



Feasibility study of biodiesel production using lipids of *Hermetia illucens* larva fed with organic waste



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ABSTRACT

Hermetia illucens larvae by nature are a decomposer which fed on organic wastes. This study explores the potential of producing biodiesel using lipids from *H. illucens* larvae. Three types of organic wastes (sewage sludge, fruit waste and palm decanter cake from oil palm mill) were selected based on considerable generation and disposal concern in the area of study as well as lack of investigations as feed for *Hermetia illucens* larvae in current literatures. Growth rate of the larvae was determined with studying the changes in the biomass per day. *H. illucens* larvae fed with fruit waste and palm decanter cake have shown growth rates of 0.52 ± 0.02 and 0.23 ± 0.09 g d⁻¹, respectively. No positive sign of growth were observed in the larvae fed with treated sewage sludge (-0.04 ± 0.01 g d⁻¹). Biodiesel as fatty acid methyl ester (FAME) was synthesized by transesterification of the larvae lipid using sulphuric acid as catalyst in methanol. FAME produced was ascertained using ATR-FTIR spectroscopy and GC-MS. The main compositions of fatty acid were found to be C12:0, C16:0 and C18:1n9c. Fatty acid composition of C12:0 fed with fruit waste, sewage sludge and palm decanter was found to be most abundant in the larvae lipid. The amount of C12:0 obtained was 76.13%, 58.31% and 48.06%, respectively. In addition, fatty acid of C16:0 was attained at 16.48% and 25.48% fed with sewage sludge and palm decanter, respectively. Based on the findings, FAME derived from larvae lipids is feasible to be used for biodiesel production.

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1. Introduction

The increase in waste generation as result of population growth is among major concerns in many metropolitan areas around the world. If the waste is not managed well, it will pose serious threats to the environment and can cause rapid deterioration in public hygiene and health (Kargbo, 2010). Foul odour, emission of green house gasses like methane gas, contamination of water due to underground leaching and diseases spread by vectors are some of the consequences from the organic waste mismanagement. On another aspect, energy demand along with the search for alternative renewable energy is rising to reduce the dependency on petroleum fuel which is non-renewable and is declining in availability. Therefore, it would be certainly an ideal solution to address both problems if the organic waste can be transformed into higher value product.

The concept of bioconversion seems to be an attractive solution that can address both these issues of organic waste management and energy scarcity concomitantly. Being a natural process,

bioconversion is sustainable where the process uses insect larvae such as *Hermetia illucens* larvae to transform organic waste. Subsequently, *H. illucens* larvae bio-convert the nutrient obtained from the waste and stored as their biomass. Furthermore, majority of the larvae biomass consists at least 30% of lipid (Sheppard et al., 1994). Hence, the bioconversion by *H. illucens* is a win-win process not only assisting in waste management but the amount of lipid obtained is upcycled into biodiesel as an alternative feedstock for renewable energy.

Other related bioconversion studies using lipid accumulation microorganisms or oleaginous microorganisms such as yeast and fungi for biodiesel production fed with various sources of wastewater have been reported. The used of this oleaginous microorganisms have drawn attentions because of their ability to accumulate humongous quantity of cellular lipids (Mondala et al., 2009; Angerbauer et al., 2008; Chen et al., 2012; Seo et al., 2013; Liu et al., 2013; Cheirsilp et al., 2011). However, there are very few studies on biodiesel production utilizing invertebrate such as insect larvae (Manzano-Agugliaro et al., 2012). *H. illucens* larvae are an ideal candidate to be considered for biodiesel production. *H. illucens*, which is commonly known as Black soldier fly, has not been reported as a vector as the adult does not have mouth

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parts to feed and relying on the food-reserve produced during the larval stage (Sheppard et al., 1994, 2002; Tomberlin and Sheppard, 2002; Newton et al., 2005). This is unlike the common housefly (*Musca domestica*) whose adult do need to feed and is a vector.

Production of biodiesel using *H. illucens* fed with dairy manure was done by Li et al. (2011). The study was conducted by extracting the lipid content obtained from *H. illucens* using three different methods such as immersing, Soxhlet and ultrasonic method. The biodiesel yield obtained when fed with dairy manure was 0.75 g d⁻¹. Their lipid profile, revealed 10 kinds of fatty acid predominantly from C10 to C22, consisting of 15.8% of saturated and 39.8% unsaturated fatty acids. Nonetheless, C15:0 and C19:0 were found, which accounted for 1% and 1.4%, respectively. The highest saturated fatty acid determined was lauric acid (35.6%) followed by palmitic acid (14.8%) while the highest unsaturated fatty acid determined was oleic acid (23.6%). Biodiesel production using restaurant food waste and rice straw as feeds for *H. illucens* larvae was investigated by Zheng et al. (2012a,b). The fatty acid components identified were found to be similar to those of Li et al. (2011). In Zheng et al. (2012a,b) study, the yield of biodiesel was 4.38 g d⁻¹. This indicates that the production of biodiesel per 1000 larvae is 21.9 g compared to Li et al. (2011) which is 13.1 g per 1000 larvae. Similar studies for biodiesel production was done by Li et al. (2012) using oriental latrine fly (*Chrysomya megacephala*) and Zheng et al. (2013) using yellow mealworm beetle (*Tenebrio molitor*) larvae. The maximum FAME yield achieved was 87.7%, transesterified using 1.6% KOH with molar ratio 6:1 of methanol to oil for 30 min (Li et al., 2012). Where else, Zheng et al. (2013) obtained 34.2 g of biodiesel from *T. molitor*. In this study the feasibility of biodiesel production using lipid from *H. illucens* larvae fed with three types of organic wastes was investigated. The selected wastes were from three different producer category namely municipal services waste (sewage sludge), horticulture waste (fruit waste), and industrial waste (palm decanter cake from oil palm mill).

2. Materials and methods

2.1. Feed preparation

Sewage sludge was acquired from a sewage treatment plant at a location in Kampar, Perak, Malaysia. Fruit waste was collected from the university cafeteria and Palm decanter cake was collected from Cahaya Muda Oil Palm Mill, Bidor, Perak, Malaysia. All the organic waste feeds were prepared in a batch stock and stored in refrigerator at -18 °C. A required portions of each fed was prepared before experiments and brought to ambient temperature.

2.2. Farming of *H. illucens* larvae

H. illucens adults used in this study were acquired from wild population. The colony was set up within the university campus at a location near to the natural tree-grown area. Adult flies were lured into a cage ($H \times W \times L$: 120 cm \times 40 cm \times 40 cm) using fruit waste. Thereafter, the *H. illucens* population was reared in captivity for at least 3 generations for consistency. The eggs oviposited by the adult flies were left to hatch until larvae emerged 4–6 days later. Finally, the larvae were allowed to feed on the fruit waste for 7 days prior to being used in the experiments.

2.3. Experimental design

Each treatment (comprising 200 larvae per container) was done in triplicates. The container was equipped with perforated lid and has the dimension of 15 cm \times 10 cm \times 7 cm. Feeds were supplied

to the larvae in three different amounts; 1, 5 and 25 g. Feeds were administered daily until the larvae reached pupation. The weights of the larvae were recorded daily using sartorius analytical balance with readability of 0.1 mg. The larvae were cleaned and blotted dry prior to weight measurement. Pre-pupae were isolated from their container, weighed and placed in another dry container to allow them to complete their life-cycle as pupa and adult. The rate of waste consumption by the larvae was evaluated in terms of waste reduction index (WRI). The efficiency of the larvae to consume and metabolize the waste was evaluated in terms of efficiency of conversion of digested food (ECD). The growth rate (GR, g d⁻¹) was determined too using the method described by Stefan et al. (2009). Eqs. (1)–(3) were used for determination of WRI, ECD and growth rate.

$$WRI = \frac{D}{t} \times 100 \quad (1)$$

where $D = \frac{(W-R)}{W}$

W = Total amount of feed applied, g

R = Residual of feed, g

D = Overall degradation

t = Duration, days

$$ECD = \frac{B}{(I - F)} \quad (2)$$

where

B = prepupae biomass, g

I = total feed offer, g

F = the residual feed leftover, g

$$GR = \frac{(\text{Final body weight} - \text{Initial body weight})(g)}{\text{Rearing durations in days}} \quad (3)$$

2.4. Statistical analysis

Results were expressed as means \pm SE ($n = 3$). The data were analyzed using Microsoft Excel 2010 for one way ANOVA with 95% confidence level to establish whether a statistical significant difference occurred between feed loading of 1, 5 and 25 g. Differences were accepted as statistically significant when $P < 0.05$.

2.5. Ultrasonic aided in-situ transesterification

The ultrasonic aided in situ transesterification was carried out using 10 g of dried larvae suspended into methanol containing 5% v/v of H₂SO₄ acid catalyst in a screw cap vessel. The experiments were carried out using 8:1 and 12:1 mass ratio of methanol to dried larvae. The vessel containing the reaction mixture was sonicated in the ultrasonic bath at 55 °C for 30, 45 and 60 min. A 25 mL of n-hexane was added to each of the reaction vessel to enhance the lipid solubility. After in-situ transesterification, 50 mL of n-hexane was added to the reaction mixture and was centrifuged at 3000 rpm for 5 min and the supernatant was transferred to a separation funnel. Further extractions were repeated 3 times for 15 min. The extracts were washed with warm distilled water to remove excess traces of acid catalyst and methanol. Two layers were formed during the washing and the upper hydrophobic layer containing n-hexane and FAME was collected, dried over anhydrous sodium sulphate and stored in a container for further analysis.

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