Pilot-scale biodegradation of swine manure via *Chrysomya megacephala* (Fabricius) for biodiesel production

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**Abstract**

Swine manure may cause environmental pollution and resource waste if not handled properly on pig farms. In this paper, the technology for pig manure biodegradation and biodiesel production using *Chrysomya megacephala* (Fabricius) is described. About 700 kg of fresh pig manure (73% moisture) can be converted within one week into 18.2 kg dried larvae biomass containing about 21.11% oil in a pilot plant. The properties of the oil extracted from the larvae meal treated with three different drying methods were compared, indicating that the drying method may affect the properties of feedstock oil. The acid value (1.9 mg KOH/g), iodine value (86.3 gI/100 g), melt point (3.1 °C) and peroxide value (0.08 meq/kg) of the oil extracted from the larvae treated with both boiling water and oven-drying were superior to the values of the larvae treated with either oven-drying or sun-drying directly. The main fatty acids of the swine manure *C. megacephala* larvae oil were found to be composed of palmitic acid (36.91%), oleic acid (27.67%), palmitoleic acid (10.89%) and linoleic acid (9.49%). Most of the properties of the biodiesel converted from the feedstock oil by alkaline-catalyst transesterification met the EN 14214 standard in terms of density (0.89 g/cm³), viscosity (5.1 mm²/s), ester content (96.6%), flash point (138 °C), cetane number (56), water content (0.02%) and acid value (0.28 mg KOH/g). This study suggests that the swine manure-grown *C. megacephala* larvae could be a feasible feedstock for a large-scale biodiesel production.

1. Introduction

In recent years, manure treatment has been a recurring problem for most pig industries in many countries [1]. With the raising tendency to intensive and concentrated pig farming activities, a huge amount of swine manure is produced every day, not only leading to the potential environmental crisis caused by pathogens, parasites and weed seeds [2], but also resulting in a suitable breeding ground for the housefly (*Musca domestica*) when not properly processed [3]. Consequently, solving this environmental headache is urgent. Swine manure can be utilized as bio-fertilizer for crops [4]. However, the continued land application for manure disposal may result in excessive nutrient loss from soil to water, causing water eutrophication and deteriorating ecosystem stability [5]. In addition, swine manure can be converted into biogas by anaerobic digestion, but it is essential to add co-substrates into manure like vegetable processing wastes or apple waste to improve the methane yield by co-digestion [6,7]. Methane production has always been relatively low using only manure for anaerobic fermentation due to factors such as the high quantity of water, and unbalanced carbon/nitrogen (C/N) ratio [8]. Another promising method is converting swine manure into biodiesel using coprophagous insects.

Biodiesel is an important form of recyclable energies, and has been considered an ideal substitute for fossil fuels [13]. However, the feedstock cost and production scale are the main obstacles preventing biodiesel from being used as a primary fuel [14]. The coprophagous insects may degrade organic matter from swine manure and transform it into biomass for oil/fat extraction. Insect oil/fat, especially extracted from the larvae of the black soldier fly...
(Hermetia illucens L.), flesh fly (Boettcherisca peregrine) and blow fly (Chrysomya megacephala) fed with solid organic wastes, has been reported as a novel feedstock for biodiesel production [9–12].

C. megacephala is an important agent for estimating the post-mortem interval (PMI) in forensic entomology and also an important economic insect in orchards [15,16]. Also, it can become a potential ideal candidate for a pilot-scale biodiesel production from swine manure. With the well-investigated biological background and high productivity [17], C. megacephala can be continuously mass-reared to produce sufficient eggs, and the content of oil in the C. megacephala larvae is 26.96%, higher than that of soybean (20%) [12]. Furthermore, C. megacephala larvae can digest the swine manure in 5–6 days, obviously more time-saving than black soldier fly (about 15 days) [10]. While the C. megacephala larvae biodiesel derived from food waste has been proved available [12], it is mainly performed in the laboratory scale and, to the best of our knowledge, little information is available about the pilot-scale biodgradation of swine manure via C. megacephala for biodiesel production.

In this study, we established a pilot-scale plant with the daily capacity to process about 100 kg fresh swine manure via C. megacephala and produce approximately 0.5 L biodiesel. In addition, the drying method of C. megacephala larval was optimized. The results indicated that most of the properties of the C. megacephala F. larval biodiesel produced in the pilot-scale plant met the EN 14214 biodiesel standard.

2. Materials and methods

2.1. Materials

The initial C. megacephala colony was established in 2010 by collecting pupae bred in pig manure from National Engineering Research Centre of Microbial Pesticides, Huazhong Agricultural University.

Two types of fresh swine manure were collected from the Pig Breeding Farm of Huazhong Agricultural University. One manure contained sawdust (about 20% of volume), which was used as bedding for the fattening pigs. The moisture was about 73%. The other manure was obtained from breeding pigs fed by a standard growing diet and contained no sawdust. The moisture was about 78%.

2.2. Rearing of adult flies

Adult flies were kept at 26 °C with a photoperiod of 12:12 (L:D) and a relative humidity of 75%. Flies were maintained in a nylon cage (100 × 100 × 100 cm), with eight loops in each angle to hold the adult shelf, as indicated in Fig. 1(a). This production cage was specially designed for the pilot scale plant, which could be loaded with up to 10,000 pupae and was used primarily for egg production. Two batches of nylon cages were used in rotation to maintain the stability of the egg production. The used cages were removed, washed and sterilized with boiling water for another batch production.

Two plastic trays (3 × 16 × 30 cm) were placed inside the cage, one used to hold food (a mixture of powdered milk and sugar at a ratio of 1:1) and the other used to hold a sponge soaked with water. Food and water were fed to adult flies ad libitum, and the trays were changed every two days. Another tray (7 × 15 × 30 cm) containing about 10,000 pupae 2–3 days before expected emergence was put inside one cage. An artificial diet (blood meal, fish meal and wheat bran at a ratio of 1:2:7, 65% moisture) was placed in a plate (10 cm diameter) and used as an ovisposition substrate to induce the adults to produce eggs. Eggs were collected and weighed at 8:00 am every day. With 1 g of eggs being weighed and counted under stereomicroscope, the volume of eggs from each adult cage was recorded daily (1 g ≈ 6500 eggs) during the egg production period.

2.3. Structure and facilities

Floor plans for the biodiesel production facilities are illustrated in Fig. 2. The pilot plant was mainly made up of five parts: adult rearing room, larval rearing room, pupae room, biodiesel production room and storeroom. In the adult rearing room, three adult shelves were placed for the egg production of the adult C. megacephala flies colony, with two layers and two nylon cages on each adult shelf (100 × 100 × 215 cm). In the larval rearing room, swine manure biodgradation was performed in six larvae shelves, with 16 plastic tanks (60 × 42 × 14 cm) on each larvae shelf (41 × 89 × 185 cm, eight layers) for manure loading. Pupae room was specially designed for the pupation of the third instar C. megacephala larvae. In the biodiesel production room, larvae were dried and ground, followed by larvae oil extraction and biodiesel production. In the storeroom, the products (biodiesel, fertilizer and feedstuff) were stored, with a corridor connecting these rooms. To prevent the adult flies fleeing away, screen doors were installed in the adult room and the pupae room; flypapers were also used in the corridor to trap the escaped flies.

2.4. Biodegradation of swine manure

Fresh swine manure was collected and transported from the pig farm every morning. As depicted in Fig. 3 and Fig. 1(b), 100 kg fresh manure was distributed averagely into the 16 plastic tanks, and each containing about 6 kg at a depth of 5 cm. Subsequently, C. megacephala eggs were incubated in the manure (0.5 g eggs/kg manure) about 1 day. After 6-day biodegradation, the third instar larvae were separated from the substrate by sieving. Six larvae shelves in the larvae room were used in rotation to ensure the daily processing capacity of 100 kg manure. Approximately 4% mature larvae were picked out and transferred to the pupae room for pupation. About 5 days later, the new eclosion adults were put into a cage for multiplication. After 7-day rearing in the cage, the adults began to produce eggs. The remaining 96% larvae were dried for oil extraction.

To evaluate the larval growth conditions in three substrates: two types of manure (sawdust manure and fresh manure) and artificial diet, the substrates (5 kg) were weighed, spread into larval tanks, and inoculated with 2.5 g eggs. Larval tanks with inoculated substrates were kept in the larval rearing room until the larvae pupated and 30 larvae were picked up randomly and weighed by electronic balance (AUW120D, SHIMADZU Japan).

2.5. Optimization of drying methods

We hypothesized that the feedstock oil (larvae oil) properties may be affected by the larvae drying method. After being separated from the manure, 1 kg third instar C. megacephala larvae were killed and dried directly in the oven (CH-0447, Jiangxi Fanqun Drying Equipment Factory China) at 60 °C for 12 h; 1 kg larvae were dried under the sunlight for about 2 days; and 1 kg larvae were killed by boiling water and then dried in the oven at 60 °C for 12 h. The three types of dried larvae were ground to pass through a 40 mesh screen, respectively. The larvae meal was then sealed in a cloth filter bag immersed in petroleum ether (bp. 60–80 °C) with stirring for oil extraction [18]. After 48 h leaching, solvent was recycled by vacuum distillation. The oil of dried C. megacephala larvae was calculated by the weight loss before and after extraction.

Properties of the three oils derived from larvae treated with three different drying methods were analyzed according to the standard methods (ASTM) in terms of acid value, iodine value,
saponification value and melt point. Oxidation stability was determined by the EN 14112 standard.

2.6. Biodiesel production

Fig. 3 outlines the procedures used to produce biodiesel from feedstock oil. As can be seen, larvae oil, methanol (molar ratio 6:1) and 1.6% KOH (Potassium hydroxide, as catalyst) were added into an autoclave for transesterification at 55 °C for 30 min, with agitation by a magnetic stirrer [12]. After the reaction, the mixture was separated by gravity. The upper layer was crude biodiesel and the lower layer was glycerin and water. Finally, the crude biodiesel were purified by distilling to remove the residual methanol and dried with sodium sulfate to remove the residual water.
2.7. Analysis

The fatty acid composition in the larvae oil was analyzed with a Thermo-Finnigan GC–MS system equipped with a polyethylene glycol phase capillary column (Agilent, USA) as previously described [19].

Biodiesel properties were measured in density (EN ISO 3675), viscosity (EN 3105), ester content (EN 14105), water content (EN ISO 12937), flash point (EN ISO 3679), and cetane number (EN ISO 5165).

The data obtained were submitted to analysis of variance, using the general linear model procedure of SPSS17.0 software. The significance of differences among treatments was tested by Duncan’s multiple-range test and a level of \( P < 0.05 \) was used as the criterion for statistical significance.

3. Results and discussion

3.1. Rearing of adult flies

Fig. 4 shows the productivity of one cage of adult \( C. \) megacephala flies, i.e., about 10,000 pupae in one adult cage (100 \( \times \) 100 \( \times \) 100 cm), and an eclosion rate of 80%. The amount of eggs collected one week after emergence was small (2.3 \( \pm \) 0.9 g), followed by a dramatically increase (71.5 \( \pm \) 4.8 g) during the second week and then the peak (114.2 \( \pm \) 13.2 g) and the secondary peak (81.9 \( \pm \) 8.8 g) during the third and the fourth week, respectively. It indicated that the old flies should be eliminated, and the cage should be prepared for another adult rearing four weeks after emergence.

Maintaining a sufficient and continuous egg production is essential for the conversion of swine manure into larvae biomass for oil extraction. Many factors can influence the oviposition of adult flies, such as fly strain, adult diet and adult population density [20–22]. But in our previous work, higher population density (>10,000 pupae) in adult cage may lead to high fatal rate (unpublished paper).

3.2. Swine manure biodegradation

As shown in Table 1, the three media (fresh swine manure, sawdust swine manure and artificial diet) were converted into \( C. \) megacephala larvae at a varying yield. Artificial diet achieved the highest larvae yield (12.39%), followed by sawdust swine manure (9.64%) and fresh swine manure (9.58%). Two previous studies reported that the yield of housefly pupae derived from pig manure in the Miloslavov pilot plant reached 4.8–8.1%, and 7.8% of the black soldier fly prepupae reared in poultry manure [22,23].

As shown in Fig. 5, the highest individual weight (0.0828 \( \pm \) 0.0015 g) for the larvae from the artificial diet occurred on the fourth day, but that for the larvae from two types of swine manure emerged on the fifth day, probably because fish meal and blood meal in the artificial diet provided sufficient nutrition for larval growth. The individual weight (0.0521 \( \pm \) 0.0027 g), probably due to the loose structure and good aeration of the former medium [24].

Oil content in larvae derived from sawdust swine manure was 21.11%, slightly higher than that of fresh swine manure (20.01%), but obviously lower than that of artificial diet (25.72%). It is reported that the oil content in \( C. \) megacephala larvae fed with food
is a common feedstock for the production of biodiesel [30]. Two fatty acids with an odd carbon number were identified from C. megacephala larvae oil: pentadecanoic acid (3.03%) and margaric acid (1.38%). The content of total odd carbon fatty acids oxide value indicates the level of rancidity during oil storage. All the data suggest that bathing live larvae in boiling water before oven-drying could improve the properties of feedstock oil for the coming biodiesel production.

In spite of substantial cost savings, the treatment of larvae with sun-drying requires enough land and more importantly depends on weather. Oven-drying larvae directly can save work, but the properties of the feedstock oil were negatively affected. Therefore, the cost effective method to process live larvae was the combined treatment with boiling-water and oven-drying, which can also be used in a large-scale larvae production.

### 3.4. Larvae oil chemical composition

Table 3 shows the fatty acids composition of the oil derived from C. megacephala larvae fed with swine manure. Totally, 10 different fatty acids were detected, and the main fatty acids identified were palmitic acid (36.91%), palmitoleic acid (10.89%), oleic acid (27.67%) and linoleic acid (9.49%). Except for the minor differences in the relative content of fatty acids, the composition of the swine manure larvae oil is similar to that of the restaurant garbage larvae oil [12].

The total saturated fatty acid composition of the C. megacephala larvae oil was 48.43%, higher than that of restaurant garbage larvae oil (43.17%) [12]. Saturated short-chain esters may be suited for biodiesel [30].

Two fatty acids with an odd carbon number were identified from C. megacephala larvae oil: pentadecanoic acid (3.03%) and margaric acid (1.38%). The content of total odd carbon fatty acids

<table>
<thead>
<tr>
<th>Composition</th>
<th>Structure</th>
<th>Swine manure</th>
<th>Restaurant garbage [12]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>14:0</td>
<td>1.90</td>
<td>3.91</td>
</tr>
<tr>
<td>Pentadecanoic acid</td>
<td>15:0</td>
<td>3.03</td>
<td>0.33</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16:0</td>
<td>36.91</td>
<td>35.48</td>
</tr>
<tr>
<td>Margaric acid</td>
<td>17:0</td>
<td>1.38</td>
<td>0.32</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18:0</td>
<td>5.21</td>
<td>2.77</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>20:0</td>
<td>–</td>
<td>0.36</td>
</tr>
<tr>
<td>Total saturated fatty acid</td>
<td></td>
<td>48.43</td>
<td>43.17</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>16:1</td>
<td>10.89</td>
<td>13.02</td>
</tr>
<tr>
<td>Hexadecenoic acid</td>
<td>16:2</td>
<td>1.31</td>
<td>0.70</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18:1</td>
<td>27.67</td>
<td>24.38</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2</td>
<td>9.49</td>
<td>15.26</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>18:3</td>
<td>0.98</td>
<td>1.25</td>
</tr>
<tr>
<td>Total unsaturated fatty acid</td>
<td></td>
<td>50.34</td>
<td>54.62</td>
</tr>
</tbody>
</table>
was 4.41%, higher than that of the restaurant garbage C. megacephala larvae oil (0.65%) [12]. The odd carbon fatty acids do not exist in oilseeds, but can be found in insects. There is no odd carbon fatty acid in rape biodiesel [31], but the content odd carbon fatty acids is 5.8% in the yellow mealworm beetle (Tenebrio molitor) biodiesel [18], and 2.4% in the B. peregrine larvae biodiesel [11]. It is notable that the fatty acid methyl esters derived from odd-carbon fatty acids have a better low temperature performance than those from even-carbon fatty acids [32].

3.5. Biodiesel properties

In this study, about 0.5L of biodiesel was produced from C. megacephala larvae fed with 100 kg of swine manure by the alkaline-catalyst transesterification method.

The swine manure larvae biodiesel properties were compared with those of the restaurant garbage larvae biodiesel in density, viscosity, ester content, flash point, cetane number and acid value, which were further compared with the EN 14214 standard [12]. Most of the fuel properties of C. megacephala biodiesel met the specifications of the EN 14214 standard, as listed in Table 4. The viscosity (5.1 mm²/s) was higher than that of the restaurant garbage larvae biodiesel (4.0 mm²/s), but lower than that of rapeseed biodiesel (6.35 mm²/s) and the black soldier fly biodiesel (5.8 mm²/s) [10,12,33]. It is reported that viscosity affects the atomization of a fuel upon injection into the combustion chamber and the structure of fatty acid alkyl esters [34]. The flash point of swine manure larvae oil-based biodiesel (138 °C) agrees well with the minimum specifications in the EN 14214 standard (120 °C), lower than that in the restaurant garbage larvae oil-based biodiesel (170 °C) [12]. However, the cetane number in swine manure larvae biodiesel (56) was higher than that in restaurant garbage larvae biodiesel (54.8). Cetane number is a dimensionless descriptor related to the ignition quality of a fuel in a diesel engine. Generally, the higher the cetane number, the better the ignition quality of the fuel and vice versa [30]. At last, oxidation stability of swine manure larvae was 4.2 h, shorter than the EN 14214 standard (6 h) but longer than that of restaurant garbage larvae oil-based biodiesel (3.6 h) [12]. Oxidation stability refers to anti-oxidation and the ability of a biodiesel or oil product to maintain the related qualities. It could be expressed by induction period (hour), which passes between the moment when the measurement is started and the moment when the formation of oxidation products rapidly begins to increase.

3.6. Economic benefit analysis

Actually, it is a project on comprehensive utilization of the swine manure via CML. About 5L (4.45 kg) biodiesel, 20 kg de-greased CML meal and 240 kg organic fertilizer (20% moisture) could be obtained from 1 ton fresh swine manure in our pilot-scale study. These products will produce about 319 RMB gross profits according to the current China market quotations. In China, about 258 million tons of swine manure are generated each year [35]. In the hypothesis of processing 50% manure with this method, approximately 0.57 million tons of biodiesel could be produced, which is equivalent to the yield of biodiesel from 2.85 million tons of soybean that cost 19.95 million RMB in China [36]. Thus it can be seen that CML biodiesel not only can bring the profits and mitigate the crisis of energy shortage but also has the potential environmental and social benefits.

4. Conclusions

A pilot-scale swine manure biodegradation system via C. megacephala (Fabricius) has been successfully established to produce larvae biomass for biodiesel production. The maximum larvae productivity reached 9.64% with a corresponding oil concentration of 21.11%. About 2 kg degreased larvae meal and 0.5 L biodiesel were produced from 100 kg fresh swine manure (73% moisture) per day in this pilot-scale plant. Larvae drying method was found to be a factor for the properties of feedstock oil and thus was optimized. The acid value (1.9 mg KOH/g), iodine value (86.3 gl/100 g), melt point (3.1 °C) and peroxide value (0.08 meq/kg) of the oil extracted from the boiling-water bathed larvae were improved compared with oven-drying larvae or the sun-drying larvae. Compared with the C. Megacephala oil derived from restaurant garbage, the swine manure larvae oil has a higher content of saturated fatty acid (48.43%) and odd-carbon fatty acid (4.4%). Furthermore, the larvae biodiesel properties met the EN 14214 standard in density (0.89 g/cm³), viscosity (5.1 mm²/s), ester contents (96.6%), flash point (138 °C) and cetane number (56). The results of this research demonstrated that C. Megacephala larvae can recycle swine manure into renewable energy, and reduce environmental pollution. This study suggests that the swine manure-grown C. megacephala larvae could be a feasible feedstock for a large-scale biodiesel production.

Acknowledgments

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References


