

# A Review on Insights for Green Production of Unconventional Protein and Energy Sources Derived from the Larval Biomass of Black Soldier Fly

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## **Abstract:**

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Review

# A Review on Insights for Green Production of Unconventional Protein and Energy Sources Derived from the Larval Biomass of Black Soldier Fly

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**Abstract:** The purpose of this review is to reveal the lipid and protein contents in black soldier fly larvae (BSFL) for the sustainable production of protein and energy sources. It has been observed from studies in the literature that the larval lipid and protein contents vary with the rearing conditions as well as the downstream processing employed. The homogenous, heterogenous and microbial-treated substrates via fermentation are used to rear BSFL and are compared in this review for the simultaneous production of larval protein and biodiesel. Moreover, the best moisture content and the aeration rate of larval feeding substrates are also reported in this review to enhance the growth of BSFL. As the downstream process after harvesting starts with larval inactivation, various related methods have also been reviewed in relation to its impact on the quality/quantity of larval protein and lipids. Subsequently, the other downstream processes, namely, extraction and transesterification to biodiesel, are finally epitomized from the literature to provide a comprehensive review for the production of unconventional protein and lipid sources from BSFL feedstock. Incontrovertibly, the review accentuates the great potential use of BSFL biomass as a green source of protein and lipids for energy production in the form of biodiesel. The traditional protein and energy sources, preponderantly fishmeal, are unsustainable naturally, pressingly calling for immediate substitutions to cater for the rising demands. Accordingly, this review stresses the benefits of using BSFL biomass in detailing its production from upstream all the way to downstream processes which are green and economical at the same time.

**Keywords:** black soldier fly larvae; protein; lipid; biodiesel; substrate; transesterification

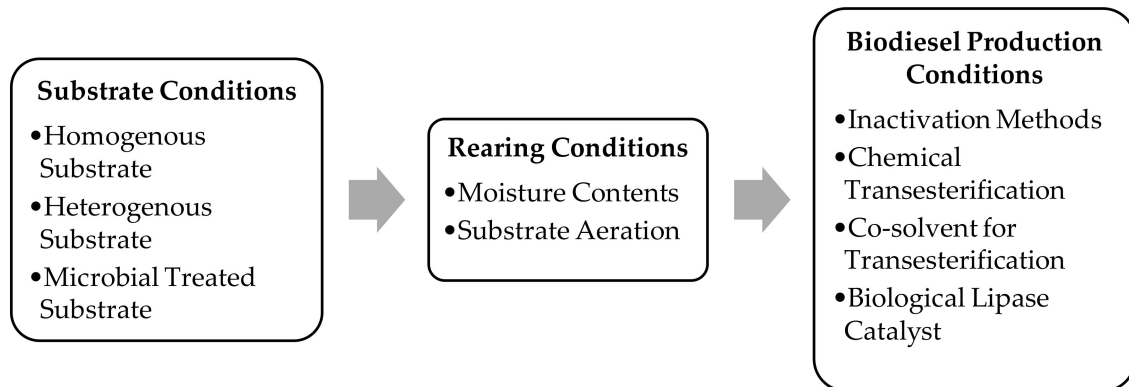
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## 1. Introduction

Fossil fuel holds the position of being the main source of energy consumed in the world. According to the World Energy Forum, the reserves of fossil-based oil, gas and coal, used mainly in the transportation, agriculture, domestic and industrial sectors, will be exhausted in less than a decade. As this main source of energy is rapidly diminishing at an alarming rate, it has accelerated the demands to find an alternative source that serves the same functions. This has lead researchers to consider renewable energy, offering not only improved energy security, but also a chance for the planet to reduce carbon emissions while providing much cleaner air. This in turn will permit the future generation to have a more sustainable green footing in regard to the environment. According to Barnwal and Sharma [1], fuels that are of biological origin, originating from vegetable oils, alcohol, biomass and biogas, are some of the alternatives presented from these past few years as sustainable fuels. Some of these fuels can be used directly, while others may need further modification before the fuels can be used. Biodiesel, one of the alternative fuels that originates from vegetable oils, animal fats and microorganisms such as microalgae, yeast, bacteria and fungi, shows promising results in becoming the main source of energy. For maximum yield, a transesterification process is carried out on the glyceride of the oily sources with alcohol in the presence of a catalyst to form fatty acid alkyl esters and glycerol [2]. However, biodiesel has challenges in implementation due to its high cost and limited availability of resources rising from the food versus fuel issue [3]. This is because the sources were limited to plant and animal feedstock, thereby competing with a food source needed for consumption. Microorganisms then became a new interest in synthesizing biofuels, making microbes such as bacteria, fungi and microalgae the next generation of biodiesel [4]. It was determined that microalgae contained the highest lipid content, over 75% measured relative to dry biomass weight [5]. However, this new source has led to the other problems such as extensive time consumption of medium preparation and intensive energy requirement for harvesting as microalgae are more buoyant and difficult to settle [6].

Thus, to generate biodiesel in a more favorable condition, researchers have suggested to derive the sustainable fuel from insects. Fuels derived from insects through insect farming allow several biochemical products and byproducts to be obtained, including proteins. Biodiesel production from insects has become more favorable since it has been found that insect breeding is economically and environmentally viable. Certain species of insects can easily degrade organic matter, converting organic waste into insect biomass. Insect breeding space is not large compared to the large land areas required for crops such as soybeans or to the large water footprint required for microalgae production. This new alternative has become more feasible, especially for countries with limited space and highly populated areas that need to devote their land for food-source production [7]. Insect larvae can accumulate lipids as their fat body and are able to stimulate the metabolic reserves needed, especially during their immature stages such as larva, pupa and nymph. Insects possesses a nutrient storage system that is used in the metamorphosis process, a structure called the "fat body". This structure is able to accumulate the lipids in the body as fat, which is used as an energy reserve and plays a role in the intermediary metabolism. From the research work conducted by Leong et al. [8], the *Hermetia illucens* larvae, or the black soldier fly larvae (BSFL), has become the ideal candidate in biodiesel production during its larval stage because the adult of the fly has been reported to be missing the mouthparts to feed and relies on food reserves, unlike common houseflies. This means that the black soldier fly is not a vector that can transmit diseases or parasites when feeding. Thus, this species of fly is not considered as a harmful pest, feeding on only kitchen waste, spoiled feed and manure. Recently, this fly, which can be commonly found in poultry- and pig-rearing units, has been found to be able to reduce unpleasant smells as it feeds on the manure or compost, efficiently reducing the polluting compounds from manures and compost. Undesirable bacteria are also reduced by the modification of the bacterial

microflora by the BSFL during feeding. The BSFL is a sustainable source for biodiesel production, as the chemical composition of this species is able to accumulate fat, depending on its feeding medium during its rearing process. Upon the lipid extraction for biodiesel production, the residual is a protein-rich larval biomass and can be used as the animal feed to replace fishmeal, which is not sustainable for the long term. Various research studies have been conducted on employing the BSFL biomass as the animal feed for farming of land animals as well as for aquaculture. Figure 1 presents the flow of the present review, starting from the BSFL substrate preparations all the way until the conditions for larval biodiesel production.



**Figure 1.** Flow of review, encompassing the larval substrate, rearing and biodiesel production conditions.

## 2. Homogenous Substrate

With a wide dietary range [9], BSFL has been evaluated for the precision and easy incorporation in formulating its diets that allow sufficient amount of lipid for biodiesel and protein production. Studies had been conducted on feeding BSFL with two different types of feeding mediums, namely, homogenous and heterogenous substrates. The homogenous substrates contain only one kind of organic matter, while the heterogenous substrates incorporate a mixture of two different types of organic matters or more before feeding the BSFL. For the homogenous substrates, there are various type of mediums that have been used to feed BSFL in order to assess its lipid content, biodiesel yield and protein content. Those single substrates are manure, animal feed/food, waste and nutritional meal.

Manure is basically an organic matter originating from the feces of animals, mainly used to fertilize crops. Different type of animals have different consumption of feeds in their diets, affecting the nutritional content of their manure. According to Li et al. [10], the use of cattle manure to feed BSFL would generate the extracted lipid content of 38.2 g, yielding 29.9% amount of fat. The biodiesel produced was 35.6 g and the BSFL that was fed with the cattle manure was able to yield 93% of biodiesel. When pig manure was used, the amount of lipid produced was 60.4 g with the yield of 29.1%, while the biodiesel produced was 57.7 g with the yield of 96%. The amount of lipid produced when chicken manure was used however gave the amount of 98.5 g with the yield of 30.1% and the biodiesel production of 91.4 g with yield of 93%. According to this study, the BSFL fat-based biodiesel fuel properties were comparable to a crop-based fuel, rapeseed. With the amount of crude fat as well as biofuel yield from the transesterification process, the results from this study show that BSFL fat has the potential as a feedstock in biodiesel production.

Other studies were also conducted by Newton et al. [11] in comparing the lipid and protein contents between poultry manure and swine manure. According to their studies, it was found that the lipid content of BSFL was slightly higher when fed with poultry manure, with the yield of 34.8%, while BSFL was able to yield 28% when fed with swine manure. The protein content of BSFL was higher when it was fed with swine manure, with yield of 43.2%, than when it was fed with poultry manure (42.1%). This study showed that BSFL contained a high concentration of oil that would yield as much energy as the methane fermentation that used the same type of manure. The difference in the

BSFL lipid and protein contents when reared by different types of manure reflects how the variation of diet affects the lipid and protein concentration, as it was tested that the other nutrients, except for phosphorus, can be found in slightly higher concentrations when fed with poultry manure. With its high level of oil in the BSFL, it would be likely best to not use BSFL as a bulk protein supplement for animal feedstock, but instead to use it as the potential energy source. According to the study conducted by Lalander et al. [9], poultry manure was fed to BSFL and the crude protein content obtained was 22.8%. It could be deduced that the development of the BSFL growth was dependent on the concentration of the protein of the BSFL. When the feed provides the BSFL with enough protein to accumulate, it will be used as part of its development, making it consume less energy from its lipid content. However, it will result in a much smaller larva. In the same study, poultry feed gave the protein content of 17.3% and dog food gave 33.9%.

Lalander et al. [9] also investigated the effects on the concentration of crude protein of BSFL when they were fed waste materials. When food waste from local restaurants was used, 22.2% was obtained. Abattoir sheep waste gave 56.3%, human feces gave 35.5%, dewatered wastewater sludge gave 16.9%, sewage sludge gave 31.5% and the digested sludge gave the protein content of 14.7%. According to the protein conversion ratio, pure abattoir waste can have the potential to obtain a higher protein ratio if more carbon was added to allow nutrients in the substrates of the waste to be balanced. The nitrogen content of the waste can also be improved with added carbon as it allows the BSFL to utilize the protein content in a much higher usage during its development. The sludge may have low protein content as it has too few volatile solids. The feeding rate that was regulated to dry matter in this investigation was affected. Human feces has a high ratio, and this may be due to its biomass conversion ratio.

Another type of homogenous substrate was flour protein, as carried out by Arango Gutiérrez et al. [12], which contains proteinic ingredients and high digestibility that has the qualities that make it suitable for providing the right nutritional value in the animal's feed. According to the analysis, it is found that when the flour protein was fed to the BSFL, the larvae had the lipid content of 18.82% with the protein content of 36.98%. This research shows that the feed has potential ingredients to provide energy content.

### 3. Heterogenous Substrate

Lately, it has been found that the oxidation from the fiber of plants or crops is important factor that contributes greatly towards the metabolic activity of the BSFL. As reported by Li et al. [13], the fibers that exist provide the black soldier fly larvae the sufficient materials and energy required for life activities. Therefore, a balanced nutrition is required in the BSFL diet to ensure that the total conversion efficiency is enhanced; this will, in turn, assist the black soldier fly larvae's digestion of the materials. With a better nutrient balance, a higher yield is due to the synergy of the biological growth established being highly positive. According to Wu Li et al. [14], when corn cob residue was soaked in restaurant wastewater at the optimal soaking condition of 75 °C for 5 h, 23.34% lipid content was able to be produced from the BSFL. The restaurant wastewater was used to soak the corn because of its acidification properties, allowing cellulose hydrolysis which allows the lignocellulose of the corn to degrade easily. Different concentrations of xylose and glucose of a fibrous plant or crops in the BSFL feed greatly influences the insect's dry weight and the lipid content [13]. With xylose being the most abundant carbohydrate derived from fibers, especially corn, it became of great importance to extract the xylose to be able to produce lipid. Without any time lag, BSFL is able to consume both xylose and glucose of a plant, easily transforming it to lipid. When a standard feed with a mixture of 8% of glucose was added, 34.31% lipid was able to be yielded from the BSFL. If 6% xylose was mixed with the standard feed, 34.60% lipid was able to be yielded. This shows that both xylose and glucose are able to yield a good amount of lipid. Thus, when 0.3% glucose and 0.8% xylose were used in the mixture with standard feed, the lipid yield became 33.78%. On the other hand, 97.3% of the glucose and 93.8% of the xylose were able to be successfully converted to lipid as the dynamics changed between the three substrates within only 14 days. Another study showed that the types of substrate that are usually fed

to the BSFL have a high concentration of cellulose, hemicellulose and lignin, as the animal's main diet consists of crops or plants. The BSFL do possess guts with microbiome symbioses that are able to digest the cellulose consumed. With the right enzymes available in the BSFL, the cellulose, hemicellulose and lignin can be degraded. The main challenge, however, is when a feed with a high amount of crude fiber is used as the main diet of the BSFL, such as dairy manure. More energy is required to break down the cellulose of the fiber materials, thus reducing the lipid yield for biodiesel production. Therefore, a lower fiber content, such as chicken manure, is used together with the dairy manure as co-digestion with different ratios for the BSFL. The study conducted by Rehman et al. [15] evaluated the performance of the BSFL digestion and with the data obtained was able to develop a co-digestion mixture between dairy manure and chicken manure. With the ratio between dairy manure and chicken manure being 40:60, it was found that this ratio of co-digestion resulted in the larvae with the richest nutrient content, enhancing waste conversion efficiency of the BSFL. This study has shown that the use of organic waste in co-digestion must focus on implementing the process of mixing high fiber content with less-fibrous materials and explore the mechanisms as well as the magnitudes of the effect on the BSFL to ensure biodiesel production.

#### 4. Microbial-Treated Substrates

A study has been conducted with the substrate mixture consisting of dairy manure, chicken manure and bacteria. The use of exogenous bacteria, *Bacillus* strains, assists the BSFL gut microbiome development in more efficiently reducing the waste capacity, utilizing the nutrients of the wastes and enhancing the production in the larval biomass. The ratio of dairy manure to chicken manure was 2:3 and this resulted in the lipid yield of 47.7% and protein yield of 53.9%. However, there was a significant increase in both of the yields when bacteria were added. The lipid yield was 67.8% while the protein yield was 71.2%. This shows that the usage of treatment with microbes utilizes a higher amount of lipid and protein compared to the controlled feed that contains only dairy manure and chicken manure. With the help of cellulose-degrading bacteria, a higher biomass promotes for a higher fat yield is promoted, as they enhance the digestion of the waste materials. Therefore, it is important for the selection of the bacteria in assisting the BSFL to ensure that the lignocellulose-rich waste is able to be managed successfully [16]. Soybean curd residue was also used as the feed of BSFL with the addition of a bacteria, *Lactobacillus buchneri*. The results shown by Somroo et al. [17] indicated that the lipid yields differed between when the feeds were only soybean curd residue (26.1%) or artificial feed (24.3%) and when bacteria were added to the feed, which resulted in an increase of the lipid yield (up to 30%). This gave a similar result for the protein yield as the insect–bacteria symbiosis increased the protein yield from 52.9% (soybean curd residue only) and 50.4% (artificial feed) to a much higher value of 55.3%. With this positive interaction, there is great benefit in the availability of the nutrients, playing a major role in the growth of BSFL, the development of the BSFL gut microbiota and the BSFL's production for digestive enzymes. This also shows that the use of symbiotic bacteria allows the success of the BSFL to adapt to new environments and new food sources while still being able to obtain positive growth and reproduction. When the treated rice straw with 39.7 g of glucose and 25.9 g of xylose underwent a fermentation process with *Saccharomyces cerevisiae*, the residues were mixed with enzymes containing hydrolyzed residues, such as lignocellulose, proteins and reducing sugars. The residue was then fed to the BSFL which underwent lipid extraction to yield a total of 5.2 g of lipid from 200 6-day-old BSFL. Additionally, 4.3 g of biodiesel was able to be produced from 200 BSFL. This shows that the nutritional source from BSFL diets consisting of lignocellulosic biomass can be another potential in lipid as well as biodiesel production. Having similar qualities as plant-based biodiesel, BSFL-based biodiesel is proven to be another alternative source of renewable energy [13]. Restaurant waste is heavily concentrated with lipids and protein. However, this substrate lacks the lignocellulose that the rice straw does not lack. If rice straws are used alone as the feed for the BSFL, the growth will be stilled because of the absence of nutrition. Therefore, using the ratio of restaurant waste to rice straw of 7:3 [18], a mixture was made. Rid-X contains natural bacteria that has the main

function of breaking down the cellulase, lipase, protease and amylase of the rice straw as well as the solid waste of the restaurant waste. This helps and increases the efficiency of the conversion for BSFL, degrading the cellulose and hemicellulose much faster. More nutrition from the both of the substrates is available for consumption, and the digestion of the food is aided by the microbes. A total of 43.8 g of biodiesel was able to be produced from 2000 BSFL. The properties of the biodiesel were also investigated, and it was found that the fatty acids of the biodiesel were similar to rapeseed-based biodiesel. Thus, it is shown that the quality of the biodiesel, despite originating from different sources, can still hold a high quality in terms of performances.

From these results, although BSFL contains the microbes that can hydrolyze the cellulose content of the feed, the amount of the microbes in the gut may not be sufficient to digest a much larger amount of feed. Research must continue to test various types of microorganisms in undergoing treatment with the feed of BSFL that contains high amount of fiber. This is to observe the conversion efficiency of the bacteria to obtain a high quality fuel for biodiesel production.

## 5. Substrate Moisture Contents

According to Barry et al. [19], the conversion of waste to biomass of BSFL can be achieved if the consumption of food waste is given a particular care and attention in ensuring its efficiency. Therefore, different parameters need to be investigated, preponderantly the moisture content of the larval feeding substrate, in pushing towards a successful bioconversion. It was found that when study was carried out using almond hull as the main medium for BSFL, alteration of the moisture content in the hull could directly impact the growth of BSFL [20]. The results showed positive effects on the dry weight (0.013 to 0.46 mg/larvae) as well as the yield of harvested larvae ( $3.7 \times 10^{-4}$  to 0.11) as the moisture content was increased from 480 to 680 g kg<sup>-1</sup>. However, it was found that the larval consumption of hull decreased (from 15% to 13%) with increasing of the moisture content. Other studies that reported the effects of manipulating the moisture content of substrates on larval development presented opposite results. The BSFL had been found to grow bigger in terms of weight and needed less medium for consumption as the moisture content was increased [21]. The larval growth rate was also greatly affected when moisture content was reduced, as reported by Cheng et al. [22]. Using almond hull, as reported by Palma et al. [20], showed much different results, perhaps due to the decrease of diffusion of oxygen into the medium. Oxygen diffusion was limited when the pores of the hull were not air-filled and were blocked by the moisture. This directly impacted the growth of bacterial activity and would disrupt the synergistic potential of microorganisms that contributed in the conversion of hull to larval biomass. Therefore, more study needs to be conducted to observe the trends that affect the larval growth and substrate consumption resulting from manipulation of the moisture content, as the role of microorganisms plays a significant role in bioconversion of insect biomass.

## 6. Substrate Aeration

Managing the substrate aeration while growing the BSFL can improve the overall larval growth through engineering to acquire the right and suitable environment for medium digestion by larvae. Significant effects when manipulating the aeration content towards the development of larvae had been determined by experiment by Palma [20], using waste from almond as the BSFL feeding medium. It was found that increasing the aeration rate gave rise to a positive fit to the nonlinear regression of the BSFL weight and yield. Accordingly, the maximum larval weight and yield could be achieved at 95% at aeration of 0.57 and 0.05 mL/min g dry weight, respectively; this also contributed greatly towards the consumption of almond hull. It could be deduced that the aeration had a direct impact towards the growth of larvae, and the bed depth of the substrate may play a major role as well. This was because the anaerobic condition occurred from the oxygen utilization by both BSFL and microorganisms surpassing the oxygen being supplied from diffusion from the bed surface. This caused the larvae to migrate elsewhere to obtain nutrients that otherwise could be obtained when the larvae migrated to a deeper depth of substrate. When aeration rate was dropped, the larval consumption of hull was negatively

affected as well, showing that the presence of microorganisms could heavily impact the environment for rearing BSFL. Therefore, the oxygen content supplied by aeration should be considered of the great importance in insuring that the growth of BSFL does not negatively affect the rate of waste conversion. Moreover, the calcium content was also investigated, and it was found that increasing oxygen content during larval growth would generally increase the larval calcium content. The consumption of hull by BSFL impacted the uptake of calcium from the almond hull and later affected the larval biomass compositions. According to Liu et al. [23], calcium as the mineral element of BSFL was needed for the cuticle formation. Another study, conducted by Wong et al. [24], showed that the harvesting of BSFL at different calcium or chitin levels could directly affect the lipid content since the accumulated body fat tissues were needed during metamorphosis.

## 7. Inactivation Methods

Various ways that the BSFL could be inactivated were reported by Larouche et al. [25]. Grinding was the first method of mechanical disruption in which the larvae were homogenized at 15,000 rpm in their study. The larvae were packed under 95% vacuum on high hydrostatic pressure with 600 MPa of pressure treatment. The next method involved heating the BSFL, i.e., via blanching, where the larvae were immersed in boiling water for 40 s. Desiccation was another method of heating the BSFL. This approach required the larvae to be located in the air oven with the temperature set to 60 °C for 30 min. The other type of larval inactivation method was freezing, where the larvae were either frozen at the temperature of −20 °C or −40 °C for one hour. Freezing the larvae also could be completed by using liquid nitrogen in a vacuum package for 40 s. Finally, asphyxiation was the last inactivation method reported by Larouche et al. [25], in which the larvae were initially vacuum packaged and subsequently stored at the temperature of 27 °C for 120 h. The atmosphere was modified either to contain 100% carbon dioxide or nitrogen gas. The larvae were then stored at the temperature of 27 °C for 120 or 144 h. The ether extract for each of the larval samples was conducted using petroleum ether as extraction solvent. It was found that the larval lipid contents were higher when the inactivation method of asphyxiation (CO<sub>2</sub> = 15.9%; N<sub>2</sub> = 16.6%; vacuum = 15.9%) was used as opposed to heating (desiccation = 13.4%; blanching = 14.5%), freezing (−20 °C = 12.8%; −40 °C = 12.4%; liquid nitrogen = 12.6%) or mechanical disruption (grinding = 11.9%; high hydrostatic pressure = 12.0%).

The inactivation method of BSFL was lately studied even further, as it was important to be explored to ensure the investigation of the composition of larval lipid could be exploited for biodiesel production and also support a higher value of uses. The characteristics of larval lipid distributions during the processing and storage of BSFL biomass must be unveiled since there is currently a lack of this information. Caligiani et al. [26] directly related the inactivation method of BSFL with extracted lipid characteristics. The inactivation methods reported were blanching and freezing. In their study, half of the first sample was provided in a frozen condition and stored at the temperature of −20 °C, while the other half was ground and freeze-dried until it reached residual moisture of 10% before being kept at the temperature of −20 °C. The next set of larval samples were obtained alive. The larvae were killed by blanching the prepupae in hot water at the temperature of 100 °C for 40 s before storing at the temperature of −20 °C prior to the lipid analyses. Another live larval sample was stored directly at the temperature of −20 °C until use. During the extraction, the Soxhlet lipid extractor that either used diethyl ether or petroleum ether as the solvent was compared with the use of chloromethane solvent. The results of the first sample inactivated by using freezing before the arrival showed that there was no significant difference of using the different extraction solvents via the Soxhlet method. The next set of samples were obtained alive, and diethyl ether was used, resulting in the lipid yield was 13.0 ± 1% when inactivated by freezing; while using blanching, the lipid yield was 13.3 ± 0.8% from BSFL biomass. This also proved that the BSFL was a good source of lipid, unlike other type of insects. However, as compared with the method employing chloromethane, the larval lipid extracted was slightly lower (9.11%). In the live larval sample that was also inactivated via freezing, it was found that both of these samples had a high free fatty acids content. This also explained that the low



lipid yield when using the chloromethane for extraction was due to the loss of fatty acid salts in the aqueous phase. However, when the live BSFL were blanched before being frozen, the loss of fatty acid was negligible. When the freezing method was applied towards the BSFL, the amount of acyl glycerols was drastically reduced, most likely due to the activation of the lipase, releasing the free fatty acids. However, the free fatty acids were not used for biodiesel production as a reaction with acyl glycerols was needed during the transmethylation process. When the BSFL was inactivated by blanching, a thermal pretreatment method, the lipid fraction was observed to be stable as it was mainly composed of triacylglycerols. This may be due to the thermal environment deactivating the lipase activity in the BSFL, as it did not damage or influence the lipid fraction conspicuously, preserving it for transmethylation process in producing biodiesel.

## 8. BSFL-Based Biodiesel

Black soldier fly larval biomass has become an attractive candidate as a renewable source of energy due to its high lipid content. Transesterification is a process for biodiesel production from larval biomass in which the extracted lipid will react with alcohol. It has become essential to ensure that the lipid conversion during biodiesel production is at its highest efficiency. Surendra et al. [27] carried out an investigation to determine the fatty acid compositions of BSFL fats or lipids for biodiesel production. It was found that the BSFL had a very high amount of lauric acid ( $44.9\% \pm 1.5\%$ ) as compared with the crop-based biodiesel such as soybean oil (negligible) and palm oil (0.1%), a trait that was significant in terms of biodiesel production. The saturated fat was found to be 67% of total fatty acid while soybean was known to only have 11% and palm oil to have only 37% of total fatty acids. On the other hand, the BSFL had a proportion of 28% fatty acids being of unsaturated fat, lesser than that of soybean (85%) and palm oil (55%). The quality of the biodiesel was known to be greatly affected by the composition of fatty acids in a substrate. In this case, the BSFL-based biodiesel was shown to have a significant amount of saturated fatty acids and a low concentration of unsaturated fatty acids, making it an ideal substrate for a high quality of biodiesel production. Thus, the biodiesel would have a viscosity with much lower value and a more stabilized property in terms of its oxidative state. Additionally, the process of transesterification of larval oil that has been extracted must be efficiently processed in ensuring the biodiesel production is of the highest quality.

## 9. Transesterification of Larval Lipids

An optimum condition for executing the transesterification of BSFL lipids was investigated by Li et al. [28]. The harvested larval biomasses were initially fed with three different substrates individually, namely, cattle manure, pig manure and chicken manure. There is a two-step process during the conversion of larval lipids into fuel. The first step was the acid-catalyzed esterification of fatty acids. This step was to decrease the amount of acids in the BSFL lipids that were extracted and that acted as the pretreatment for the conversion process. The next step was the typical alkaline-catalyzed transesterification. One of the reaction conditions that was optimized was the esterification temperature for 1 h of reaction time using the methanol to larval lipid ratio of 8:1. It was found that as the temperature was increased from 55 to 85 °C, the conversion of fatty acids in the crude lipids to biodiesel increased from 73% to 92%. This demonstrated a positive relationship between temperature and conversion of fatty acids, as it could be directly related to the efficiency of mass transferred with increasing temperature that caused the crude lipid to be more soluble. Accordingly, the temperature of 75 °C was found to be optimal temperature for the esterification process. Another reaction condition investigated was the molar ratio of methanol to larval crude lipid. It was found that the maximum conversion of fatty acids was achieved at 90% when the optimum molar ratio used was 8:1. When a much lower ratio was used instead, the conversion was found to be incomplete. The reaction time was another reaction condition investigated, and the converted fatty acids were found to be 73% to 90% at the reaction times of 30 and 60 min, respectively. The biodiesels produced from the crude lipids of BSFL fed with chicken manure, pig manure and cattle manure were 91.4%, 57.8% and 35.6%, respectively, through the

optimum transesterification condition of 30 min at 65 °C with molar ratio of methanol to lipid at 6:1 while using 0.8% NaOH as the catalyst of the reaction. The biodiesel was tested, and it contained a high percentage of saturated fatty acids at 67.6%. This meant the biodiesel produced would have a high oxidative stability value, an excellent trait for biodiesel storage. The optimal transesterification conditions were further tested and used during the experimentation with BSFL-based biodiesel derived from waste grease of restaurants [29]. The two-step process which consisted of the acid-catalyzed esterification and alkaline-catalyzed transesterification was carried out using 1% H<sub>2</sub>SO<sub>4</sub> as the reaction catalyst with reaction temperature set at 75 °C, molar ratio of methanol to lipid at 8:1 and 1 h of reaction time. For the alkaline-catalyzed transesterification, the methanol-to-lipid ratio was kept at 6:1 with 0.8% NaOH as the reaction catalyst. The biodiesel produced was 23.6 g from 1000 g of solid residual fraction of restaurant waste fed to 1000 BSFL. The conversion rate of free fatty acids attained was 91.9%, with the total yield of biodiesel of 2.4%. Li et al. [10] also investigated the conversion of BSFL fat to biodiesel using the dairy manure as the main larval substrate. After conducting the two-step transesterification process, 15.8 g of biodiesel was able to be produced from 1.2 kg of dairy manure. The larval biodiesel also contained 58.2% saturated fatty acids and 39.8% of unsaturated fatty acids with overall quality satisfying the EN 14214 standard.

## 10. Co-Solvent for Transesterification of Larval Lipids

The conventional way of biodiesel production has generally presented some problems, such as consuming a high amount of energy, that make the process costly. Therefore, a direct transesterification involving fewer steps was suggested and investigated by Nguyen et al. [30]. Methanol was used in prior studies as both the solvent for lipid extraction and reactant for lipid transesterification. However, an excess amount of methanol could weaken the function of catalyst, reducing the yield of biodiesel. In the study by Nguyen et al. [30], co-solvents such as n-hexane, n-pentane, chloroform, acetone and petroleum ether were individually mixed with methanol during the direct transesterification process. With the capability to dissolve long chain triglycerides, these co-solvents showed high potential efficiency in extracting the larval lipid, yielding higher amounts of biodiesel. This would prevent lipid loss during the process as less solvent and energy were consumed. When the solvents were mixed with the methanol at the volume ratio of 1.17:1, a high yield of biodiesel was observed. The use of the mentioned solvent should bring positive effects, as the co-solvents are generally capable in dissolving the lipid and short-chain alcohol used as the homogenous catalyst. It was found that by using n-hexane as the co-solvent, the highest yield of biodiesel (63.37%) could be obtained as compared with acetone (54.83%), chloroform (48.50%) and petroleum ether (35.67%). The effects of volume ratios between the n-hexane and methanol were also investigated and it was observed that biodiesel could be yielded at a higher amount when a much lower volume ratio was used. This was probably because the high methanol content in the reaction would lead to a higher molar ratio between the methanol and lipid, leading to the higher reaction yield. Thus, the optimum volume ratio of n-hexane to methanol was determined to be 1:2. The methanol to biomass ratio of the reaction would increase, promoting the conversion yield. However, the frequency of collision between the lipid and methanol would decrease if excess solvent was used in the reaction. This would, overall, result in the increase of heat and mass transfer resistance, decreasing the conversion yield. Other than that, in a similar study with the presence of free fatty acids from the BSFL fat, the catalyst selected was sulfuric acid. As the catalyst loading was increased from 0.4 to 1.2 mL, the yield of biodiesel had also increased from 48.93% to 65.87%, respectively. The polymerization of unsaturated fatty acids was activated with the presence of excess sulfuric acid at high reaction temperature and long reaction time. The increase in reaction temperature would result in the increase of biodiesel yield (87.67% at temperature of 130 °C). The optimal temperature was found to be 120 °C as, although the extraction efficiency increased with enhancement of reaction rate, there was no significant difference between 120 and 130 °C. The biodiesel yields also increased with the reaction time and managed to reach the equilibrium state at reaction time longer than 90 min. Therefore, the highest biodiesel yield was able to be obtained at 94.14% with

the reaction temperature of 120 °C using n-hexane:methanol volume ratio of 1:2, the solvent at 12 mL, the catalyst loaded at 1.2 mL and reaction time of 90 min. The biodiesel yield was also observed to be increased from 4.73% with no co-solvent to 63.37% when n-hexane was used. Therefore, n-hexane was proven to be the suitable co-solvent for the reaction involving larval lipid transesterification.

### 11. Biological Lipase Catalyst for Transesterification of Larval Lipids

The two-step process of transesterification involves acid-catalyzed esterification and alkali-catalyzed transesterification. However, some complications have been presented when sulfuric acid and sodium hydroxide were used in the process, such as damaging the equipment and complications in removing the dissolved catalysts. Therefore, in the study by Nguyen et al. [31] for biodiesel production, methanol catalyzed with lipase was used during the transesterification of BSFL lipid. In the biodiesel production, several lipases were tested such as Novozym 435, Lipozyme TL-IM, Lipozyme RM-IM and lipase PS. Transesterification was then carried out using 10% lipase mixed with methanol and lipid at the molar ratio of 3:1 and reaction temperature and time of 20 °C and 4 h, respectively. According to the results, the Novozym 435 presented a biodiesel yield of 56.78% as opposed to Lipozyme RM-IM (42.18%), porcine pancreas lipase (23.46%), *Candida rugosa* lipase (22.82%), *Rhizopus oryzae* lipase (21.56%), amano lipase G (13.85%) and amano lipase PS (12.56%). This showed that Novozym 435 had the highest catalytic conversion property and also could be used repeatedly. With the enzyme loading at 20%, the maximum yield for biodiesel could be achieved with the methanol:lipid molar ratio of 6.33. Lipase deactivation, however, may occur when the methanol level exceeded the required amount and would cause a reversal in the aforementioned trend. The yield of the reaction would then decrease. In terms of the reaction temperature, the highest yield could be achieved at 40 °C, as higher temperatures would deactivate the activities of enzymes. Enzyme loading played another part in the reaction, as low loading would affect the temperature and later the biodiesel yield. On the other hand, at high level of enzyme loading the temperature did not bring any significant effect towards the yield. Therefore, any problem rising from the temperature could be overcome by increasing the concentration of enzyme in the reaction. In conclusion, to obtain a maximum yield of biodiesel of 97.65%, the molar ratio of methanol to lipid was set at 6.33:1, with 20% enzyme loading and 26 °C as the reaction temperature for 9.48 h. This experiment had shown positive results, and this should be an encouragement in using the green enzyme-catalyzed process to produce biodiesel.

However, a high level of methanol and ethanol would still cause the deactivation of lipase functions. This was because the absorption of alcohol on the surface immobilized the lipase. Therefore, to overcome this problem, Nguyen et al. [32] investigated the effects of methyl acetate, which is an acyl acceptor that would increase the rate of reaction during transesterification. It was observed that using a high amount of methyl acetate had no effects on the stability as well as the activity of enzymes in replacing the alcohol. To obtain a high amount of biodiesel yield using lipase-catalyzed transesterification (Novozym 435 chosen as catalyst) using methyl acetate as the acyl acceptor for BSFL in the production of biodiesel, several reaction conditions were tested and observed for their effects. The reaction conditions observed were the molar ratio of methyl acetate to lipid and enzyme loading. Biodiesel yield decreased with low ratio, as this increased the loading of enzyme in the reaction. This was because it caused the polymer beads to aggregate with the immobilized enzymes, and this disrupted the mass transfer, enabling the enzyme to react with the oil–water interface flexibly. The conversion yield would be lowered as a result. However, at a higher molar ratio, the enzyme loading would increase, thus increasing the biodiesel yield. The temperature of the reaction was also observed, and it was found that there was no significant increase of biodiesel yield with the increase in temperature. The optimal ratio of methyl acetate to lipid on the other hand was found to be 12:1. In between the enzyme loading and temperature, the yield would increase as the temperature was increased with any amount of loaded catalyst. However, the deactivation of enzyme occurred at high temperature. Therefore, for Novozym 435, biodiesel yield was produced at the highest amount at 39.5 °C reaction temperature. Concisely, in order to obtain a maximum yield of biodiesel, 12 h

of reaction time, 14.64:1 molar ratio of methyl acetate to lipid, 17.58% enzyme loaded and 39.50 °C reaction temperature should be used in a lipase-catalyzed transesterification reaction using Novozym 435 as the catalyst. With the proven high biodiesel yield using the optimized conditions mentioned, BSFL lipid has become a reliable source of energy and can be further developed in future [33,34].

## 12. Conclusions

In short, this review has demonstrated that the BSFL biomass can be the source of protein and lipid for energy. The rearing conditions of BSFL can be systematically optimized to allow the accumulation of more larval protein and lipid in the fat body. Upon the harvesting, the lipid in the form of larval fat can be extracted and transesterified for producing a mixture of fatty acid methyl esters of biodiesel. In this regard, the extraction and transesterification processes can be optimized as well to maximize the BSFL-based biodiesel production. The residual BSFL biomass after the lipid extraction is a protein-rich larval biomass and can be served as the feedstock for animal feed production. In determining the optimum various larval processing conditions, the preparation of substrates, rearing of BSFL and eventually biodiesel production are vital in ensuring the maximum yield from BSFL biomass. The mixture of substrate added with 6% of xylose prompted 34.60% lipid content from the BSFL biomass. Microbial-treated substrate such as dairy manure and chicken manure mixture inoculated with *Bacillus* strains would yield 67.8% lipid and 71.2% protein. Addition of Rid-X to a mixture of restaurant wastes and rice straw could get 43.8 g of biodiesel from 2000 BSFL. To ensure the optimum conditions during rearing period, the moisture content of the substrate should be in the range of 480 to 680 g/kg. The increase in moisture content would result in the decrease in feed consumption. The aeration of the substrate should also be at 95% to achieve maximum larval weight and yield of dry weight as well as a positive growth of the larvae. To inactivate the mature BSFL for maximum yield, it has been shown that if petroleum ether was used as the extraction solvent during lipid extraction, asphyxiation would result in higher lipid content. If diethyl ether was used in the Soxhlet method, both blanching and freezing inactivation methods could be employed. The optimum transesterification conditions were determined to be at 30 min at 65 °C with molar ratio of methanol to lipid at 6:1 while using 0.8% NaOH as the catalyst in the reaction. During direct transesterification, hexane was recommended as the co-solvent, and sulfuric acid as the catalyst. The reaction temperature should be at 120 °C using hexane:methanol volume ratio of 1:2, the solvent at 12 mL, the catalyst loaded at 1.2 mL and reaction time of 90 min. Novozym 435 can be added in a lipase-catalyzed transesterification reaction with methyl acetate added to replace the methanol and ethanol. Studies have shown that with 12 h of reaction time, 14.64:1 of molar ratio between methyl acetate to lipid, 17.58% enzyme loaded and 39.50 °C of reaction temperature could ensure obtaining the maximum yield of biodiesel. Therefore, since the detailed laboratory-proven information with regard to the upstream and downstream of BSFL biomass production is currently accessible, the mass production of this feedstock at industrial scale should be assessed to unveil its feasibility concerning the cost and long-term environmental sustainability. Lastly, the authors of this review believe that the BSFL biomass could potentially arise as the new and unconventional feedstock for protein in replacing the traditional fishmeal if it is not used for biodiesel.

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## References

- Barnwal, B.K.; Sharma, M.P. Prospects of biodiesel production from vegetable oils in India. *Renew. Sustain. Energy Rev.* **2005**, *9*, 363–378. [\[CrossRef\]](#)
- Boocock, D.G.B.; Konar, S.K.; Mao, V.; Sidi, H. Fast one phase oil-rich processes for preparation of vegetable oil methyl esters. *Biomass Bioenergy* **1996**, *11*, 43–50. [\[CrossRef\]](#)
- Atabani, A.E.; Silitonga, A.S.; Badruddin, I.A.; Mahlia, T.M.I.; Masjuki, H.H.; Mekhilef, S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renew. Sustain. Energy Rev.* **2012**, *16*, 2070–2093. [\[CrossRef\]](#)
- Sitepu, I.R.; Setric, R.; Ignatia, L.; Levin, D.; German, J.B.; Gillies, L.A.; Almada, L.A.G.; Boundy-Mills, K.L. Manipulation of culture conditions alters lipid content and fatty acid profiles of a wide variety of known and new oleaginous yeast species. *Bioresour. Technol.* **2013**, *144*, 360–369. [\[CrossRef\]](#) [\[PubMed\]](#)
- Xin, L.; Hong-ying, H.; Ke, G.; Ying-xue, S. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresour. Technol.* **2010**, *101*, 5494–5500. [\[CrossRef\]](#) [\[PubMed\]](#)
- Pinzi, S.; Leiva, D.; López-García, I.; Redel-Macías, M.D.; Dorado, M.P. Latest trends in feedstocks for biodiesel production. *Biofuels Bioprod. Biorefining* **2014**, *8*, 126–143. [\[CrossRef\]](#)
- Manzano-Agugliaro, F.; Sanchez-Muros, M.J.; Barroso, F.G.; Martínez-Sánchez, A.; Rojo, S.; Pérez-Bañón, C. Insects for biodiesel production. *Renew. Sustain. Energy Rev.* **2012**, *16*, 3744–3753. [\[CrossRef\]](#)
- Leong, S.Y.; Kutty, S.R.M.; Malakahmad, A.; Tan, C.K. Feasibility study of biodiesel production using lipids of *Hermetia illucens* larva fed with organic waste. *Waste Manag.* **2016**, *47 Pt A*, 84–90. [\[CrossRef\]](#)
- Lalander, C.; Diener, S.; Zurbrugg, C.; Vinnerås, B. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *J. Clean. Prod.* **2019**, *208*, 211–219. [\[CrossRef\]](#)
- Li, Q.; Zheng, L.; Qiu, N.; Cai, H.; Tomberlin, J.K.; Yu, Z. Bioconversion of dairy manure by black soldier fly (*Diptera: Stratiomyidae*) for biodiesel and sugar production. *Waste Manag.* **2011**, *31*, 1316–1320. [\[CrossRef\]](#)
- Newton, L.; Sheppard, C.; Watson, D.W.; Burtle, G.; Dove, R. *Using the Black Soldier Fly, Hermetia Illucens, as a Value-Added Tool for the Management of Swine Manure*; North Carolina State University: Raleigh, NC, USA, 2005.
- Arango Gutiérrez, G.P.; Vergara Ruiz, R.A.; Mejía Vélez, H. Analisis composicional, microbiológico y digestibilidad de la proteína de la harina de larvas de *Hermetia illucens* L (*Diptera: Stratiomyiidae*) en Angelópolis-An-tioquia, Colombia. *Revista Facultad Nacional de Agronomía-Medellín* **2004**, *57*, 2491–2500.
- Li, W.; Li, M.; Zheng, L.; Liu, Y.; Zhang, Y.; Yu, Z.; Ma, Z.; Li, Q. Simultaneous utilization of glucose and xylose for lipid accumulation in black soldier fly. *Biotechnol. Biofuels* **2015**, *8*, 117. [\[CrossRef\]](#)
- Li, W.; Li, Q.; Wang, Y.; Zheng, L.; Zhang, Y.; Yu, Z.; Chen, H.; Zhang, J. Efficient bioconversion of organic wastes to value-added chemicals by soaking, black soldier fly (*Hermetia illucens* L.) and anaerobic fermentation. *J. Environ. Manag.* **2018**, *227*, 267–276. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rehman, K.U.; Cai, M.; Xiao, X.; Zheng, L.; Wang, H.; Soomro, A.A.; Zhou, Y.; Li, W.; Yu, Z.; Zhang, J. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). *J. Environ. Manag.* **2017**, *196*, 458–465. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rehman, K.U.; Rehman, R.U.; Somroo, A.A.; Cai, M.; Zheng, L.; Xiao, X.; Rehman, A.U.; Rehman, A.; Tomberlin, J.K.; Yu, Z.; et al. Enhanced bioconversion of dairy and chicken manure by the interaction of exogenous bacteria and black soldier fly larvae. *J. Environ. Manag.* **2019**, *237*, 75–83. [\[CrossRef\]](#) [\[PubMed\]](#)
- Somroo, A.A.; Rehman, K.U.; Zheng, L.; Cai, M.; Xiao, X.; Hu, S.; Mathys, A.; Gold, M.; Yu, Z.; Zhang, J. Influence of *Lactobacillus buchneri* on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*) for food and feedstock production. *Waste Manag.* **2019**, *86*, 114–122. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zheng, L.; Hou, Y.; Li, W.; Yang, S.; Li, Q.; Yu, Z. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy* **2012**, *47*, 225–229. [\[CrossRef\]](#)
- Barry, T. Evaluation of the economic, social, and biological feasibility of bioconverting food wastes with the black soldier fly (*hermetia illucens*). Ph.D. Thesis, University of North Texas, Denton, TX, USA, 2014.
- Palma, L.; Ceballos, S.J.; Johnson, P.C.; Niemeier, D.; Pitesky, M.; VanderGheynst, J.S. Cultivation of black soldier fly larvae on almond byproducts: Impacts of aeration and moisture on larvae growth and composition. *J. Sci. Food Agric.* **2018**, *98*, 5893–5900. [\[CrossRef\]](#) [\[PubMed\]](#)

21. Cammack, J.A.; Tomberlin, J.K. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *Insects* **2017**, *8*, 56. [[CrossRef](#)] [[PubMed](#)]
22. Cheng, J.Y.K.; Chiu, S.L.H.; Lo, I.M.C. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Manag.* **2017**, *67*, 315–323. [[CrossRef](#)] [[PubMed](#)]
23. Liu, X.; Chen, X.; Wang, H.; Yang, Q.; Ur Rehman, K.; Li, W.; Cai, M.; Li, Q.; Mazza, L.; Zhang, J.; et al. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS ONE* **2017**, *12*, e0182601. [[CrossRef](#)] [[PubMed](#)]
24. Wong, C.-Y.; Rosli, S.-S.; Uemura, Y.; Ho, Y.C.; Leejeerajumnean, A.; Kiatkittipong, W.; Cheng, C.-K.; Lam, M.-K.; Lim, J.-W. Potential protein and biodiesel sources from black soldier fly larvae: Insights of larval harvesting instar and fermented feeding medium. *Energies* **2019**, *12*, 1570. [[CrossRef](#)]
25. Larouche, J.; Deschamps, M.-H.; Saucier, L.; Lebeuf, Y.; Doyen, A.; Vandenberg, G.W. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*hermetia illucens*) larvae. *Animals* **2019**, *9*, 182. [[CrossRef](#)] [[PubMed](#)]
26. Caligiani, A.; Marseglia, A.; Sorci, A.; Bonzanini, F.; Lolli, V.; Maistrello, L.; Sforza, S. Influence of the killing method of the black soldier fly on its lipid composition. *Food Res. Int.* **2019**, *116*, 276–282. [[CrossRef](#)] [[PubMed](#)]
27. Surendra, K.C.; Olivier, R.; Tomberlin, J.K.; Jha, R.; Khanal, S.K. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renew. Energy* **2016**, *98*, 197–202. [[CrossRef](#)]
28. Li, Q.; Zheng, L.; Cai, H.; Garza, E.; Yu, Z.; Zhou, S. From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel* **2011**, *90*, 1545–1548. [[CrossRef](#)]
29. Zheng, L.; Li, Q.; Zhang, J.; Yu, Z. Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. *Renew. Energy* **2012**, *41*, 75–79. [[CrossRef](#)]
30. Nguyen, H.C.; Liang, S.-H.; Li, S.-Y.; Su, C.-H.; Chien, C.-C.; Chen, Y.-J.; Huong, D.T.M. Direct transesterification of black soldier fly larvae (*Hermetia illucens*) for biodiesel production. *J. Taiwan Inst. Chem. Eng.* **2018**, *85*, 165–169. [[CrossRef](#)]
31. Nguyen, H.C.; Liang, S.-H.; Doan, T.T.; Su, C.-H.; Yang, P.-C. Lipase-catalyzed synthesis of biodiesel from black soldier fly (*Hermetica illucens*): Optimization by using response surface methodology. *Energy Convers. Manag.* **2017**, *145*, 335–342. [[CrossRef](#)]
32. Nguyen, H.C.; Liang, S.-H.; Chen, S.-S.; Su, C.-H.; Lin, J.-H.; Chien, C.-C. Enzymatic production of biodiesel from insect fat using methyl acetate as an acyl acceptor: Optimization by using response surface methodology. *Energy Convers. Manag.* **2018**, *158*, 168–175. [[CrossRef](#)]
33. Lim, J.W.; Mohd-Noor, S.N.; Wong, C.Y.; Lam, M.K.; Goh, P.S.; Beniers, J.J.A.; Oh, W.D.; Jumbri, K.; Ghani, N.A. Palatability of black soldier fly larvae in valorizing mixed waste coconut endosperm and soybean curd residue into larval lipid and protein sources. *J. Environ. Manag.* **2019**, *231*, 129–136. [[CrossRef](#)] [[PubMed](#)]
34. Mohd-Noor, S.N.; Wong, C.Y.; Lim, J.W.; Mah-Hussin, M.I.A.; Uemura, Y.; Lam, M.K.; Ramli, A.; Bashir, M.J.K.; Tham, L. Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. *Renew. Energy* **2017**, *111*, 646–654. [[CrossRef](#)]

