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Exploring the potential of grease from yellow mealworm beetle (*Tenebrio molitor*) as a novel biodiesel feedstock

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HIGHLIGHTS

- ▶ Yellow mealworm beetle grease could be a potential feedstock for biodiesel.
- ▶ Most of the properties of yellow mealworm beetle biodiesel met the EN 14214 standard.
- ▶ This study further indicated the promising use of insect fat to produce biodiesel.

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ABSTRACT

Biodiesel has been considered as one of the promising non-fossil fuels, but its development also have promoted a drastic debate due to its current production status, such as oilseeds dependency, arable land requirement, high cost and long-term impact on food prices. Therefore alternative resources with considerable lower cost that could be used for biodiesel production have been studied. Immature life stage of some insects is able to consume various organic wastes for fat accumulation. This high fat containing insect has the potential to serve as biodiesel feedstock. In this study, larval grease extracted from yellow mealworm beetle (*Tenebrio molitor* L.) (YMB), a post-harvest scavenger, was investigated for finding its potential as a substitute of oilseeds. Decayed vegetables were used to feed YMB and after 9 weeks, then the grease was extracted for biodiesel production. About 34.2 g biodiesel was obtained from 234.8 g dried YMB larval biomass. The main fatty acids of YMB biodiesel were linolenic acid (19.7%), palmitic acid (17.6%), linoleic acid (16.3%) and stearic acid (11.4%). Most of the properties of the YMB biodiesel fed on decayed vegetables met the standard EN 14214, including ester content (96.8%), density (860 kg/m^3), flash point (127 °C), cetane number (58), water content (300 mg/kg), and methanol content (0.2%). From comprehensive analysis on the effect to society, economy and environment, it can be concluded that YMB can recycle organic wastes into clean energy with low cost.

1. Introduction

With the rapid development of the global economy, energy crisis and environmental degradation have become a severe challenge that needs our whole human being to face together. For the last decades, many countries and scientific and technical workers have been involved in the effort to discover biofuels, such as biodiesel [1] and bioethanol [2]. Biodiesel has been increasingly recognized as one of the most promising renewable biofuels [3]. However, biodiesel also has aroused a drastic debate due to its current production status, such as oilseeds dependency, arable land requirement, high cost and long-term impact on food prices. Presently, biodiesel is mainly from edible oils; for example rapeseed oil is the major material for producing biodiesel in Europe, soybean oil in the USA, palm oil in Southeast Asia [4,5]. Large-scale production of biodiesel from edible oil could cause global imbalance to the food supply and bring more serious problem to the world's famine [6]. The main obstacle of biodiesel application is still the high cost in which about 75% comes from the feedstock, causing a tremendous restriction of its economic feasibility [7]. To mitigate this situation and reduce the biodiesel cost, many studies have been conducted to find new nonfood and cheap feedstock for biodiesel production,

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such as *Jatropha curcas* [8], *Chinese tallow* [9], and microalgae [10,11]. However, most of the researches are yet at the laboratory scale, due to the problem of high costs or unsolved technical barriers.

Insecta, which are rated as the most diverse animal group, are distributed extensively in nearly every corner of the world, from the equator to the poles. Insecta have attracted the attention of researchers for its potential as energy resource in recent years, in which the scavengers that can recycle organic wastes for fat accumulation are the most being investigated. Black soldier fly larvae (BSFL), *Hermetia illucens* L. which can colonize a wide range of organic matters including animal manure, domestic and municipal refuse, can use the waste nutrition for development in which high protein and fat were synthesized. This larval grease is a low cost, promising, non-food feedstock for biodiesel [12]. The grease derived from BSFL which is converted from different kinds of organic wastes has been proved a novel and available feedstock for biodiesel [13–15]. BSFL can convert organic wastes into biodiesel and protein feedstuff, which is in accordance with the concept of circular economy in short lifecycle, while the energy plants need long lifecycle and plenty of land which do avoid conflict with food necessary for human beings [16]. These scavengers may make waste profitable and provide a new choice for the feedstock of biodiesel. However, more candidate insects need to be evaluated based on their different feeding habits of all wastes.

Yellow mealworm beetle (YMB), *Tenebrio molitor* L. which is an important post-harvest scavenger and distributed all over the world, with preference to eat decayed grain or milled cereals in damp and poor conditions; in some cases they even infest stored products. Each female adult YMB lays about 300 eggs, which could be hatched into larvae in 10–14 days. YMB is very voracious and highly resistant to low temperature [17,18]. It is routinely used as traditional protein feedstock especially in aquiculture. However, no documents had reported the application of YMB grease as the raw material for biodiesel. In this study, YMB was fed with decayed vegetables, and then the grease was extracted to produce biodiesel. The results indicated that biodiesel could be produced with lower cost from the organic wastes by YMB.

2. Materials and methods

2.1. Biomass

The YMB colony used in this study was maintained in the National Engineering Research Centre of Microbial Pesticides, Huazhong Agricultural University, Wuhan, China. The decayed vegetables, such as carrots, lettuce seeds and Chinese leaves, were obtained from the local market of Huazhong Agricultural University and used to feed YMB larvae. The main components in the decayed vegetables were cellulose (32.5%), hemicellulose (21.8%), lignin (8.6%), soluble sugar (8.7%), protein (1.2%), fat and grease (0.7%) and impurities (19.6%). The cultivation was conducted in the insectaries at 25–30 °C, humidity (60–75%). Decayed vegetables were provided to YMB as needed. After 9 weeks, YMB were harvested and inactivated at 105 °C for 5 min, and then dried at 60 °C for 3 days. After being grounded with micro-mill, the dried YMB biomass was stored at 4 °C until grease extraction could be performed.

2.2. Grease extraction from YMB biomass

To increase the grease yield from YMB larvae, preliminary trials were conducted to evaluate the extraction efficiency of different organic solvents including ethanol, acetone, and petroleum ether with Soxhlet extractor. It was found that petroleum ether (bp. 60–80 °C) hold the highest efficiency and lower energy input. Hence the grease was extracted from the dried YMB biomass in a Soxhlet extractor with petroleum ether. After 8 h of refluxing, the solvent was distilled off. The crude grease of dried YMB biomass was calculated by the weight loss before and after extraction.

2.3. Refinement of the YMB grease

The crude grease contained various kinds of impurities including phospholipids, and solid impurities. After extraction, the crude grease was treated with 1–1.5% (V/V) of concentrated H_3PO_4 (85%, V/V) at 30 °C and 2–4% of softened water, and mixed subsequently. YMB grease was gently stirred, settled for precipitation, and filtered to remove the pectin. After this refinement process, YWB grease with high purity was obtained in the end.

2.4. Characterization of YMB grease

The properties of grease, such as iodine number, saponification value, and peroxide value, were determined according to the standard method. Iodine number indicates the amount of unsaturation in the YMB grease. Saponification value is represented by the milligrams of KOH, and is used for the measurement of the average molecular weight of the YMB grease. Melt point refers to the freezing point of the grease. Peroxide value indicates the level of rancidity during storage, while acid value reflects the amount of free fatty acid in the grease. Biodiesel was derived from different sources, such as fats and oils; YMB grease was suitable for biodiesel production.

2.5. Production of biodiesel from YMB grease

Biodiesel was produced by a two step process because of the content of free fatty acids in the YMB grease [13]. The reaction was carried out in a reactor equipped with a reflux condenser, provided with thermometer, mechanical stirring and sampling out let. The free fatty acids were changed into biodiesel to decrease the acidity of the crude grease, which was reduced to less than 0.1% after acid-catalyzed esterification. The mixture was poured into a funnel for separation. Then the upper layer was transferred to a reactor for alkaline-catalyzed transesterification by a previously reported procedure, in which a 6:1 M ratio of methanol and 0.8% (w/w) NaOH were added. The 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 in a funnel. The upper layer was then separated from the lower and purified by distilling at 80 °C to remove the residual methanol.

2.6. Analysis methods

The fatty acid composition of the YMB biodiesel was analyzed by GC/MS (Agilent, USA) equipped with a polyethylene glycol phase capillary column (Agilent, USA) [14]. The acid value, kinematic viscosity, cetane number and cloud point were determined according to the standard methods (ASTM). Oxidation stability was determined by EN 14112 method.

3. Results and discussion

3.1. Extraction of the grease from YMB

About 4000 YMB larvae (0.017 g, individual weight) were fed with 5000 g decayed vegetables (1.25 g/larva) within 9 weeks. About 704.1 g fresh YMB larvae (0.176 g, individual weight) were obtained after cultivation and 234.8 g of dried biomass was ob-