed to fully utilize biodiesel's potential. In this study, the larvae of a high fat containing insect, black soldier fly (*Hermetia illucens*) (BSFL), was evaluated for biodiesel production. Specifically, the BSFL was grown on organic wastes for 10 days and used for crude fat extraction by petroleum ether. The extracted crude fat was then converted into biodiesel by acid-catalyzed (1% H_2SO_4) esterification and alkaline-catalyzed (0.8% NaOH) transesterification, resulting in 35.5 g, 57.8 g and 91.4 g of biodiesel being produced from 1000 BSFL growing on 1 kg of cattle manure, pig manure and chicken manure, respectively. The major ester components of the resulting biodiesel were lauric acid methyl ester (35.5%), oleic acid methyl ester (23.6%) and palmitic acid methyl ester (14.8%). Fuel properties of the BSFL fat-based biodiesel, such as density (885 kg/m^3), viscosity (5.8 mm^2/s), ester content (97.2%), flash point (123 °C), and cetane number (53) were comparable to those of rapeseed-oil-based biodiesel. These results demonstrated that the organic waste-grown BSFL could be a feasible non-food feedstock for biodiesel production.

1. Introduction

Since the last century, fossil fuels have been the major resource fulfilling energy needs for our economic development. However, concerns of climate change and the decline of non-renewable fossil resources have motivated the development of renewable environmentally friendly energy such as, solar energy, wind energy, tidal energy, and bioenergy from biomass [1,2]. Of those "renewable clean energies", liquid biofuels such as bioethanol and biodiesel are ready to be produced and can be used directly in existing engines used for transportation [3–8].

Although biodiesel has the potential to become a primary fuel for heavy duty trucks and farm tractors, currently, biodiesel is only used as a diesel fuel additive to minimize releasing particulates, carbon monoxide, and hydrocarbons [3,4]. Production cost is the main obstacle preventing biodiesel from being used as a primary fuel [9,10]. Cost analysis shows that 75% of biodiesel cost is derived from the feedstock, primarily crop-oil such as soybean oil, rapeseed oil and sunflower oil [11]. In addition to its economic constraints, crop oil is a limited food resource. Using limited food resources for biodiesel production is not a feasible option for developing countries such as China. To overcome these challenges, non-food feedstocks such as *Jatropha curcas* [12], Chinese tallow [13], microalgae [11,14–16], and others [17] are being developed for biodiesel production. However, these alternative feedstocks face their own challenges, such as, long lifecycle, competing for crop land, and competing for limited water resources.

On the other hand, organic wastes such as animal wastes, residential wastes (e.g., household), commercial wastes (e.g., from stores, markets, shops, hotels, etc.), and institutional wastes (e.g., schools, hospitals, etc.) are generated in large quantities in developing countries. These organic wastes can cause environmental pollution and become potential health hazards if not managed properly. In this study, we report the use of organic wastes as a resource for biodiesel production. Specifically, cattle manure, pig manure, and chicken manure were evaluated in their ability to raise a high fat containing insect, black soldier fly (*Hermetia illucens*) larvae (BSFL) [18,19], which was then used for biodiesel production. The fuel properties of the resulting biodiesel were comparable to those of rapeseed-based biodiesel, and met the European biodiesel standard, EN14214.

2. Materials and methods

2.1. Organic wastes, insect species and growth conditions

The organic wastes (cattle manure, chicken manure and pig manure) were obtained from the Breeding Farm of Huazhong

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Agricultural University, Wuhan, PR China. The insect used in this study, black soldier fly (*H. illucens*) larvae (BSFL), was kindly provided by Dr. Jeffery K. Tomberli from Texas A&M University, Texas, USA. The BSFL had been raised for more than 10 generations at Huazhong Agricultural University before being used in this study. BSFL biomass was produced as follows: BSFL was inoculated into cattle manure, pig manure, and chicken manure at a ratio of 1000 larvae per kg of waste, and were incubated for 10 days at room temperature with 65–70% moisture. The BSFL were then harvested by sterile forceps, washed by distilled water, inactivated at 105 °C for 5 min, dried at 60 °C for 2 days, and stored at 4 °C after being ground with a micro-mill.

2.2. Extraction of crude fat

To extract crude fat from the BSFL tissue, the grounded BSFL biomass was put into a filter bag and soaked in petroleum ether for 48 h at room temperature. After the filter bag being removed, the BSFL crude fat contents were then obtained by evaporating the petroleum ether using a rotary evaporator.

2.3. Production of biodiesel

Biodiesel production was carried out in a 100 ml reactor equipped with a reflux condenser, a thermometer, a mechanical stirrer, and a sampling outlet. Biodiesel production was accomplished using a two step process: acid-catalyzed esterification of free fatty acids (FFA) (to decrease the acidity of the crude fat), and alkaline-catalyzed transesterification [20,21].

2.3.1. Acid-catalyzed esterification

The acid-catalyzed esterification step was a pretreatment used to convert free fatty acids in the crude fat into biodiesel, and to decrease the acidity of the crude fat. Specifically, 16 sets of 30 g of crude fat were pretreated to esterify the free fatty acid with methanol using 1% H_2SO_4 (w/w) as the catalyst at the following conditions: four sets were pretreated (methanol to fat ratio 8:1; time 1 h) at a temperature of 55 °C, 65 °C, 75 °C, or 85 °C, respectively; four sets were pretreated (temperature 75 °C, time 1 h) with a methanol to fat ratio of 6:1, 8:1, 10:1, or 12:1, respectively; four sets were pretreated (methanol to fat ratio 8:1, temperature 75 °C) with a reaction time of 30 min, 60 min, 90 min, or 120 min, respectively. During esterification, 3 ml samples were withdrawn periodically to determine the free fatty acid conversion. After pretreatment, the reaction mixture was poured into a funnel, and was allowed to separate by gravity. The upper layer (crude fat and biodiesel) was then transferred to a reactor for alkaline-catalyzed transesterification.

2.3.2. Alkaline-catalyzed transesterification

The upper layer (crude fat and biodiesel) obtained from the above acid-catalyzed esterification was mixed with methanol (methanol to fat ratio of 6:1) and the catalyst NaOH (0.8%, w/w). This mixture was placed in a 65 °C water bath for 30 min, with agitation by a magnetic stirrer. After the reaction, the mixture was separated by gravity. The upper biodiesel layer was then separated from the lower layer and purified by distilling at 80 °C to remove the residual methanol.

2.4. Analysis

The fatty acid methyl ester compositions were determined by a GC/MS (Thermo-Finnigan, USA) equipped with a polyethylene glycol phase capillary column (Agilent, USA). The acid value (AV) of the BSFL crude fat was determined by titration with potassium hydroxide. Free fatty acid (FFA) conversion (%) was calculated using the following formula:

$$\text{FFA conversion } (\%) = (AV_i - AV_t)/AV_i \times 100.$$

While AV_i represents initial acid value, AV_t represents the acid value at reaction time *t*.

3. Results

3.1. Conversion of organic waste into insect biomass

Black soldier fly larvae (BSFL), is able to grow on organic waste, a process that converts organic waste into insect biomass. Interestingly, ~30% of BSFL biomass is composed of fat, which could be a valuable non-food feedstock for biodiesel production, provided that the organic waste could be converted into insect biomass at a high yield. To test this hypothesis, cattle manure, pig manure and chicken manure were evaluated for efficient production of BSFL biomass. Specifically, BSFL was inoculated at a ratio of 1000 larvae per kg of organic manure, and incubated at 27 °C with 60–75% humidity. After 10 days, the organic waste conversion yield and crude fat content were determined. As shown in Table 1, the three types of organic wastes were converted into BSFL biomass at varying yield. Chicken manure achieved the highest yield at 32.8%, followed by pig manure at 20.4% and cattle manure at 12.8%. Therefore, chicken manure could be the most valuable resource used to culture BSFL for biodiesel production, provided that the fuel properties of BSFL fat-based biodiesel are comparable to those of crop oil-based biodiesel.

3.2. Production of biodiesel

Ten day old BSFL biomass was subjected to petroleum ether extraction, resulting in 98.5 g, 60.4 g and 38.2 g of crude fat for BSFL grown on chicken manure, pig manure and cattle manure, respectively (Table 1). These crude fats, with an acid value of ~7.3 (mg of KOH per g of fats), were used for biodiesel production by a two step process. To ensure an efficient conversion of high acid value crude fat into biodiesel, an acid-catalyzed esterification (1% H_2SO_4 catalyzed) was initially used to convert the free fatty acids present in the crude fat into biodiesel, and to lower the acid value. Then, an alkaline-catalyzed transesterification (0.8% NaOH catalyzed) was applied for conversion of fat into biodiesel.

3.2.1. Conversion of free fatty acids into biodiesel

Reaction temperature, methanol to fat ratio and reaction time were optimized for efficient esterification of free fatty acids. First, esterification temperature (55 °C, 65 °C, 75 °C and 85 °C) was optimized using a methanol to fat ratio of 8:1 and with a 1 h reaction time. As shown in Fig. 1A, when temperature increased from 55 °C to 85 °C, free fatty acid conversion increased from 73% to 92%,

Table 1
Yields of biomass, crude fat and biodiesel from waste-grown BSFL.

BSFL[a]	Cattle manure	Pig manure	Chicken manure
Biomass (g)	127.6	207.4	327.6
Biomass yield (%)	12.8	20.7	32.8
Crude fat (g)	38.2	60.4	98.5
Fat yield (%)	29.9	29.1	30.1
Biodiesel (g)	35.6	57.8	91.4
Biodiesel yield (g)	93	96	93

[a] Biomass was BSFL dry weight; biomass yield was the BSFL biomass produced from 100 g of organic waste used. Crude fat was the total fat extracted by petroleum extraction; fat yield was the percentage of fat accounted for total biomass. Biodiesel yield was the conversion rate of crude fat into biodiesel.

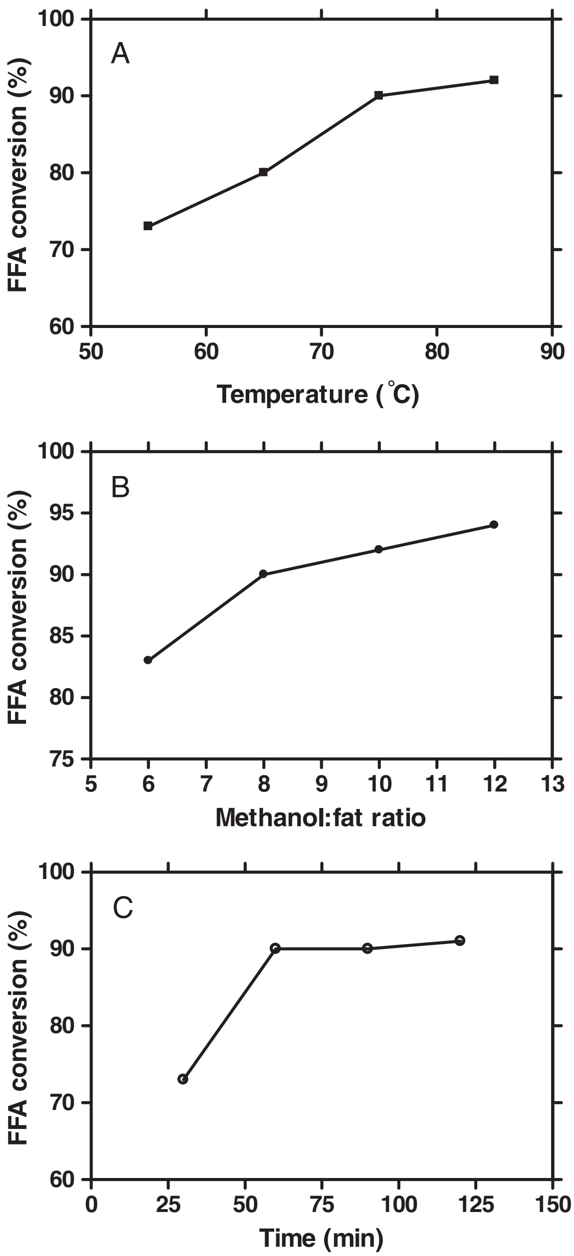

Fig. 1. Optimization of esterification conditions for (A) temperature (with a fixed methanol to fat ratio of 8:1, and a 1 h reaction time); (B) methanol to fat ratio (with fixed temperature of 75 °C and a 1 h reaction time); and (C) reaction time (with a fixed methanol to fat ratio of 8:1 and 75 °C). All reactions were carried out using 1% H_2SO_4 as the catalyst.

showing a positive relationship between temperature and free fatty acid conversion. This temperature dependent conversion could be attributed to mass transfer efficiency, a high solubility of crude fat required for efficient mass transfer [22]. However, when temperature was higher than 75 °C, the conversion increased only slightly. Therefore, 75 °C was chosen as an "optimal" esterification temperature.

Next, the methanol to fat molar ratio was optimized at 75 °C with a 1 h reaction time. The stoichiometry for esterification requires one molar methanol per molar fatty acid, yielding one molar fatty acid methyl ester and one molar water. A higher methanol to fatty acid ratio, however, could result in increased ester formation, but could also interfere with the separation of glycerin in later stages. Therefore, methanol to fat ratios of 6:1, 8:1, 10:1 and 12:1 were evaluated for esterification. As expected, the methanol to fat ratio had a positive impact on free fatty acid conversion (Fig. 1B); the higher the ratio, the higher the conversion. Nevertheless, a methanol to fat ratio of 8:1 achieved 90% free fatty acid conversion. Ratios lower than 8:1 resulted in incomplete conversion, while ratios higher than 8:1 resulted in slightly improved conversion. A methanol to fat ratio of 8:1 was, therefore, selected for esterification.

Finally, the reaction time was optimized using a methanol to fat ratio of 8:1 and a temperature of 75 °C. As shown in Fig. 1C, free fatty acid conversion increased dramatically (from 73% to 90%) when reaction time increased from 30 min to 60 min. However, reaction time longer than 60 min did not increase free fatty acid conversion. As a result, reaction time of 60 min should be used for esterification.

Table 2
Comparison of fatty acid methyl ester composition of BSFL fat-based biodiesel and rapeseed oil-based biodiesel.

Composition	BSFL fat-based biodiesel[a] (%)	Rapeseed oil-based biodiesel[b] (%)
Capric acid methyl ester	3.1	n/a
Lauric acid methyl ester	35.6	n/a
Myristic acid methyl ester	7.6	n/a
Palmitoleic acid methyl ester	3.8	n/a
Palmitic acid methyl ester	14.8	3.5
Oleinic acid methyl ester	23.6	64.4
Linoleic acid methyl ester	5.8	22.3
Linolenic acid methyl ester	nd	8.2
Stearic acid methyl ester	3.6	0.8
Noadecanic acid methyl ester	1.4	n/a

[a] The nd stands for not detected.
[b] The data of rapeseed oil-based biodiesel was from Ref. [11]. The n/a stands for not reported.

Table 3
Comparison of fuel properties of BSFL fat-based biodiesel, rapeseed oil-based biodiesel, and the standard EN14214.

Properties	EN14214	BSFL biodiesel[a]	Rapeseed biodiesel[b]
Density (kg/m^3)	860–900	885	880
Viscosity at 40 °C (mm^2/s)	1.9–6.0	5.8	6.35
Sulfur content (wt.%)	0.05	nd	<0.01
Ester content (%)	96.5	97.2	n/a
Water content (mg/kg)	<0.03	0.03	0.03
Flash point (°C)	120	123	n/a
Cetane index	48–60	53	45
Acid number (mg KOH/g)	<0.8	1.1	0.3
Methanol or ethanol (m/m)	0.2%	0.3%	n/a
Distillation (°C)	n/a	360	352

[a] The nd stands for not determined.
[b] The data for rapeseed oil was from Ref. [11]. The n/a stands for not reported.

3.2.2. Conversion of triglycerides (fat) into biodiesel

The above optimized conditions, 75 °C, a methanol to fat ratio of 8:1 and a 60 min reaction time, were applied for pretreatment of the crude fats extracted from the BSFL grown on three types of organic manure. After the pretreatment, the reaction mixture was settled for 2 h, resulting in two separate layers. The upper layer, containing triglycerides and fatty acid methyl esters (biodiesel) derived from pretreatment, was transferred into a reactor for transesterification for 30 min at 65 °C using a methanol to fat ratio of 6:1 and 0.8% NaOH as the catalyst. This reaction converted the triglycerides into fatty acid methyl esters (biodiesel). The reaction mixture was allowed to settle for 2 h. The upper layer containing the fatty acid methyl esters was then purified by distillation (at 80 °C) to remove the residual methanol, resulting in 91.4 g, 57.8 g and 35.6 g of biodiesel being produced from the BSFL crude fat grown on 1 kg of chicken manure, pig manure and cattle manure, respectively (Table 1).

3.3. Fuel properties of BSFL fat-based biodiesel

The chemical compositions of BSFL fat-based biodiesel were analyzed using a GC/MS. As shown in Table 2, 10 different fatty acid methyl esters were detected, with lauric acid methyl ester (35.6%), oleinic acid methyl ester (23.6%), and palmitic acid methyl ester (14.8%) being the main methyl esters. The fuel properties of the BSFL fat-based biodiesel were then determined (Table 3). Remarkably, most fuel properties of the BSFL fat-based biodiesel, such as density, viscosity, flash point, cetane number and ester contents, were comparable to those of rapeseed oil-based biodiesel and met the European biodiesel standard, EN14214. Moreover, the BSFL fat-based biodiesel had a higher percentage (67.6%) of saturated fatty acid methyl esters than rapeseed oil-based biodiesel (4.3%) [11]. Since saturated fatty acid methyl ester is more oxidative stable than unsaturated fatty acid methyl ester, the BSFL fat-based biodiesel may have higher oxidative stability than rapeseed oil-based biodiesel.

4. Discussion

With limited crop land and the world's largest population, China cannot afford crop-oil based biodiesel production. It is imperative to develop non-food feedstocks for biodiesel production. In this study, we evaluated the potentials of using livestock organic wastes to raise a high fat-containing insect, black soldier fly (BSFL), for biodiesel production. Among the organic wastes evaluated, chicken manure was the best one for maximal BSFL growth (327.6 g), which resulted in 98.5 g of crude fat after petroleum ether extraction. An optimized two step conversion process was performed, yielding 91.4 g of biodiesel with a biodiesel yield of 93% from crude fat contents. Importantly, the fuel properties of the BSFL fat-based biodiesel were comparable to those of rapeseed oil-based biodiesel and met the European biodiesel standard, EN14214. These results demonstrated that BSFL-fat could be a valuable feedstock for biodiesel production.

The BSFL fat-based biodiesel has at least two advantages over those of crop oil-based biodiesel. First, it does not compete with food resources or land use; second, it maximizes the benefits of waste management by using "waste nutrients" for insect growth. To maximize the economics of BSFL fat-based biodiesel, however, additional research is needed to (1) evaluate the minimal growth time for maximal BSFL biomass; (2) evaluate the number of cycles of organic waste that can be used for efficient BSFL growth; (3) evaluate the fat free BSFL biomass as a potential high protein animal feed.

5. Conclusions

A high fat containing insect, black soldier fly, can be used to serve two objectives: organic waste management and biodiesel production. Among the organic wastes evaluated, chicken manure appears to be the best one for achieving maximal BSFL biomass. About ∼30% of the waste-grown BSFL biomass can be extracted as crude fat, which can be used for the production of biodiesel. The fuel properties of the BSFL fat-based biodiesel, such as density (885 kg/m^3), viscosity (5.8 mm^2/s), and cetane number (53) are comparable to those of rapeseed oil based biodiesel.

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