Green Production from BSFL

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The purpose of this entry 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

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 the 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. 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.

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