Blended Sewage Sludge?Palm Kernel Expeller to Enhance the Palatability of Black Soldier Fly Larvae for Biodiesel Production

Authors:

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Abstract:

Black soldier fly larvae (BSFL) have been employed for valorizing organic waste materials as the larvae are able to consume organic waste and transform it into valuable larval biomass. In this study, BSFL were found to potentially reduce blended sewage sludge. The addition of palm kernel expeller (PKE) fortified the protein and lipid content in blended sewage sludge substrates, leading to larval growth enhancement. In addition, the larval weight also influenced the lipid yield and fatty acid methyl ester (FAME) profile. However, the optimum ratio of sewage sludge to PKE had to be determined as excess PKE content could become a threat to larval growth by contributing to the reduction of non-fiber carbohydrates content in the feed, thereby resulting in the decrease in lipid yield and FAME content. In this work, a sewage sludge to PKE ratio of 2:3 proffered the highest larval weight gained at 46.99 ± 2.09 mg/larva. Meanwhile, a proportion of 3:2 of sewage sludge to PKE was able provide the highest lipid yield of 17 ± 1.77%. Furthermore, the FAME profile revealed the presence of a significant amount of saturated and monosaturated fatty acids, indicating a good quality biodiesel. Thus, BSFL-based biodiesel fed with blended sewage sludge and PKE could be utilized for producing a high quality biodiesel. However, further improvement on the amount of lipid yield and FAME content should be further investigated.

Article

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Abstract: Black soldier fly larvae (BSFL) have been employed for valorizing organic waste materials as the larvae are able to consume organic waste and transform it into valuable larval biomass. In this study, BSFL were found to potentially reduce blended sewage sludge. The addition of palm kernel expeller (PKE) fortified the protein and lipid content in blended sewage sludge substrates, leading to larval growth enhancement. In addition, the larval weight also influenced the lipid yield and fatty acid methyl ester (FAME) profile. However, the optimum ratio of sewage sludge to PKE had to be determined as excess PKE content could become a threat to larval growth by contributing to the reduction of non-fiber carbohydrates content in the feed, thereby resulting in the decrease in lipid yield and FAME content. In this work, a sewage sludge to PKE ratio of 2:3 proffered the highest larval weight gained at 46.99 ± 2.09 mg/larva. Meanwhile, a proportion of 3:2 of sewage sludge to PKE was able provide the highest lipid yield of 17 ± 1.77%. Furthermore, the FAME profile revealed the presence of a significant amount of saturated and monosaturated fatty acids, indicating a good quality biodiesel. Thus, BSFL-based biodiesel fed with blended sewage sludge and PKE could be utilized for producing a high quality biodiesel. However, further improvement on the amount of lipid yield and FAME content should be further investigated.

Keywords: black soldier fly larvae; palm kernel expeller; sewage sludge; lipid; biodiesel

1. Introduction

The increase in environmental concerns, especially climate change, has become an awareness issue for industries at the present time to reduce the amount of greenhouse gases emitted into atmosphere [1,2]. Combustion of fossil fuels stemming from myriad human activities, such as transportation, coal-fired power plants, and large industries, can pollute the air by producing greenhouse gases, particularly nitrogen oxides and ammonia. The presence of excessive nitrogen oxides in the atmosphere can contribute to environmental problems via the formation of smog and acid rain [3]. Thus, the utilization of biodiesel

instead of fossil fuels is considered clean in addition to its renewability, since biodiesel can be generated from biomass materials [1]. According to the United States Environmental Protection Agency, biodiesel can reduce the emission of greenhouse gases by 86% [4]. The increasing biodiesel consumption worldwide, i.e., approximately 429.9 thousand barrels per day, has led to the investigation of sustainable raw materials to produce biodiesel [5]. The production of biodiesel from edible crops, which were regarded as the first generation of biodiesel feedstock classification, can result in a shortfall of food sources. Lignocellulosic biomass has been studied for producing the second generation of biodiesel. However, advanced equipment is needed for the operational process, leading to high investment cost [6]. Although the third generation of biofuel has been investigated to replace the lignocellulosic biomass for producing biodiesel from microalgae and cyanobacteria, an expensive harvesting cost is inevitably required. Although these microorganisms contain a high level of lipid contents, i.e., over 75% in microalgae, ample time and intensive energy are needed for handling a large volume of microalgal feedstock [7–9]. To overcome the challenges, lipid derived from insect larvae has become an attractive source for biodiesel production as the larvae are considered an economical feedstock, easily grown, and can bio-convert organic waste materials into its biomass through biotransformation process [10–13].

Insect larvae can consume organic wastes, such as food waste, fruit and vegetable waste, animal manures, and sludge, then bio-convert the wastes into more valuable larval biomass for further applications [14–17]. Lipid from insects in the form of fat is a predominant biomass composition that has been studied to produce a sustainable biofuel [11]. Larval lipid is stored in its body to be used during the non-feeding period of the larval lifecycle [9,18]. Among the various insect species such as flesh fly, superworm, mealworm beetle, housefly, latrine blowfly, soldier fly, and ants, *Hermetia illucens* larvae, or black soldier fly larvae (BSFL), are commonly selected for producing biodiesel since BSFL can valorize a variety of organic wastes; contain a high level of lipid content (around 50%); the adults are not a pest; they can cope with a wide range of environmental conditions, e.g., pH, temperature, and humidity; and they need less workforce for mass rearing [19–28]. The fatty acid profile from BSFL lipid had been found mainly comprising of C12:0 (38.43 wt%), C16:1 (15.71 wt%), and C14:0 (12.33 wt%), which are the essential compositions in biodiesel. Addtionally, the high C12:0 in fatty acid methyl esters (FAMEs) of biodiesel could contribute to the low density and kinematic viscosity, and high oxidation stability of the produced biodiesel [29,30]. Leong et al. [31] confirmed that the FAMEs derived from extracted lipid of BSFL were mainly composed of C12:0 at 76.1%, 58.3%, and 48.1% when individually fed with fruit waste, sewage sludge, and palm decanter, respectively.

Sewage sludge, an organic waste from wastewater treatment plants, is expected to continue growing worldwide due to the rising number of households connected to central treatment plants [32]. The presence of untreated sewage sludge can afflict the surrounding environments since the sewage sludge's general compositions are recalcitrant organic pollutants. Furthermore, sewage sludge is also laden with degradable organic compounds, macronutrients, micronutrients, non-essential trace metals, organic micro-pollutants, microorganisms, and pathogens, which can cause difficulties for the disposal [33–35]. A proper management of sewage sludge is required, as the mismanagement of sewage sludge via agricultural use, landfill, and incineration have afflicted human health and environments [36]. Valorization of sewage sludge by BSFL is perceived as economical and environmentally friendly, since the larvae have a potential to assimilate the organic sewage sludge. Moreover, the lipid extracted from larval fat to produce biodiesel through transesterification was proved not to be contaminated with heavy metals from sewage sludge [37,38]. However, the sewage sludge's improper nutritional properties can retard larval development, such as slow growing time or small prepupal size impinging its mortality [17,31]. Thus, blending sewage sludge with low-cost and nutritionally rich feeding substrate was considered to improve the nutritional constituent in the feeding substrate, prior to administering it to BSFL. In this work, the challenge of sewage sludge management via exploiting BSFL to assimilate and produce more valuable chemicals, i.e., a

good quality BSFL-base biodiesel, was materialized. Accordingly, the aim was to use palm kernel expeller (PKE) as a nutritional substrate to blend with sewage sludge in improving the feeding substrate nutrition for enhancing larval development and larval biodiesel quality. This low-cost agrifeed comprises 14.5–19.6% dry matter of crude protein, which is a significant nutrient for spurring overall animal growth [39]. However, PKE alone had been found retarding BSFL growth, as the presence of excessive protein would create a deleterious environment for the growing larvae. Thus, it was evidenced that blending it with sewage sludge could synergistically enrich the substrate alimentation for the rearing of BSFL, targeting the production of lipid for the conversion into BSFL-based biodiesel. Upon completing the research, the projection for large-scale production of BSFL-based biodiesel was also unveiled, paving the way for its real application.

2. Materials and Methods

2.1. Preparation of Blended Substrates

The dewater sewage sludge and palm kernel expeller (PKE) were collected from the local municipal wastewater treatment plant and palm oil mill, respectively. The collected dewater sewage sludge possessed an initial moisture content of approximately 90%, while the PKE was already in the powdered and dry form. The sewage sludge was blended with PKE at various ratios: namely, sewage sludge to PKE at 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5 (signified as G1, G2, G3, G4, G5, and control, respectively). The total dry weight for each blended substrate was fixed at 10 g; for example, G2 was composed of 8 g of sewage sludge and 2 g of PKE, both measured in terms of dry weight. All substrates' moisture content was maintained around 70% before administering to the BSFL.

2.2. Experimental Set Up for BSFL Rearing

Fresh black soldier flies' eggs were bought from MLF Ingredient Sdn Bhd located in Johor, Malaysia. Upon arrival, the eggs were transferred into clean container and left in incubator at 27 °C until eclosion. The newly hatched BSFL were collected and reared using fresh coconut endosperm waste until 6 days old, prior to the employment for experiments. A total of 20 6-day old larvae were then handpicked and reared in a plastic container with the diameter of 8 cm, and height of 10 cm, as well as equipped with perforated lid. The blended feeding substrates at different ratios were individually administrated to each of the 20 BSFL. The rearing period was ended once 80% of BSFL had reached their 5th instar stage. The mature BSFL were then harvested via individual separation from the residual feed, washed with distilled water, and deactivated in freezer at −20 °C for 10 min before being dried in an oven at 65 °C until a constant weight was obtained. The duration of larval rearing from six days old until the day on which they were deactivated, and final total weight of larvae were recorded. The feed residue was also recorded to determine the overall degradation (OD) and waste reduction index (WRI) as presented by Equations (1) and (2), respectively. The larvae's digestibility of consumed feed was represented in terms of efficiency of conversion of digested feed (ECD) as shown in Equation (3). Finally, the metabolism of consumed feed (g) was calculated based on mass balance [15] as shown in Figure 1.

$$\text{Overall degradation (OD)} = \frac{\text{Total dry feed offered (g)} - \text{Dry residue remained (g)}}{\text{Total dry feed offered (g)}} \times 100\% \quad (1)$$

$$\text{Waste reduction index (WRI)} = \frac{\text{Total dry feed offered (g)} - \text{Dry residue remained (g)}}{\text{Rearing duration (day)}} \quad (2)$$

$$\text{Efficiency of conversion of digested feed (ECD)} = \frac{\text{Larval biomass (g)}}{\text{Total dry feed offered (g)} - \text{Dry residue remained (g)}} \times 100\% \quad (3)$$
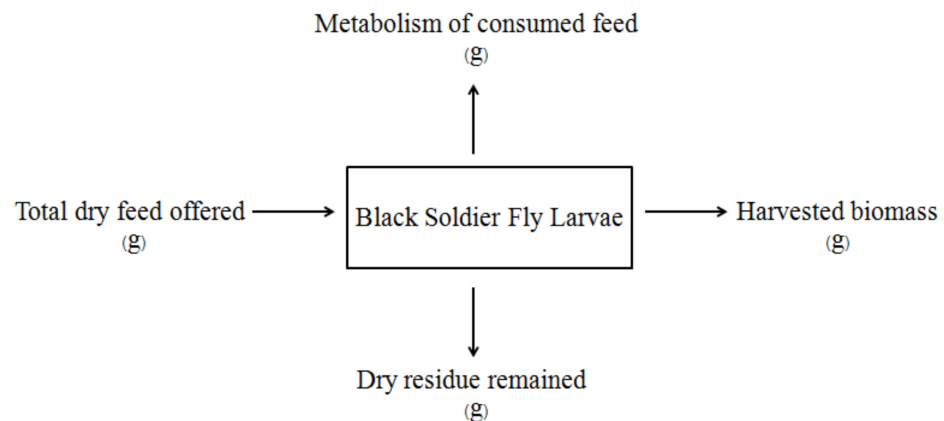
Figure 1. Mass balance model of valorization of blended sewage sludge with palm kernel expeller (PKE) via bioconversion into the biomass of black soldier fly larvae.

2.3. Lipid Extraction from BSFL Biomass

The dry larvae were mashed until becoming a homogenized form. A 100 mg of homogenized larval biomass was then introduced to a cylindrical glass bottle with a diameter of 23 mm, and height of 96 mm. A 20 mL of petroleum ether was then added into the same bottle and mixed on an orbital shaker operated at 300 rpm for 24 h. After that, the mixture of BSFL biomass and petroleum ether was filtrated with a filter paper. The petroleum ether was then evaporated from the filtrate under a blow of compressed air. Finally, the extracted lipid was introduced into an oven at 105 °C for 30 min to ensure the leftover moisture was as well evaporated. The lipid yield from prepupae in dry weight basis was then computed by using Equation (4).

$$\text{Lipid yield} = \frac{\text{Extarcted larval lipid (mg)}}{\text{Homogenized larval biomass (mg)}} \times 100\% \tag{4}$$

2.4. Transesterification of Larval Lipid into BSFL-Based Biodiesel

The extracted lipid was introduced with 1 mL of chloroform as a co-solvent to assist the lipid dissolution. A methanol containing 1 wt% of potassium hydroxide was then added to carry out a base-catalyzed transesterification process. The mixture was heated to 65 °C by heating mantle and stirred at 200 rpm for 1 h. Once the reaction had finished, the solvent consisting of methanol and chloroform was then evaporated at 70 °C. The fatty acid methyl esters (FAMEs) mixture had with 2 mL of petroleum ether added, and was washed 2 times with 2 mL of saline water (10% sodium chloride in methanol). FAMEs mixture was retrieved by using 1 mL of petroleum ether and transferred into a pre-weighed glass vial. Petroleum ether was then dried under a compressed air blow. Sample was introduced into oven at 105 °C for 30 min to ensure that the residue moisture was completely evaporated. Weight of FAMEs was measured once the glass vial was cooled down to room temperature.

Characterization of the FAME profile of BSFL-derived biodiesel was prepared by adding 1 mL of methyl heptadecanoate (C17:0) in hexane as an internal standard at the concentration of 1.012 mg/mL into a pre-weighed FAMEs sample. The FAME profile was analyzed by using the Shimadzu GC-2010 plus (Manufactured by Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and a polyethylene glycol capillary column BPX-BD20 (30 m × 0.32 mm × 0.25 μm). Helium gas was used as a carrier gas, and the inlet was operated in a split mode (1:50) at a temperature of 250 °C. The column temperature was programmed at the ramping mode. The percentage of FAME composition in BSFL-derived biodiesel was calculated as presented in Equation (5) [40].

$$\text{FAMEs content (\%)} = \frac{A_{FAME}}{A_{ISTD}} \times \frac{C_{ISTD} \times V_{ISTD}}{m} \times 100\% \tag{5}$$

where A_{FAME} is the peak area of specific FAMEs; A_{ISTD} is the peak area of internal standard (C17:0); C_{ISTD} is the concentration of internal standard (C17:0), which was 1.012 mg/mL; V_{ISTD} is the volume of internal standard (C17:0), which was 1.0 m; and m is the mass of sample used to mix with internal standard (C17:0).

3. Results and Discussion

3.1. Effect of Different Ratios of Blended Sewage Sludge on BSFL Growth

The growth of BSFL was studied in terms of rearing duration and final larval dry weight when fed with various blended sewage sludge (Figure 2). The larval weight was significantly influenced by the blended feed compositions [15]. In this study, the larval weight was the lowest with G1, i.e., the larvae fed with sewage sludge only (14.38 ± 0.34 mg/larva). This could be rationalized by the presence of extracellular polymeric substances formed by bacterial cells as a protective shield to prevent the digestion process by other microorganism colonies. Therefore, similarly, this also hindered the digestion by BSFL in assimilating the nutrients in sewage sludge, leading to the retardation of larval growth [31,41]. The larval weight was then found to be increasing when the PKE was blended into sewage sludge in G2 and G3 mediums and reached the highest value, i.e., 46.99 ± 2.09 mg/larva, in the G4 medium containing sewage sludge to PKE at a ratio of 2:3. As the proportion of PKE increased, it raised the protein content in blended feed, which was vital for larval growth to undergo pupation. The presence of more PKE would fortify the nutritional balance in the larval feed as well as enhance the buffer capacity in facilitating the BSFL digestion process [42–44]. However, the larval weight was noticed to be dropping conspicuously with a further increase in PKE in the G5 medium, primarily due to the presence of excess energy compositions in the feed. The major compositions in PKE were protein (14.4%), lipid (10%), and fiber (16%) [45]. The presence of a high protein level in the blended medium would contribute to a high energy input in BSFLs' diet. Thus, the excess protein content stemming from the increase in PKE proportion could retard the larval growth as too high an energy input from the feed would stimulate the BSFL to execute the detoxification of the proteinogenic nitrogen process within the larval digestion system [42,46,47]. Furthermore, the lipid constituent in PKE also offered a good source of metabolizable energy, increasing the energy density of feed to enhance the larval weight [48,49]. However, the excess amount of lipid content also could impede the larval development, since the unutilized lipid would hinder the digestion process by BSFL for assimilating nutrients [26]. The excess lipid could retard the fiber digestion process, since it inhibited the microbial activities within the larval digestive tract that are involved in cellulose digestion, thereby further decreasing the larval palatability towards feed [50,51]. The presence of high fiber content with the increase in PKE proportion in blended sewage sludge also could be associated with small larval weight since the fiber could not be easily digested and assimilated by the growing BSFL [17].
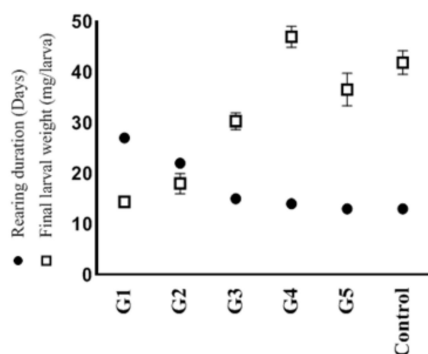

Figure 2. Effect of different ratios of blended sewage sludge on larval rearing durations and final black soldier fly larvae (BSFL) weights.

In a similar way, the rearing duration of BSFL was shorter when the amount of the propotion of PKE in the blended feeding substrate increased, i.e., it took 27 days from the larval rearing period for 80% to emerge from the 5th instar stage when fed with sewage sludge, but only 13 days whilst using G5 and control mediums. This could be ascribed by the effect of food nutrients on larval weight and rearing duration at the critical developmental stage [52,53]. The limitation of larval development to a defined time until pupation stage is affected by a shift in hormonal level at a critical developmental stage condition [54,55]. In addition, the larvae's critical weight, i.e., the larval final weight, was also relatively constant once the larvae had stopped feeding in preparation to enter pupation [56]. The rearing duration required for larvae to reach the critical weight was dependent on the nutrition availability, since the imbalanced nutrients in feed would result in a longer time entailed for feed accumulation to compensate for nutrient deficiency [52,57,58].

Table 1 shows the overall degradation (OD) and waste reduction index (WRI) investigated in the current study to prove the potential of BSFL to valorize solid organic wastes associated with the bioconversion process. The WRI increased with the increase in PKE. Generally, the higher the WRI, the higher the amount of waste being valorized per time period [9]. It was found that the capability of BSFL to valorize organic waste materials via assimilation for growth was dependent on the properties of feed as shown in terms of efficiency of conversion of digested feed (ECD). The optimum ratio of blended feeding substrate could proffer a high conversion of substrate into BSFL biomass. In this study, the ECD was in a range of 1–5%. The ECD values rose up with the increase in PKE and reached the highest in the G4 medium. The lower ECD indicated insufficient nutrition in blended sewage sludge for feeding of BSFL [15]. The ECD was then slightly decreased in the G5 medium, and this could be due to the presence of excess fiber that was difficult to be digested by BSFL [17].

Table 1. Valorization of various blended sewage sludge ratios by BSFL.

Parameter	Blended Sewage Sludge Medium					
	G1	**G2**	**G3**	**G4**	**G5**	**Control**
Overall degradation (%)	73.34 ± 5.41	67.39 ± 2.68	62.45 ± 4.31	75.91 ± 3.30	84.85 ± 3.03	85.74 ± 0.20
Waste reduction index (g/d)	0.54 ± 0.04	0.61 ± 0.02	0.83 ± 0.06	1.08 ± 0.05	1.31 ± 0.05	1.32 ± 0.00
Efficiency of conversion of digested feed (%)	1.14 ± 0.32	1.94 ± 0.23	2.71 ± 1.20	5.11 ± 0.22	3.65 ± 0.13	4.26 ± 0.29

The proportion of feed converted into larval biomass, feed used for metabolism, and feed residue for each ratio of blended mediums for BSFL feeding are shown in Figure 3. The increase in feed residue when fed BSFL with G1 to G3 could be ascribed to the insufficient nutrients presented in blended sewage sludge that afflicted the palatability of BSFL. The presence of only sewage sludge in the G1 medium would cause the BSFL to prioritize the allocated energy budget in its body to maintain homeostasis by increasing metabolism of consumed feed, reducing excretion, and increasing the amount of consumed feed, leading to a lower amount of feed residue as opposed to the G2 and G3 mediums [15]. The further increase in PKE in the G2 and G3 mediums led to the higher amount of feed nutrition than required for larval growth. Thus, the feed was left over as a feed residue [15]. However, the feed residue then decreased when employing the G4, G5 and control mediums. This could be due to the imbalance of nutrition in blended feed, resulting in higher metabolism of consumed feed for these mediums. The observation was in conformity with the discussion provided for the final larval weight parameter in the presence of excess lipid and protein from PKE (Figure 2).
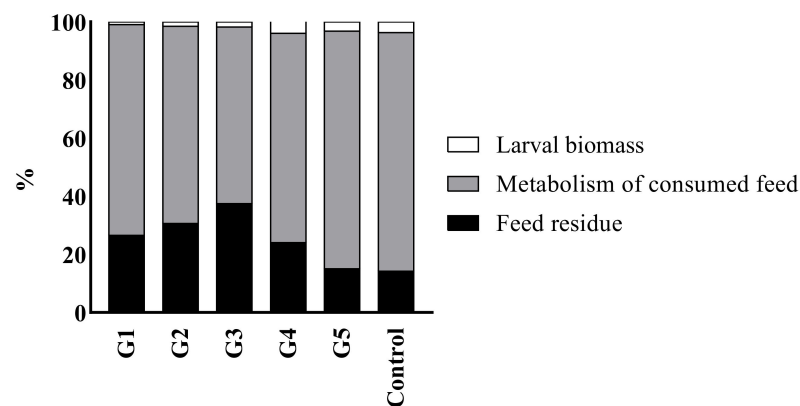
Figure 3. Proportion of feed converted into larval biomass, feed used for metabolism, and feed residue for each ratio of blended sewage sludge employed for BSFL rearing.

3.2. Effect of Different Ratios of Blended Sewage Sludge on BSFL Lipid Yield

The lipid yields from BSFL fed with various blended sewage sludge ratios are shown in Figure 4. The BSFL fed with sewage sludge only offered a lipid yield of merely 8.61 ± 0.18%. The values of lipid yield increased from 8.61 ± 0.18% to 12.81 ± 0.00% when the PKE was added into the sewage sludge and reached the highest at 17 ± 1.77% when fed with sewage sludge to PKE at a ratio of 3:2 in the G3 medium. The higher larval weight resulted in higher level of lipid yield [42]. However, the further increase in PKE caused the decrease in BSFL lipid yield due to the presence of more non-fiber carbohydrate (NFC) composition. The highest larval weight was obtained with the G4 medium, while the highest lipid yield was with the G3 medium. Apart from the effect of larval weight on lipid yield, the NFC content in feed was also considered as a factor that affected the larval lipid yield. The NFC content decreased once the proportion of PKE was increased [59]. As NFC content would affect the lipid accumulation in the larval body, the lower NFC level led to a lower lipid yield as reported by Danieli et al., (2019) [60] since carbohydrates would be converted into lipids in the larval body [39]. Hence, the appropriate protein and NFC composition in feed should be considered, as the protein supply played a significant role in larval weight and lipid yield. Nevertheless, the higher protein and lower NFC condition also led to lower lipid yields from harvested BSFL biomass [39].
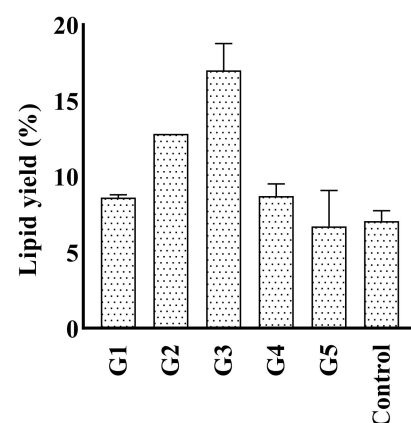
Figure 4. Lipid yields from BSFL fed with different ratios of blended sewage sludge.

3.3. BSFL-Based Biodiesel

The FAME profiles derived from larval lipids fed with various ratios of blended sewage sludge are presented in Table 2. The composition in feed mediums would affect the FAME compositions and subsequently the biodiesel qualities [9,61]. In this study, the highest percentage component in the FAME mixture was C12:0, followed by C18:1, C14:0,

and C16:0. The quantity of C12:0 and C18:1 contents corresponded with the presence of PKE, i.e., C12:0 content increased with higher PKE proportions and reached the highest in G3 before decreasing with further increase in PKE, while C18:1 decreased once PKE was added in feed.

Table 2. Percentage of FAME content of BSFL-derived biodiesel fed with various ratio of blended sewage sludge.

FAMEs	Blended Sewage Sludge					
	G1	**G2**	**G3**	**G4**	**G5**	**Control**
C10:0	0.86	5.56	4.48	18.90	16.35	5.73
C12:0	19.56	24.28	53.36	21.37	7.91	16.92
C14:0	10.26	16.35	12.52	6.62	16.06	11.01
C14:1	8.93	7.18	2.79	14.15	7.85	16.11
C16:0	15.34	10.24	9.89	16.13	11.93	5.28
C16:1	12.83	1.37	4.40	6.26	5.24	5.46
C18:0	-	2.56	1.23	8.67	5.82	10.30
C18:1	24.37	20.29	9.22	5.0	18.56	11.91
C18:2	7.86	12.17	2.11	2.89	10.30	17.28

Another point to notice was that the BSFL-derived biodiesel fed with various ratios of blended sewage sludge offered an essential composition to produce high quality of biodiesel. The FAME profiles revealed the dominating content of saturated fatty acid (SFA) and low in polyunsaturated fatty acid (PUFA) as shown in Figure 5 [62]. The content of SFA also provided a similar pattern of lipid yield and C12:0, i.e., the highest SFA content was obtained in G3, which was 81.48% before the percentage of SFA dropped with the increase in PKE proportion. The FAME profile could be ascribed to the effect of larval weight and NFC content in feeding substrate on FAME composition, since a larger larval weight would lead to more accumulation of C12:0 and SFA. However, the further increase in PKE would reduce the NPC content, which influenced the C12:0 component as BSFL would synthesize C12:0 from carbohydrates presented in feed as reported by Ewald et al. (2020). The high SFA and low PUFA contents could lower a cold plugging point that affects cold flow properties. Nonetheless, the higher molecular weight of FAMEs would increase viscosity and density, and the performance of oxidative stability was good for a better storability of biodiesel [42,61–64]. Hence, the optimum proportion of blended sewage sludge should be considered to determine the optimum proportion of SFA and PUFA for producing a good quality biodiesel. Furthermore, biodiesel with a high SFA level indicates a higher cetane number than biodiesel with a high level of unsaturated fatty acid, i.e., better fuel combustion inside a compression engine [65].
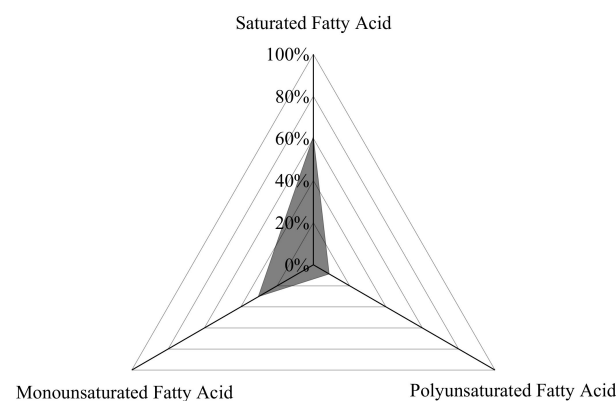

Figure 5. Degree of unsaturation of fatty acid methyl esters (FAMEs) profile in BSFL-based biodiesel fed with various ratios of blended sewage sludge.

3.4. Projection for Large-Scale Production of BSFL-Based Biodiesel

With regard to the worthiness of valorizing blended sewage sludge by BSFL for the subsequent projection to industrial scale, the economical calculations were estimated based on lab-scale data whilst using discounted cash flow and benefit per cost analysis. For an industrial scale, the calculations can be generally divided into 4 parts: namely, (1) investment cost, (2) operating cost, (3) transportation cost, and (4) product cost, i.e., biodiesel cost. First, the investment cost encompasses installation and equipment cost, and this expense is usually incurred once, such as industrial reactor plants. Next, the operating cost which will be expensed annually is calculated from electricity, chemicals, water system, and other utilities used in the plant. The transportation cost (T) will mainly depend on the distance between the sewage sludge and PKE collection points and the BSFL-based biodiesel processing line (D), the number of loads (N), and the rate of the truck (r) as shown in Equation (6) [66]. Finally, the product cost, i.e., biodiesel in this case, is influenced by the feedstock cost, plant size, and value of glycerin by-product. Both the sewage sludge and PKE employed in the current study for feeding of BSFL were considered economical feedstock, since those are organic wastes generated abundantly from wastewater treatment plants and palm oil mills, respectively [67]. Moreover, the BSFL used as mediator is also cost-favorable since this species is abundant in tropical regions, and the larval rearing process is not complex [28].

$$T = DNr \qquad (6)$$

The estimation would be based on the calculations that focused on feedstock, chemicals, and main product (biodiesel) prices for the present study. Indeed, the worthiness investigation for larval biodiesel production should also cover the calculations of the whole industrial scale that can be simulated using ASPEN PLUS software [68]. Furthermore, both investment and transportation costs could not be precisely determined in this study, since the investment cost depends on each plant, and transportation cost will be calculated based on the agreement between plant and transporter [66]. In the case of product cost, the biodiesel price would be calculated based on B7 (7% blended biodiesel) price since this is a current biodiesel mandate in Malaysia's industrial scale [69]. Accordingly, Malaysia has six refineries with a production capacity of approximately 596,700 barrels per day. In this study, it was assumed that the production of biodiesel was 99,450 barrels per day per plant, i.e., 15,811,286 L per day per plant [70]. The biodiesel price is based on palm methyl ester price at fuel station which was about 2.28 MYR/liter or 0.56 USD/liter [71]. Thus, the biodiesel could be produced about 5534 million liters per year and the revenue from biodiesel was around 3099 million USD/year. Those values were calculated with the assumption that the plant starts running after 15 days, since the BSFL offered the highest yield with G3 blended sewage sludge with 15 days of rearing. The process was also assumed to be running every day of the year with no shutdown. As the highest lipid yield in this study was 17% while employing the G3 medium, the quantity of sewage sludge and PKE used as a feedstock were 18,340,162 and 12,226,774 tons/year, respectively. Even though the sewage sludge was assumed to have no cost, in fact, the storage tank or transportation costs of sewage sludge are usually needed; while PKE was priced at about 119 USD/metric ton. Hence, the feedstock price was estimated at around 1320 million USD/year. In addition, the chemicals costs used for lipid extraction and transesterification processes, namely, petroleum ether, chloroform, 1 wt% KOH/MeOH, and 10% NaCl/MeOH, were obtained based on the prices by KSFE Company. In this regard, the estimated total cost of chemicals expense annually was around 78,623 million USD. Even though the BSFL-based biodiesel rendered a good quality of biodiesel, the lipid yield was still low, leading to unprofitable cost, with a benefit per cost ratio of less than 1.0. As the operating cost was significantly spent on chemicals, and 90% of total cost was incurred by the feedstock, our research used organic waste which was considered an inexpensive feedstock cost. Thus, once the industrial grade chemicals could be precisely determined with improvement on large-scale extraction and transesterification processes, investment in BSFL feedstock should be an interesting alternative to produce biodiesel [72]. In addition, in terms of environmental values, biodiesel can emit

CO_2 content, particulate matters, carbon monoxide, and hydrocarbons to a lesser extent than combustion of diesel fuel, which contributes to the reduction of total impact cost of particulates [73]. Currently, the lipid extraction method, which influences biodiesel quality used on a large scale, is still scarce. However, the supercritical carbon dioxide extraction method seems to be a suitable method for lipid extraction in upscaling industries at the moment, as this method leaves less effects on environment, i.e., emitting less CO_2, and the residual biomass can be used for agricultural application [74]. The alkali-catalyzed transesterification to convert lipid into biodiesel and alkyl oxide solutions of sodium methoxide or potassium methoxide in methanol was confirmed as the most widely used for large continuous-flow production processes [72,75].

4. Conclusions

Black soldier fly larvae (BSFL) had a potential for valorizing blended sewage sludge and converting into valuable larval biomass through biotransformation process. The feed composition had been found to significantly affect larval growth, lipid yield, and fatty acid methyl esters (FAME) composition. The addition of palm kernel expeller (PKE) could enhance larval growth and biomass weight due to the presence of more protein and lipid content in feed. However, excess PKE content could negatively affect larval rearing since too high an energy and fiber content would retard larval growth. Furthermore, the larval weight would affect the lipid yield and FAME profile. Nevertheless, the proper proportion of sewage sludge and PKE should be considered, as excess PKE composition would lower the non-fiber carbohydrate content in feed, contributing to lesser lipid yield and FAME content. In this study, the optimum ratio of sewage sludge to PKE was obtained at 3:2, to provide the highest lipid yield and FAME content. Moreover, the FAME profile presented a significant composition that resulted in a good quality of biodiesel. Hence, the BSFL fed with blended sewage sludge had a potential to serve as the feedstock for producing biodiesel. However, the improvement of feeding substrate should be further investigated to enhance the quantity of lipid yield and FAME content.

Conflicts of Interest: The authors declare no conflict of interest.

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