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Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production

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ABSTRACT

Biodiesel is a promising alternative diesel fuel which has increased worldwide public interest in a number of countries including China. But the high cost of producing biodiesel from feedstock, predominately food grade oils, limited its economic feasibility. An alternative of using grease extracted from restaurant waste to produce biodiesel is a potential low cost approach. However, this approach generates a significant large quantity of solid residual fraction which required proper disposal. This study was conducted to evaluate the potential of a secondary biodiesel production from the solid residual fraction of restaurant waste after typical grease extraction (SRF) employing a high fat containing insect, black soldier fly, *Hermetia illucens*. The SRF was sampled and fed to black soldier fly larvae. The resulting larval biomass was used for crude grease extraction by petroleum ether. The extracted crude grease was then converted into biodiesel by acid-catalyzed (1% H_2SO_4) esterification and alkaline-catalyzed (0.8% NaOH) transesterification. About 23.6 g larval grease-based biodiesel was produced from approximately 1000 larvae grown on 1 kg of SRF. The weight of SRF was reduced by about 61.8% after being fed by the black soldier fly larvae for 7 days. The amount of biodiesel yield from restaurant waste was nearly doubled (original restaurant waste grease, 2.7%; larval grease, 2.4%). The major methyl ester components of the biodiesel derived from black soldier fly larvae fed on SRF were oleinic acid methyl ester (27.1%), lauric acid methyl ester (23.4%), and palmitic acid methyl ester (18.2%). Most of the properties of this biodiesel met the specifications of the standard EN 14214, including density (860 kg/m^3), viscosity (4.9 mm^2/s), flash point (128 °C), cetane number (58) and ester contents (96.9%). These results indicated that black soldier fly larval biomass obtained from larvae reared on SRF could potentially be used as a non-food feedstock for biodiesel production. This approach not only enhances the efficiency of biodiesel production from restaurant waste, it also helps to better manage and significantly reduce the large quantity of solid residual fraction produced during the process of biodiesel production using restaurant waste.

1. Introduction

With the world population growth and economic development, energy consumption and demand surge worldwide. The reserves of fossil fuel which is a nonrenewable but major energy resource for nearly every country is progressively decreased and predicted to be exhausted in the near future [1]. Thus, considerable amount of studies have been promoted to identify viable alternative fuel sources. In this context, biofuel has attracted great attention in both developed and developing countries due to its renewability, possible better emissions, non-toxic and biodegradability.

As a key member of biofuel family, biodiesel, a mono alkyl ester (methyl or ethyl ester) of long chain fatty acids derived from renewable lipid such as vegetable oils and animal fats, can be used as a substitution fuel for traditional diesel in any compression ignition (diesel) engines with little or no modification [2–4]. The superiority of biodiesel relies on its sulfur content, aromatic content and flash point [5]. Presently, about 95% of the world biodiesel was produced from edible oil in which about 84% was from rapeseed oil, 13% from sunflower oil and 3% from palm oil, soybean oil and others, leading to the production cost of biodiesel of approximately 1.5 times higher than that for petroleum diesel [6,7].

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Moreover, continuous and large-scale production of biodiesel from food grade oil may cause global imbalance to the food supply and demand market [1,8]. The competition between using lands for growing crops for biofuel production and for food consumption is a major concern [9,10]. To address the land usage competition issue and to reduce the cost of biodiesel, a range of research has been done on finding new technology for biodiesel production. One of the main approaches is using waste cooking oil (WCO) [5,11–15]. Other non-edible plant oils also have been investigated such as jatropha, madhuca and rubber seed oil [16,17]. In addition, efforts have been taken to find biodiesel feedstock from solid organic waste. It is reported that *Lipomyces starkeyi* has potential to grow on and convert sewage sludge for lipid accomulation [18]. But a pretreatment of the sluge by alkaline or acid hydrolysis, thermal or ultrasonic treatment was needed for the growth of *L. starkeyi* which is less environmental friendly and high energy cost. Rice bran, a byproduct during milling to produce polished rice, containing about 15–23% oil, was also investigated for biodiesel production [19,20]. An “in situ esterification” was suggested for high purity and recovery of biodiesel, however, excess alcohol and acid were still needed. Kuchkina et al observed that dredging sediments of lakes or eutrophic reservoirs, a cost-free material containing relevant amounts of algal lipid, can be utilized as a novel feedstock for biodiesel production [21]. A high biodiesel yield may not be expected due to the low lipid content of the sediments (0.24% in studied sediments), but it likely helps to manage this pollutant.

Another important candidate is restaurant waste grease which has attracted considerable amount of research [2,22]. Waste grease derived from restaurant waste is often considered as a potential feedstock for biodiesel production because it is very low in value which will help to reduce biodiesel cost. Restaurant waste is generated in large quantities in densely populated cities and countries. For example, in Wuhan China, above 1000 tons of restaurant waste is accumulated daily. The potential of using restaurant waste as biodiesel feedstocks was reported by Canakci [2]. However, this process generates a huge quantity of solid residual fraction after grease extraction for biodiesel production. This solid residual fraction of restaurant waste after typical grease extraction (SRF) is mostly consisted of food residues which is high in starch, fat, protein and fibre. It is a potential hazardous material to environment without proper disposal. This study reports an approach to manage SRF and a secondary production of biodiesel by feeding SRF to a non-pest insect, black soldier fly, *Hermetia illucens*. It had been confirmed that one can use larval biomass of black soldier fly which fed on animal manure for biodiesel production. About 15.8 g biodiesel was produced from about 1200 black soldier fly larvae fed on dairy manure in a 21 days period. Fat content accounted for about 30% of the black sodier fly larvae biomass fed on manures [23,24]. A higher biodiesel yield and shorter conversion period was hypothesized due to the more rich nutrition in SRF. This study further demonstrates that the nutrient in SRF can be utilized by black soldier fly larvae to convert into its larval biomass. Crude grease extracted from this high fat black soldier fly larval biomass can be further processed into biodiesel, therefore, resulting in enhanced biodiesel production efficiency from restaurant waste. Compositions and properties of the biodiesel produced from larval biomass from black soldier fly reared on SRF were then analyzed and reported.

2. Materials and methods

2.1. Source of fly

The black soldier fly, *H. illucens*, in Huazhong Agricultural University (HZAU) was originated from Dr. Jeffery K. Tomberlin, Texas A&M University, TX, USA. The larvae were fed for about 8 days on standard colony diet before being used in this study [25].

2.2. Solid residual fraction of restaurant waste after typical grease extraction (SRF)

Raw restaurant waste was collected from restaurants in Wuhan city, China, by Hubei Tianji Bioengineer Co. Ltd., a government authorized biodiesel processing plant located in Huazhong Agricultural University. Waste grease was extracted from the raw restaurant waste in the processing plant and used for biodiesel production. At this point, about 3% (w/w) of the raw restaurant waste was separated as waste grease which was further processed into biodiesel with an overall biodiesel yield of 2.7% (w/w) from raw restaurant waste (Unpublished data from Tianji Bioengineer Co. Ltd.). The SRF was then used to rear black soldier fly larvae. Grease was then extracted from the larval biomass of the black soldier fly. The larval grease was used for conversion into biodiesel.

2.3. Bioconversion for insect biomass

Based on preliminary trials, rearing 1000 black soldier fly larvae in 1 kg of SRF is the optimal condition. In this study, about 1000 of 8 days old black soldier fly larvae were inoculated into 500 g SRF. Another 500 g SRF was replenished about three days later, making the total amount of 1000 g of SRF per 1000 larvae. This process was conducted in a greenhouse at 26–29 °C with 65–75% air moisture. When approximately 50% of the larvae reached prepupal stage, the larvae were harvested for grease extraction and biodiesel conversion. The larvae were separated from the SRF medium residue, water washed, and dried at 70 °C (about 2 days) until constant weight was obtained. After being ground with micro-mill, the larval biomass was stored at 4 °C until grease extraction could be performed.

In this study, three batches of SRF was obtained from Hubei Tianji Bioengineer Co. Ltd.. Before feeding the SRF to the 8 days old black soldier fly larvae, the water content, organic fraction, fat concentration and protein of SRF was determined. Two batches of black soldier fly larvae were reared in each SRF sample (1000 larvae per Kilogram of SRF per batch). A total of 6 batches of black soldier fly larvae were reared on SRF in this study. The weight and the moisture of the remaining SRF after feeding to black soldier fly for 7 days were determined.

2.4. Crude grease extraction

Black soldier fly larvae grease was extracted using a modified method described by Li et al. [23]. Briefly, ground larval biomass was placed into a filter bag and soaked in petroleum ether (200 ml) for 48 h twice at room temperature. Resulted leach liquor was combined. Crude larval grease was then obtained by evaporating petroleum ether with a rotary evaporator.

2.5. Production of biodiesel

To achieve maximal biodiesel yields, in this study, a two step approach with acid-catalyzed esterification and sequential alkaline-catalyzed transesterification was used for biodiesel production to ensure an efficient conversion of free fatty acids (FFA) into biodiesel [26]. This is because of the high acid value of the larval crude grease (~7.1 mgKOH/g). Reaction temperature, methanol to fat ratio and reaction time have profound impacts on transesterification. An optimized reaction procedure described by Li et al. [23], was used in this study. This process was carried out with a system equipped with a 100 ml reactor, a thermometer, an electromagnetic stirrer, a reflux condenser, and a sample outlet.

Briefly, as a pretreatment to convert free fatty acids in the crude grease into biodiesel, acid-catalyzed esterification was conducted at the following conditions: 1% H_2SO_4 as the catalyst, 75 °C, methanol to fat ratio of 8:1, and reaction time of 1 h, to decrease the acid value of the crude grease. Resulted mixture was poured into a funnel for separation. The upper layer containing crude grease and biodiesel was further transferred into a new reactor for alkaline-catalyzed transesterification at the following conditions: methanol to fat ratio of 6:1, 0.8% NaOH (w/w) as the catalyst, 65 °C, and reaction time of 30 min. After separation with a funnel, the biodiesel layer was distilled at 80 °C to remove residual methanol.

2.6. Analyses

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 larval crude grease and biodiesel was determined by titration with potassium hydroxide according to EN 14104. Other characteristics of biodiesel such as density (EN ISO 3675), viscosity (EN 3105), ester content (EN 14105), water content (EN ISO 12937), flash point (EN ISO 3679), cetane index (EN ISO 5165) were also measured. FFA conversion rate (%) 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 and discussion

3.1. Solid residual fraction (SRF) conversion into insect biomass

The SRF media before being fed to black soldier fly larvae was determined to have 81.2% water content, 13.6% organic fraction, 3.4% fat concentration, 4.6% protein content, and 1.2% cellulose. Black soldier fly larvae were able to develop normally in SRF media. This shows that the nutrients in SRF are suitable for black soldier fly larval growth and development, and to achieve optimal larval biomass which is rich in fat and protein. The larvae reared on SRF appeared to develop faster to prepupal stage as compared to previous studies conducted using animal manure. In this study, 50% of the 8 days old larvae developed into prepupal stage in 7 days. Myers et al. [27] reported that black soldier fly larvae required significantly longer duration to reach prepupal stage when reared on animal manure. Faster development indicated that SRF may be a better diet for black soldier fly larvae as compared to other types of media such as animal manure.

Table 1 shows the yields of biomass, crude grease and biodiesel production from black soldier fly reared on SRF. An average of 64.9 g insect biomass (dry matter, DM) was obtained from about 1000 larvae fed on1 kg of SRF, and the average percent yield is about 6.5%. Meanwhile, as shown in Table 2, an average weight of 382.5 g digested SRF was left after being fed by black solider fly larvae for 7 days. The weight of SRF was reduced by approximately 61.8%. The percent dry matter was increased from 19.8% to about 29.1%. In addition, it was determined that the average moisture of SRF was also reduced from 81.2% to 70.9%.

Table 1
Yields of biomass, crude grease and biodiesel (Mean ± SE) from black soldier fly larvae fed on solid residual fraction of restaurant waste (SRF).

	Insect biomass[a]	Crude grease	Biodiesel
Weight (g)	64.9 ± 2.3	25.4 ± 3.5	23.6 ± 0.5
Yield (%)	6.5 ± 0.2	39.2 ± 1.9	93.1 ± 0.78
Total yield (%)[b]	2.4 ± 0.1		

[a] Insect biomass was based on larval dry weight per 1000 black soldier fly larvae fed on 1000 g of SRF; biomass yield was larvae dry weight produced from 100 g SRF. Crude grease was total grease extracted from 1000 black soldier fly larvae fed on 1000 g of SRF; crude grease yield was the percentage of grease accounted for total biomass. Biodiesel yield was conversion rate of crude grease into biodiesel.
[b] Total yield was biodiesel produced from 100 g SRF.

Table 2
Variations of selected factors of solid residual fraction of restaurant waste (SRF) after conversion by black solider fly.

	SRF	Digested SRF
Weight (g)	1000.0	382.5 ± 2.3
Dry matter (%)	19.8 ± 0.7	29.1 ± 1.1

When black soldier fly larvae feed on a diet media, it changes the physical/chemical characteristics of the media. It has been reported that the cellulose crystallinity of diet media can be reduced, making the residual media more fermentable and susceptible to enzymes treatment [24]. In this study, it was observed that the remaining SRF after being fed by black soldier fly larvae for 7 days became less compact, and the moisture content was reduced by about 10%. The remaining SRF after being fed by black soldier fly larvae potentially can be a good resource for further production of other forms of energy, such as ethanol by enzymatical hydrolysis [28], syngas by pyrolysis [29], hydrogen gas by fermentation [30].

3.2. Crude grease extraction

Petroleum ether extraction was conducted twice for each batch of insect biomass. On average, about 25.4 g of crude grease was extracted, and fat content in this insect biomass accounted for about 39.2% (Table 1). This fat content is much higher than the 30% reported by Li et al. [21] when black solider fly was fed with animal manure, and by Sheppard & Newton [31] and Diener [32]. The physical and chemical properties of the larval grease such as iodine number, saponification value, melt point, peroxide value and acid value were presented in Table 3. The results in Table 3 show that black soldier fly larval grease has the proper properties for biodiesel production.

3.3. Biodiesel production

Using the two steps process, an average of about 23.6 g biodiesel was produced after removal of methanol by distillation at 80 °C (Table 1) from larval grease fed on SRF. Conversion rate of FFA and crude grease into biodiesel reached about 91.9% and 93.1%, respectively. The mean total yield of biodiesel was about 2.4%. The amount of biodiesel production from restaurant waste therefore nearly doubled by feeding SRF to black soldier fly larvae (Initial waste grease 2.7%, larval grease 2.4%).

3.4. Biodiesel chemical compositions

Table 4 shows the chemical compositions of the larval grease-based biodiesel determined using a GC/MS. One can see from Table 4 that 9 different fatty acid methyl esters were detected and

Table 3
Physical and chemical properties of crude larval grease (Mean ± SE) extracted from black soldier fly larvae reared on solid residual fraction of restaurant waste (SRF).

Iodine value (gI/100 g)	Acid value (mgKOH/g)	Saponification value (mgKOH/g)	Melt point (°C)	Peroxide value (meq/kg)
89.7 ± 3.9	7.1 ± 0.3	146.6 ± 8.2	4 ± 0.5	0.04 ± 0.01

Table 4
Comparison of fatty acid methyl ester composition of biodiesel derived from black soldier fly larvae fed on solid residual fraction of restaurant waste (SRF) and larvae fed on manure.

Composition	Larval grease - based biodiesel (fed SRF) (%)	Larval grease - based biodiesel (fed manure) (%)[a]
Capric acid methyl ester (C10:0)	1.8	3.1
Lauric acid methyl ester (C12:0)	23.4	35.6
Myristic acid methyl ester (C14:1)	3.7	7.6
Palmitoleic acid methyl ester (C16:1)	9.4	3.8
Palmitic acid methyl ester (C16:0)	18.2	14.8
Oleinic acid methyl ester (C18:1)	27.1	23.6
Linoleic acid methyl ester (C18:2)	7.5	5.8
Stearic acid methyl ester (C18:0)	5.1	3.6
Nonadecanoic acid methyl ester (C19:1)	3.3	1.4

[a] The data was from Ref. [23].

identified. The main methyl esters identified were oleinic acid methyl ester (27.1%), lauric acid methyl ester (23.4%), and palmitic acid methyl ester (18.2%). Except for the minor differences in the relative content of methyl esters, the compositions of larval grease-based biodiesel from larvae fed on SRF is very similar to those of larval grease-based biodiesel from larvae fed on animal manure (Table 4). This may indicates that the fatty acid profile of the black soldier fly larvae may not be affected by the types of diet media they were fed on.

3.5. Fuel properties

The properties of this larval grease based-biodiesel were then determined according to European biodiesel standard, EN14214. The results are presented in Table 5. The fuel properties of biodiesel processed from larvae fed on SRF were similar to the biodiesel processed from larvae fed on animal manure. Most of the properties of the biodiesel from larvae fed on SRF met the specifications of EN 14214, including density (860 kg/m^3), viscosity (4.9 mm^2/s), flash point (128 °C), cetane number (58) and ester contents (96.9%). Properties of biodiesel are strongly influenced by the characteristics of the individual fatty esters in biodiesel [33]. Technical problems with biodiesel include oxidative stability and cold flow. In this situation, methyl oleate has been proposed as a suitable major component of biodiesel, but methyl palmitoleate has advantages as compared to methyl oleate, especially with regards to low-temperature properties [34]. This larval grease based-biodiesel which has a relative high content of methyl oleate (27.1%) and methyl palmitoleate (9.4%) may have a good performance under low-temperature conditions. Besides, saturated fatty acids methyl esters, which are more oxidative stable, accounted for about 50% in the larval grease based-biodiesel. As compared to rapeseed oil-based biodiesel, higher oxidative stability could be expected.

Table 5
Comparison of fuel properties of biodiesel derived from black soldier fly larvae fed on solid residual fraction of restaurant waste (SRF), manure and the standard EN14214.

Properties	Larval grease - based biodiesel (fed SRF)	Larval grease - based biodiesel (fed manure) [b]	EN14214[c]
Density (kg/m^3)	860	885	860–900
Viscosity at 40 °C (mm^2/s)	4.9	5.8	3.5–5.0
Sulfur content (wt.%)	nd[a]	nd	0.02max.
Ester content (%)	96.9	97.2	96.5
Water content (mg/kg)	0.02	0.03	0.03max.
Flash point (°C)	128	123	120min.
Cetane index	58	53	51min.
Acid number (mg KOH/g)	0.6	1.1	0.5max.
Distillation Temperature (°C)	360	360	n/a

[a] The nd stands for not determined.
[b] The data was from Ref. [23].
[c] The n/a stands for not reported.

4. Conclusion

Black soldier fly, a no-pest and high fat containing insect, can be used as a new biomass feedstock, reducing SRF accumulation and increasing the overall biodiesel yield from restaurant waste. Crude grease which can be processed into biodiesel accounted for about 39.2% of the resulting biomass. About 23.6 g of larval grease-based biodiesel was produced per kg SRF and the yield of biodiesel from restaurant waste was nearly doubled (Initial waste grease, 2.7%; larval grease, 2.4%). The properties of the biodiesel derived from black soldier fly larvae fed on SRF which has a desirable methyl ester profile met the standard EN 14214 including density (860 kg/m^3), viscosity (4.9 mm^2/s), flash point (128 °C), cetane number (58) and ester contents (96.9%). In addition to doubling the biodiesel production, this technique also provides an alternative to better manage and reduce the accumulation of solid residual fraction of restaurant waste after removal of grease for biodiesel production. The remaining SRF after being fed by black soldier fly larvae potentially can be further used for production of other forms of energy, such as ethanol by enzymatical hydrolysis, syngas by pyrolysis, hydrogen gas by fermention. Moreover, further studies should focus on developing technologies to increase unit yield of insect biomass and conversion rate of crude grease, and evaluating the potential of fat free larval biomass rich in protein to be used as an animal feed additive which will bring additional benefit to biodiesel processing plant.

To fulfill the worldwide energy demand and target of reduction of greenhouse gas (GHG) emissions, renewable energy sources need to be developed. Biodiesel as well as other biofuel appeared to be promising but has been challenges because of controversial in farmland competition for food, rise in international food price, and energy inputs in the refining steps [35,36]. Restaurant waste grease is one of those cheap feed stocks which have the potential to overcome the limitations of biodiesel. The results of this study further demonstrate the potential of using restaurant waste as a cheap feedstock for biodiesel production.

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