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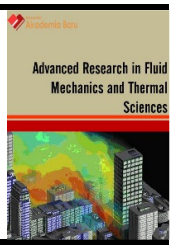

Lipid and Protein from Black Soldier Fly Larvae Fed with Self-Fermented Coconut Waste Medium

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ABSTRACT

This study explored the potential of black soldier fly larvae (BSFL) in producing lipid and protein as well as its ability to treat the organic waste by rearing at different fermentation periods of coconut waste medium (0, 2, 4, 6 and 8 weeks). Growth rate of larvae was determined by studying the changes in the larvae biomass weight per rearing duration. The BSFL fed with 4 weeks of feed showed the highest growth rate and weight followed by week 6 and week 8. Week 4 attained the highest value for lipid (42.74 ± 2.06)% and week without fermentation had the lowest value of lipid (32.96 ± 1.99)%. Protein content obtained from the BSFL was increasing with fermentation period. The highest protein content was larvae fed with 8 weeks fermentation (18.63 ± 0.18)%. The lowest protein content was also larvae fed with without fermentation medium (10.81 ± 0.11)%. Waste reduction rate (WRR) was the highest when the larvae were fed with medium without fermentation that was (0.024 ± 0.001) g/d. The lowest WRR was when the larvae were fed with 8 week fermentation medium (0.015 ± 0.001) g/d. The highest Efficiency of Converted of Digested Food (ECD) value was found in sample of 4 week fermentation medium (0.093 ± 0.003). The lowest ECD value was found in the sample without fermentation (0.063 ± 0.002).

Keywords:
Black soldier fly larvae, coconut waste, protein, lipid

1. Introduction

Since the last century, the world is experiencing crises regarding to the depletion of fossil fuel and its consequential usage impacts on the environment. The burning of fossil fuels has engendered many environmental issues with particular global warming that transpires from the unabated injection of greenhouse gases to the atmosphere. Therefore, the necessities for the searches of more environmentally friendly and sustainable renewable energy sources have to be urgently addressed with serious actions follow.

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Accordingly, significant studies have led to the development of practical option fuel sources [1]. Alternative fuels, known as non-conventional or advanced fuels, are any material or substance that can be utilize as fuel, other than conventional fuels like petroleum, coal and natural gas [2]. Some well-known alternative fuels include biodiesel, hydrogen, non-fossil methane, non-fossil natural gas, vegetable oil and other biomass sources [3]. Biodiesel is considered to be one of the most promising alternatives to fossil fuels due to its environmentally friendly, sustainable and renewable properties [2]. Biodiesel is also conventionally recognized to own combustible potential comparable with fossil fuels. Biodiesel is a mono-alkyl esters of long chain fatty acids derived from renewable resources [4] which typically satiating the requirements decreed by either European (EN 14214) Standard or American Society for Testing and Materials (ASTM D-6751) Standard. Other standards such as DIN Standards, National Standards of Canada for biodiesel, etc. are as well regionally applicable. Alternative feedstocks for biodiesel production can be utilized from edible oils to non-edible oils including BSFL oil, animal fats, waste cooking oil and recent innovative algae and microbial oils [5]. The advantages of using biodiesel as fuel are its portability, ready availability, renewability, high combustion efficiency, low sulphur and aromatic content, high cetane number and biodegrability [6]. Ninety-five percent of global biodiesel production was made from edible vegetable [7]. The cost of these materials accounts for 60-75% of the total cost of biodiesel production [7]. Thus, non-food feedstock such as microalgae and sludge are being developed for biodiesel production [8]. However, this alternative faces their own challenges such as long life cycle and limited water resources [8]. The alternative feedstock should be technically feasible, economically competitive, environmental acceptance and easily available [8]. Insects belong to the phylum Arthropoda, an invertebrate animal having an exoskeleton. Naturally, insect larvae will enhance its metabolic reserves to accumulate lipids in fat body. Fortunately, many insect's larvae showed high efficiency to degrade organic waste and use it as their nutrition supply [6]. *Hermetia illucens L.* (Diptera: Stratiomyidae), known as black soldier fly (BSF), is inclined to live in the outdoors and often associated with livestock, usually around decaying organic wastes such as animal waste or plant material [9]. The BSF prepupae consume large quantities of organic matter in a short period of time, reducing pathogens and converting excreta into organic fertiliser. The organic materials were converted into soluble organic molecules, and then incorporated into its biomass with high level of protein and grease [11]. Grease from black soldier fly larvae (BSFL) is a low cost biodiesel feedstock, and the bioconversion of organic wastes into biodiesel has a beneficial effect on the environment [12]. The aims of this study were to determine whether protein and lipid accumulations of BSFL differ when fed with varying self-fermented feed mediums, as well as to evaluate the organic waste treatment by BSFL reared at different fermented mediums.

2. Materials and Methods

Black soldier fly used in this study was acquired from wild population. The colony was set up within vegetation area of Universiti Teknologi PETRONAS. The female flies were lured by using coconut waste. The eggs were then collected in a corrugated cardboard [8] held in outdoor colony housed in a cage. Neonate larvae was held for 4-6 days to reduce its mortality from handling and then 20 larvae were distributed into five different self-fermentation treatments that are 0, 2, 4, 6 and 8 weeks by putting the coconut waste into a tightly closed cap bottles. Then, the larvae weight was measured by using Sartorius analytical balance with readability of 0.1 mg.

A. Biodiesel Production

1) Preparation of Sample: The larvae was washed with distilled water and inactivated in boiling water for 10 seconds and then dried at 105˚C to the constant weight. Then, the samples were blended to get the powdered form. The biomass sample was stored at 4˚C until lipid extraction could be performed [9].

2) Extraction of lipid: The extraction of lipid was following Bligh and Dyer method. Approximately 1.6 ml of distilled water was added into 1 mg of dried sample in a glass vial. Then, 6 ml of mixture of chloroform and methanol (1:2) was added and the sample was vortex until it mixed well. A 2 ml of chloroform and 2 ml of distilled water each was added into the sample and put in the sonicator for 30 minutes at 50˚C. Then, the sample was centrifuged at 3500 rpm for 15 minutes. The lipids were collected by using micropipette and stored to be used for transesterification process.

3) Extraction of protein: The homogenized dried prepupae of BSFL biomass ground by micro-mill was initially weighed to 0.5 g and introduced into the kjeldahl flask. The biomass was then mixed with 10 mL of concentrated sulfuric acid and added with mixed catalysts containing 0.1 g of CuSO4.5H2O and 0.025 g of TiO^2 and a few boiling chips. The biomass mixture in kjeldahl flask was digested at about 380°C until the copious white fumes disappeared and the digestion continued until the transparent pale blue solution appeared approximately 30 min later. This solution was neutralized and its ammonium-nitrogen (NH^+_4-N) concentration was determined via distillation method. A 20 mL of filtered sample was transferred to the distillation flask and added with 2.5 mL of borate buffer solution and 3 drops of 6 N NaOH solution. The distillation flask was then placed into the distillation unit with the tip of the delivery tube situated below the surface of 10 mL of indicating boric acid solution in a 100-mL Erlenmeyer flask. Under a hot alkaline condition, the NH^+_4-N species in the sample was distilled and trapped in the boric acid solution. The distillation rate was set at 30 mL/min which enable 90 mL of distillate was collected in a period of 3 min, reaching a total volume of 100 mL in the Erlenmeyer flask. The concentration of NH^+_4-N (in mg/L) in the sample was determined by titrating with 0.008 N standard H_2SO_4 titrant until the colour of indicator was turned from green to pale lavender in the Erlenmeyer flask.

B. The Formulation

Growth Rate (GR)

$$= \frac{(F-I)}{D} \quad (1)$$

where,
F= Final body weight (g)
I = Initial body weight (g)
D= Rearing duration (days)

Waste Reduction Rate (WRR)

$$= \frac{(A-B)}{(D)(N)} \quad (2)$$

where,
A= Dry weight of feed applied (g)
B= Dry weight of feed after (g)
D= Rearing duration (days)
N= Number of larvae

Efficiency of Conversion of Digested food (ECD)

$$= \frac{(F-I)}{(A-B)} \quad (3)$$

where,
A= Dry weight of feed applied (g)
B= Dry weight of feed after (g)
F= Final body weight (g)
I = Initial body weight (g)

Overall Degradation (D)

$$= \frac{(A-B)}{A} \quad (4)$$

where,
A= Dry weight of feed applied (g)
B= dry weight of feed after (g)

Lipid content

$$= \frac{C}{(C+D)} \times 100\% \quad (5)$$

where,
C= weight of lipid (g)
D= weight of biomass (g)

Protein content = percentage of N ×6.25 (6)

Percentage of N

$$= \frac{N \times V}{M} x100 \quad (7)$$

where,
N= NH_4^+-N concentration (mg/ml)
V= Digested solution volume (ml)
M= Initial biomass used (mg)

3. Results and Discussions

A. Effect of Feed Fermentation on Weight and Growth Rate of BSFL

The weight gained by the larvae was studied through different fermentation weeks (Fig. 1). The weight gained by the larvae was found increasing with fermentation weeks where the increment showing 4 week fermentation had the highest weight gain and the weight was noticed declining when the larvae were fed with feed fermented for more than 4 weeks. The lowest weight obtained by BSFL was in medium without fermentation (0.4280 ± 0.0042) g and its weight increased until week 4. Week 4 was showing the highest weight obtained (0.6727 ± 0.0445) g and the weight decreased following week 6 (0.5718 ± 0.0350) g and week 8 (0.5213 ± 0.0471) g, respectively.

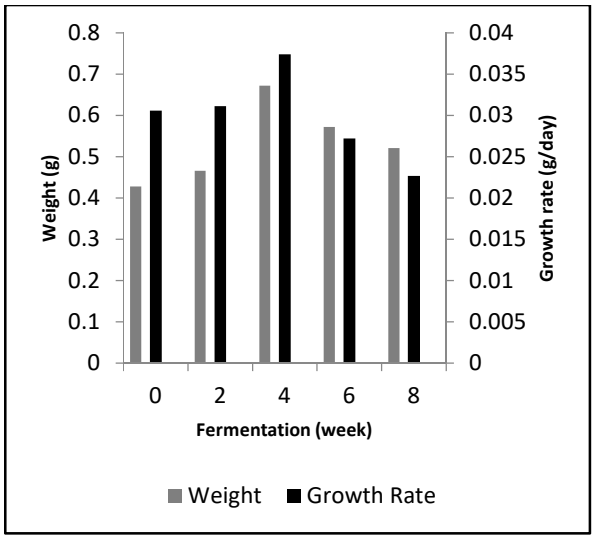

Fig. 1. Growth rate and weight performances by BSFL fed on fermented feed

The highest growth rates of BSFL were observed when the larvae were fed with 4 weeks fermentation medium (0.0374 ± 0.002) g/d. The Fig. 1 shows the growth rate of the larvae was progressively increasing when fed with medium without fermentation (0.0306 ± 0.000) g/d and 2 weeks fermentation (0.0311 ± 0.000) g/d and the increment ended at week 4. This was due to the maturity of the medium [9]. At week 4 fermentation, the medium was matured enough where it reach its highest nutrient level. After week 4, possibly the microbes in the medium died and it decreased the nutrient content in the medium [10]. This condition supported the deceleration of the larvae metabolism, affecting the growth rate. This was in accordance with the lower growth rate in week 6 (0.0272 ± 0.002) g/d and week 8 (0.0227 ± 0.002) g/d. During this study, smaller prepupae were observed when they were fed with shorter period of fermentation medium. Besides, shorter fermentation period showing the fastest maturity duration. The maturity duration for prepupae fed with medium without fermentation was 14 days followed by week 2, 4, 6 and 8 which are 15, 18, 21 and 23 days, respectively.

B. Effect of Fermentation of Medium on Protein and Lipid Contents of BSFL.

The different weeks of fermented medium were used to rear BSFL in order to determine its protein and lipid contents. From the Fig. 2, it shows that week 4 had the highest value for lipid (42.74 ± 2.06)% and week without fermentation had the lowest value of lipid (32.96 ± 1.99)%. Protein content obtained from the BSFL was increasing with fermentation. The highest protein content was larvae fed with 8 weeks of fermentation (18.63 ± 0.18)% followed by 6 weeks fermentation medium (18.17 ± 0.79)%. Nevertheless, the 6 weeks and 8 weeks protein contents were almost the same. The lowest protein content was larvae fed with without fermentation medium (10.81 ± 0.11)%. The Fig. 2 shows the protein content was increased rapidly until week 6 fermentation and increased inconspicuously to week 8 fermented medium. This was due to the biochemical processes that required for the development of necessary nutritional content desired for the larvae were achieved during 6 weeks fermentation [11].

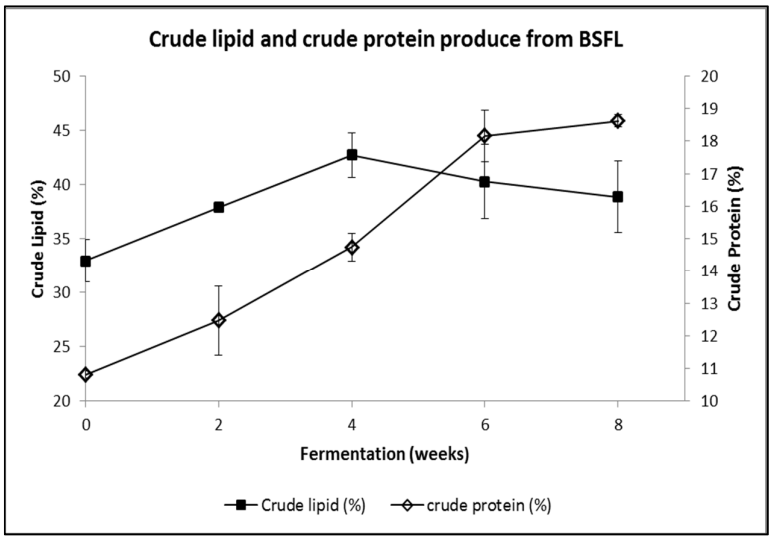

Fig. 2. The lipid and protein produce from BSFL biomass

C. Effect of Feed Fermentation on Waste Treatment.

Table 1 shows week 4 (0.362 ± 0.024) had the highest overall degradation and the overall degradation (D) of the waste was decreasing in week 6 (0.349 ± 0.034) and week 8 (0.346 ± 0.026). The lowest overall degradation was week without fermentation (0.342 ± 0.009). Overall degradation was related to the weight of the larvae attaining the highest weight when fed with 4 week fermented feed. As the fermentation week increased, the larvae were actively consuming the waste. However, because of low nutrient content from week 6 and week 8, the overall degradation was decreasing.

Waste reduction rate (WRR) was the highest when the larvae were fed with medium without fermentation (0.024 ± 0.001) g/d. The lowest WRR was when the larvae were fed with 8 week fermentation medium (0.015 ± 0.001) g/d. The efficiency of conversion of digested waste (ECD) values was found to be the highest in sample of 4 week fermentation medium (0.093 ± 0.003). The lowest ECD value was found in the sample without fermentation (0.063 ± 0.002). The value of ECD was increasing until 4 weeks fermentation and steadily decreased from 6 weeks to 8 weeks

fermentation. The process of organic waste decomposition in anaerobic conditions that produce NH_3 and CH_4 may also inhibit the waste consumption process by the BSF larvae [13-15].

Table 1
Waste Treatment by BSFL

Fermentation (weeks)	D	WRR (g/d)	ECD
0	0.342 ± 0.009	0.024 ± 0.001	0.063 ± 0.002
2	0.357 ± 0.000	0.024 ± 0.000	0.065 ± 0.001
4	0.362 ± 0.024	0.020 ± 0.001	0.093 ± 0.003
6	0.349 ± 0.034	0.017 ± 0.001	0.083 ± 0.008
8	0.346 ± 0.026	0.015 ± 0.001	0.075 ± 0.003

5. Conclusions

Black soldier fly fed with 4 weeks (0.6727 ± 0.0445) g feed heralding the highest growth rate and weight. The weight was decreased following week 6 (0.5718 ± 0.0350) g and week 8 (0.5213 ± 0.0471) g. The growth rate of the larvae was progressively increasing when fed with medium without fermentation (0.0306 ± 0.000) g/d and 2 weeks fermentation (0.0311 ± 0.000) g/d and the increment end at week 4. The lowest overall degradation was week without fermentation (0.342 ± 0.009) and week 4 (0.362 ± 0.024) had the highest overall degradation. Waste reduction rate (WRR) was the highest when the larvae were fed with medium without fermentation that was (2.441 ± 0.063) %. The lowest WRR was when the larvae were fed with 8 week fermentation medium (1.506 ± 0.112) %. The highest ECD value was found in sample of 4 weeks fermentation medium (0.093 ± 0.003). The lowest ECD value was found in the sample without fermentation (0.063 ± 0.002). Week 4 has the highest value for lipid (42.74 ± 2.06)% and week without fermentation has the lowest value of lipid (32.96 ± 1.99)%. Protein content obtained from the BSFL was increasing with fermentation. The highest protein content was larvae fed with 8 week fermentation (18.63 ± 0.18)% followed by 6 week fermentation medium (18.17 ± 0.79)% as 6 week and 8 week protein content was almost the same. The lowest protein content was larvae fed with without fermentation medium (10.81 ± 0.11)%. The profile of fatty acid methyl profile analysis will be determined in future study to confirm the biodiesel quality.

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