# A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources

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## Abstract

The increase of annual organic wastes generated worldwide has become a major problem for many countries since the mismanagement could bring about negative effects on the environment besides, being costly for an innocuous disposal. Recently, insect larvae have been investigated to valorize organic wastes. This entomoremediation approach is rising from the ability of the insect larvae to convert organic wastes into its biomass via assimilation process as catapulted by the natural demand to complete its lifecycle. Among the insect species, black soldier fly or *Hermetia illucens* is widely researched since the larvae can grow in various environments while being saprophagous in nature. Even though black soldier fly larvae (BSFL) can ingest various decay materials, some organic wastes such as sewage sludge or lignocellulosic wastes such as waste coconut endosperm are destitute of decent nutrients that could retard the BSFL growth. Hence, blending with nutrient-rich low-cost substrates such as palm kernel expeller, soybean curd residue, etc. is employed to fortify the nutritional contents of larval feeding substrates prior to administering to the BSFL. Alternatively, microbial fermentation can be adopted to breakdown the lignocellulosic wastes, exuding essential nutrients for growing BSFL. Upon reaching maturity, the BSFL can be harvested to serve as the protein and lipid feedstock. The larval protein can be made into insect meal for farmed animals, whilst the lipid source could be extracted and transesterified into larval biodiesel to cushion the global energy demands. Henceforth, this review presents the influence of various organic wastes introduced to feed BSFL, targeting to reduce wastes and producing biochemicals from mature larvae through entomoremediation. Modification of recalcitrant organic wastes via fermentation processes is also unveiled to ameliorate the BSFL growth. Lastly, the sustainable applications of harvested BSFL biomass are as well covered together with the immediate shortcomings that entail further researches.

**Keywords:** Black soldier fly larva; Waste management; Blended substrate; Fermentation; Biochemical

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## 1. Introduction

Lately, the quantity of organic wastes generated worldwide continues to increase substantially in order to enliven human consumptions. The discarded organic wastes include food wastes and undesired byproducts from various industries, namely, sewage sludge from wastewater treatment plants, animal manure from agricultural farms, soybean curd residue from tofu manufacturing process, etc. Globally, approximate 2.01 billion metric tons/year of municipal solid wastes is generated, and this amount is expected to reach 3.40 billion metric tons/year by 2050 (Ellis, 2018). Worse still, the generated organic wastes have inevitably led to large carbon dioxide emissions, i.e., 1.6 billion metric tons of carbon dioxide equivalents worldwide. The emission is unabatedly projected to increase to 2.6 billion metric tons by year 2050 (Ellis, 2018). Furthermore, the mismanagement of organic wastes can as well contribute to the broad environmental menaces and economic woes (Ferronato and Torretta, 2019). Thus, a proper planning of waste disposal and reduction is required in a bid to manage the enormous organic wastes safely and sustainably with a minimum spewing of greenhouse gasses. In this regard, the employment of insect larvae for organic wastes reduction has been proven effective and environmentally friendly since the larvae can ingest organic wastes, transforming it into larval biomass through the assimilation process without harming humans and the surrounding environments. Black soldier fly or *Hermetia illucens* is considered as an ideal insect species since the larvae (BSFL) can bioconvert various decay matters, survive among variety of surrounding conditions, inhibit the untoward microbe's growths, and most importantly, the adult is not a pest (Caruso et al., 2014; Nguyen et al., 2013; Tomberlin and Cammack, 2017; Yu et al., 2011). The essential component in larval feeding substrates is protein, in which could have a significant positive impact on the BSFL development to complete its lifecycle (Gold et al., 2020). Nevertheless, some organic wastes exploited as the feeding substrates, such as sewage sludge or lignocellulosic waste coconut endosperm, contain insufficient nutrients to support the BSFL growth (Leong et al., 2016; Mohd-Noor et al., 2017). The lignocellulosic wastes composing of lignin, cellulose and hemicellulose can significantly hinder the digestion process of BSFL, and subsequently retarding its growth measured in terms of slow larval development time or small prepupal weight and size. Therefore, blending with nutrient-rich low-cost substrates (soybean curd residue, palm kernel expeller, etc.) or fermenting in the presence of exo-microbes (*Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus buchneri*, etc.) via *in-situ* and *ex-situ* conditions can consequentially enrich the feeding substrate, prompting the palatability of BSFL. Once the BSFL mature, its biomass can be harvested to serve as the feedstock for protein, lipid and other biochemicals productions. The protein from BSFL biomass can be used to substitute the traditional aquatic meal and poultry feed, tapering off the cost of protein sources derived from soybean or fish meals. The lipid extracted from BSFL biomass is valuable for producing high quality biofuel to sate the rising demand of energy consumptions. Moreover, the utilization BSFL-based lipid as a sustainable raw material is virtually regarded as a new generation of biofuel production.

## 2. Current management of solid organic wastes

Today, various consumer products have been manufactured qualitatively and quantitatively in order to satiate the demands of increasing population in many ways. Along the manufacturing processes, vast organic waste materials have been generated in the form of by-products. This has directly given rise to the inevitable challenges for disposal as the organic fraction from solid waste stream can spew excessive greenhouse gases during degradation (Hoornweg and Bhada-Tata, 2012). The municipal solid wastes have reached 2.01 billion tons/year worldwide recently and it is projected to ratchet up to 2.59 billion tons/year by 2030 (Fig. 1a) (Kaza et al., 2018). The largest composition of municipal solid wastes is the organic waste materials, i.e., encompassing more than 44% (Fig. 1b) (Hoornweg and Bhada-Tata, 2012).

### *2.1 Major organic wastes*

A major source of organic wastes is in the form of food waste. Globally, one-third of foods generated are wasted, i.e., about 1.3 billion tons/year of foods are not consumed as reported by FAO, 2020 (Fig. 2). In Malaysia, on average, a family will discard around 0.5-0.8 kg of foods everyday (Bong et al., 2017). Moreover, Malaysia was reported to have achieved about 15 000 tons/day of food wastes by the Statistics from Solid Waste Corporation of Malaysia (Hoornweg and Bhada-Tata, 2012). The enormous solid organic wastes can afflict human health, in addition to the basic services such as waste management, district facilities, water supply and transport basic structure in any country. Also, this is very costly to the municipal budget in managing and disposing the solid organic wastes (Liyala, 2011). Alas, as a consequence of solid organic wastes disposal, the emission of carbon dioxide gas was estimated had reached 1.6 billion tons in 2016 and this figure is expected to grow to 2.38

billion tons/year by 2050 if the disposal method is still through landfilling or open dumping without a proper gas collection system (Kaza et al., 2018). The greenhouse gas emissions from three different classes for solid waste disposal that have been adopted worldwide are landfilling, incineration without energy recovery and other waste treatment methods. Among the classes, 95% of greenhouse gas was emitted from landfilling disposal mode. The other two classes had merely recorded a total of 5% of greenhouse gas emissions ( Fig. 3) ( EEA Greenhouse gas data viewer., 2014) . This indicates that landfilling has incontrovertibly crowded out as the major factor contributing to the global warming. To make matters worse, the landfilled organics can as well release a large amount of methane gas, leading to the infrared radiation absorption whilst accelerating the global warming and climate change phenomena (Move for Hunger, 2015; Recycle Bank, 2006).

Apart from food wastes, sewage sludge, a by-product generated from biological wastewater treatment plants, is also considered as a major solid organic waste material. The productions of sewage sludge in Europe, United States and China had been reported to be 10 million tons, 49 trillion liters and 20 million tons/year, respectively. Locally in Malaysia, sewage sludge is generated by around 3 million metric tons annually and expected to reach 7 million metric tons in the year of 2020 (Oladejo et al., 2019; Roslan et al., 2013). Although sewage sludge contains some valuable nutrients, its utilizations such as reuse or disposal to landfills, composting and storage are limited by the heavy metals and toxic components laden into sewage sludge during the discharge from industries or traffic related pollutions (Mateo-Sagasta et al., 2015). As of now, virtually, all countries are depending on landfilling to manage the generated sewage sludge, e.g., EU-27, United States and China were disposing 12%, 30% and 80% of sewage sludge to the landfill sites, respectively. This traditional solid waste management method is, indeed, not sustainable while enlarging the carbon footprint insidiously (Mateo-Sagasta et al., 2015). In Malaysia, the estimated cost of management is about US$ 0.33 billion per year to dispose the sewage sludge due to its high contents of pathogens, micro-pollutants, heavy metals and other hazardous substances, depending on the origin of wastewaters (Kadir and Velayutham, 1999).

In addition, other solid organic waste, namely, animal manure from agricultural farm, has increased in terms of capacity to the tune of over 1500 million tons/year in the EU-27 alone (FAO, 2003). Consequentially, 10% of greenhouse gases, 65% of $N_2O$ and 64% of $NH_3$ are emitted globally with the origin from agricultural activities, primarily via animal manure productions (Gómez-Brandón et al., 2013; Steinfeld et al., 2006). The current management strategy for animal manure is through land application, i.e., apportioned into the soil.

However, the major challenge is the presence of high level of nitrogen and phosphorus compounds contaminating the soil and later bringing about to the environmental pollution. Thus, the management of animal manure as well as agricultural waste via recent technology such as compaction or composting has considered. However, these treatment methods require a high expense (Szogi et al., 2015). Moreover, the mismanagement of animal manures can result in the emergence and spread of contagious diseases since the manures are generally hosting various dangerous and infectable microorganisms. The methane gas is also released from the fresh animal manure that has passed through the enteric fermentation of farmed animals; because of that, it intensifies the greenhouse gas emission. Meanwhile, the release of ammonia gas has mightily polluted the environment by causing Eutrophication to the natural water bodies (Aguirre-Villegas and Larson, 2017; Gómez-Brandón et al., 2013; Lim et al., 2016; Loyon, 2018; Malomo et al., 2018). The land application to dispose the generated animal manures will introduce excessive phosphorus sources into the soils, saturating the capacity of soils to retain phosphorus and subsequently, leaching the soluble phosphorus species via continuous surface runoff and erosion (Zhang and Schroder, 2014). Following the ammonia, the presence of phosphorus sources in natural water bodies will as well hasten the Eutrophication menace. Thus, to sum up, the solid organic wastes entail an inclusive management for the sake of mitigating the environmental risks and reducing the long-term costs of disposal (Attiogbe et al., 2019).

### *2.2 Management of organic wastes*

The traditional methods up to the recent approaches that have been employed to handle the solid organic wastes are landfilling, incineration or combustion, recovery and recycling, plasma gasification, composting, energy recovery and avoidance or waste minimization (Fig. 4). The broadly applied method to dispose the solid organic wastes and other garbage is dumping into landfill sites, i.e., encompassing over 60% of global waste disposal and 80% of was reduction in Malaysia, due to convenient and low-cost (Hoornweg and Bhada-Tata, 2012; Kaza et al., 2018). The procedure of landfilling method begins with solid wastes being buried beneath the trash layer. The organic fraction is then biodegraded under the aerobic condition until all the oxygen has depleted in the subsurface. Then, it will pave the way for the anaerobic biodegradation of remnant organics to transpire while spewing methane gas into atmosphere which is 25 times more powerful than $CO_2$ to cause global warming (Amritha and Anilkumar, 2016). In fact, landfilling has crowded out as the third largest source of methane emissions globally (Amritha and Anilkumar, 2016; Zhao et al., 2019). Other problem relates to landfilling is a leachate production, carrying the soluble compounds from the site and contaminating the adjacent water sources, especially the groundwater. In accounting this unsustainable approach, Malaysia has pledged to decrease the disposal of solid wastes via landfilling to 65% by 2020 (Hoornweg and Bhada-Tata, 2012; Malek and Shaaban, 2008; Sauve and Van Acker, 2020). Other resemblance method is open dumping. The receivers of open dumping can be lands or water bodies in which occupying a fraction of 33% (Kaza et al., 2018). Similar to the landfilling, the open dumping can also accelerate the global warming due to the emission of greenhouse gases especially methane that is generated from decomposing of biodegradable organics under anaerobic condition (Couth and Trois, 2009; Isibika et al., 2019).

Besides landfilling, anaerobic digestion and composting are considered as the alternative ways to stabilize solid organic wastes. Anaerobic digestion is a process to decompose organic waste materials in the absence of oxygen, whilst composting is a process to promote decomposition of organic waste materials under the aerobic condition (Kaza et al., 2018). Indeed, composting has accounted 55% of solid organic wastes treatment worldwide. The product derived from both the anaerobic digestion and composting processes can be utilized as a fertilizer for agriculture or landscaping purposes (Aggelides and Londra, 2000; Cheng et al., 2007; Lim et al., 2016). Nevertheless, the treatment of solid organic wastes by both methods needs to be managed adequately since it can contribute to the environmental problems like landfilling (Pace et al., 2018). For instance, the application of digestate, a product after anaerobic digestion process, directly in land can give rise to the uncontrolled greenhouse gas emissions since degradable substrates, phytotoxins and methanogenic microbiota remain in the digestate. Thereby, continuing the anaerobic process to further decompose the remnant organics or volatile fatty acids into methane and other greenhouse gasses (Kirchmann and Bernal, 1997; Kirchmann and Lundvall, 1993).

Incineration is one of the rapid solid waste reduction techniques using oxygen for combusting during the process. The amount of solid wastes stabilization by incineration in worldwide is about 11.1%. In the case of China, the employment of incinerations to stabilize solid wastes had increased dramatically from 3.7 million tons to 61.7 million tons between 2003 and 2015, i.e., the later had amounted 32.5% of solid wastes reduction in China (Hong et al., 2017; National Bureau of Statistics of the People's Republic of China, 2015). Even though incineration can generate heat and electrical energy from solid wastes while reducing a large volume of wastes, it requires professional managements in dealing with air pollutants emission such as $CO_2$, $NO_x$ and ash residue during as well as after the incineration process, respectively (Beylot et al., 2018; Wang et al., 2018). Moreover, incineration also incurs investment to compensate the potential losses from incineration, e.g., decreasing of biodiversity, injuring public health and accessing of land (Wang et al., 2018).

The sewage sludge disposal through sewer ( underground closed pipes) by water carriage system, i.e., water will carry sewage sludge to the disposal place, has been exploited globally at the present since this new method is suitable for the management of sewage sludge. In addition, the maintenance cost is not expensive while utilizing a small footprint of lands since most of the pipes are hidden underground without directly interfering the land developments. Nonetheless, this system requires a high initial cost for investment and 99% of water carrier will be eventually wasted (Engineering Articles, 2015).

In considering the sustainability aspects of currently employed techniques to reduce the solid organic wastes, it seems precarious and not promising for long-term applications. Thus, biological conversion or bioconversion of solid organic wastes by insect larvae has been investigated recently to overcome the setbacks experienced by the currently employed techniques. The outcomes concluded from the studies had confirmed the feasibility of a novel bioconversion technique to stabilize the waste organics via valorization, while benefitting the environment. Various insect or its larval species had been selected to bioconvert organic wastes into valuable biomass such as yellow meal worm, i.e., *Tenebrio molitor L.* in Coleoptera order, black soldier fly, i.e., *Hermetia illucens L.* in Diptera order, face fly (*Musca autumnalis L.*) , flesh fly (*Sarcophaga carnaria L.*) and house fly (*Musca domestica L.*) (Čičková et al., 2015; Wang et al., 2017b). The yellow meal worm larvae contain about 23% - 47% of fat content and have the ability to consume decayed vegetable as a feeding substrate (Alves et al., 2016; Veldkamp et al., 2012; Zheng et al., 2013). Apart from that, house fly larvae also can grow in solid organic waste materials and manure diets (Čičková et al., 2015). Even though house fly presents a rapid reproduction rate and easy for rearing, it is a pest and can widely spread diseases and parasites (Förster et al., 2007; Förster et al., 2009; Hogsette and Farkas, 2000). Next, face fly can grow by feeding organic substrates, especially cattle manure. However, face fly is a threat for cattle and horse since it can transmit diseases to these animals such as pink eye or thelaziasis. The flesh fly can be also reared with waste organic feeds; but it is difficult to identify its larval and adult stages, leading to the obstruction amidst experiments (Čičková et al., 2015). Among all the insect species, black soldier fly larvae (BSFL) is considered as a potential insect since it can consume a variety of solid organic waste materials. Also, the mature BSFL contain about 20% - 40% of fat content. Thus, the BSFL biomass can be exploited as the protein and lipid feedstock for poultry feed and biodiesel production, respectively (Dierenfeld and King, 2008; Oonincx et al., 2015). The significant strengths of BSFL are not only it can assist to control the oviposition of house flies that can inflict human and animals health, but also the adults are not a pest and the larvae are saprophagous in nature, capable of valorizing various and large amount of solid organic wastes (Čičková et al., 2015).

## 3. Lifecycle of black soldier fly

Black soldier fly (BSF) or *Hermetia illucens* is a common tropical and sub-tropical insect. This species has gained increasing interests among the fervent researchers recently to serve as the potential feedstock for larval lipid and protein productions. During the growing stage, the larvae of black soldier fly (BSFL) can accumulate various essential biochemical contents within the structural space between its organs for the uses amidst non-feeding period in its lifecycle, i.e., to undergo pupation (Manzano-Agugliaro et al., 2012). Moreover, the BSFL biomass also has higher content of saturated fatty acids as compared with other species of insects (Ramos-Bueno et al., 2016). Apart from being saprophagous and polyphagous in assimilating myriad organic waste materials such as fruit and vegetable waste or animal manure (Nguyen et al., 2013), the BSFL also can live in various environments, inhibit the growth of untoward microbes while serving as an animal feed and the adult fly is not a pest in nature (Caruso et al., 2014; Yu et al., 2011). The lifecycle of BSF takes about 40 to 50 days (Fig. 5). It begins with the female fly ovipositing eggs near the decomposing organic matters, rendering as a food source for the neonates of BSF. Then, the female fly will die thereafter its energy is exhausted. After about 4 days, the eggs will hatch and the neonates of BSFL will emerge. The larvae which have a creamy color will ingest the surrounding decomposed organics as its food source. This larval stage is the only feeding period for BSF that will extend until reaching a 5$^{th}$ instar stage, i.e., approximately 4 weeks, depending on the quality and availability of organics that can be ingested. Thereafter, the BSFL will undergo eclosion into the prepupae in achieving its 6$^{th}$ instar stage, viz. the last stage of larval form when its light brown color is darkened. During this period as well, the BSFL will stop ingesting organics and its mouthpart will be transformed into hook-shaped structure in aiding the prepupae for moving away from organics to ensconce in a dry place for pupation (Dortmans et al., 2017). The pupa will finally transform into a fly whereby the mature BSF will start spreading its wings and flying off from the cocoon. The pupation is the last eclosion process for BSF and it will consume ca. a week. The emerged BSF will live averagely for 4-6 days for mating and ovipositing eggs in continuing its lifecycle.

## 4. Feeding substrates for BSFL

The prime characteristic of BSF is the larvae can ingest-cum-valorize various organic materials inclusive of decomposable byproducts and wastes for growth until reaching prepupae. In fact, the larval stage is the only feeding duration in BSF lifecycle, i.e., the BSFL need to accumulate sufficient nutrients such as lipids and proteins prior to the pupation and subsequent emergence into adult flies. Thus, the quality of larval feeding substrates especially protein and carbohydrate contents can significantly affect the BSFL growths, organic bioconversion efficiencies, prepupal weights and nutritional contents of mature BSFL in which are generally consisting of approximately 40% of larval protein and 30% of larval lipid (dry weight basis) (Barragan-Fonseca et al., 2017; Kinasih et al., 2018). Moreover, the presence of large amount of larval feeding substrates could assist the BSFL to partially overcome the low quality of nutrients composition. Because of that, shortening the larval development time even small pupal sizes were eventually harvested (Kinasih et al., 2018). The feeding substrates for BSFL can be conventionally categorized into 2 types. The simplest larval feeding substrate consists of a single organic material and it is used directly for feeding of BSFL upon receiving. Conditioning of two or more single substrates via blending or fermenting through inoculation by the various microorganisms has as well been exploited, targeting to offset the shortcomings suffered from the use of some single substrates to grow BSFL.

### *4.1 Single substrate*

There were many completed studies demonstrating the administration of various single substrates to feed BSFL. The commonly studied single substrates were chicken feed, animal manure, food waste, fruit and vegetable residue and sludge. In general, the growth of BSFL in concert with larval body nutritional contents vary with the type of substrates having been ingested during the rearing period. The BSFL body nutritional contents encompass proteins, minerals, amino acids, fats, etc. with the alimentation values directly depending on the physical properties and chemical compositions of the feeding substrates (Table 1) (Kinasih et al., 2018). This section reviews the impacts of feeding BSFL with various single substrates on larval development time from neonates to the first prepupa emergence, prepupal weight as well as harvested BSFL biomass nutritional composition (Table 2).

Among all the single substrates studied thus far, the use of chicken feed had given rise to the shortest larval development time as compared with animal manure, restaurant waste and fruit and vegetable residue. The rearing period prior to the mature BSFL harvesting only entailed 12 days in which the first prepupa could be observed (Spranghers et al., 2017). The highest total larval biomass was also attained while using the chicken feed to grow BSFL. Kinasih et al. (2018) had also reported that the BSFL would require about 20 days for the first prepupa to emerge while controlling the larval feeding rate at 100 mg chicken feed/larva/day (typical feeding rate accepted by many researchers). The highest prepupal weight was recorded at approximately 130 mg/larva using this feeding control. It could be concluded that the chicken feed contained sufficient nutrients to enhance the palatability of BSFL in promoting its growth. Accordingly, it was found that the protein content in chicken feed was measured at about 175 g/kg dry weight (~18%) in which playing a pivotal role to spur the BSFL growth at the shortest time (Li et al., 2012; Spranghers et al., 2017; Tschirner and Simon, 2015). However, using the chicken feed for growing BSFL and later feeding the farmed chicken with mature BSFL is not an economical approach; unless, the harvested larval biomass has other commercial uses. In this regard, other low-cost single substrates were exploited to grow BSFL in order to curtail the production cost for producing BSFL biomass while valorizing the organics.

Besides chicken feed, animal manure is the next palatable substrate for BSFL feeding since it can as well spur the larval growth and body nutritional content comparably. Shumo et al. (2019) had revealed that the BSFL fed with chicken manure would obtain 41.1% of larval crude protein which was in conformity with the reported works by Sheppard et al. (1994), accentuating 42% of larval crude protein was measured while using a similar substrate. In addition, the harvested BSFL biomass was also found to contain high values of calcium mineral, i.e., 3.2 g/kg dry weight, and ash, i.e., 9.3 g/kg dry weight. The epidermis layer or outer layer of BSFL's skin could accumulate calcium mineral in the form of calcium carbonate, leading to the high level of calcium and ash contents in prepupae when early fed with chicken manure (Shumo et al., 2019). Moreover, some of the calcium and ash contents may be lost in the form of exuviae when the BSFL were undergoing stepwise eclosions in their early instars before harvesting. In other study, Newton et al. (2005) had analyzed the larval phosphorus content and found that the level was higher when the BSFL were fed with poultry manure as opposed to swine manure. Evidently, the different substrates, even in the form of manure, can significantly alter the BSFL body nutritional composition. For comparison, Kinasih et al. (2018) fed the BSFL with horse manure at the feeding rate of 100 mg/larva/day. The first prepupa was found emerging about 25 days later with the prepupal weight of merely 25 mg/larva, which was significantly lower than the BSFL having been fed with chicken feed (130 mg/larvae). Also, the BSFL generally entailed longer rearing periods for emerging into prepupae when fed with animal manure than chicken feed. This was plausibly stemming from the destitute nutritional content of animal manure in which the BSFL would intrinsically ingest more substrate amount to accumulate minimum nutrients for eclosion into prepupae (Barry, 2004; Lee et al., 2004; Nijhout, 2003; Simpson et al., 2006; Wright et al., 2003); thereby, prolonging the rearing period. Furthermore, ur Rehman et al. (2017a) had found that, it was best if the BSFL feeding substrate was comprising of high total organic carbon. Although, the daily manure fulfills the larval diet need, the conversion efficiency into BSFL biomass is still low due to the presence of large amount of lignin, cellulose, hemicellulose biopolymers. These are not facilely digested biopolymers to BSFL and will ubiquitously lead to slow growth and small prepupae (Lalander et al., 2019).

The utilization of restaurant waste and fruit and vegetable residue as the BSFL feeding substrates had been investigated lately by many researchers. Spranghers et al. (2017) presented that the rearing period for BSFL fed with restaurant waste was 19 days for the first larva to emerge as the prepupa. The duration was shortened to 15 days when the larvae were administered with fruit and vegetable residue. The slow growth was primarily due to the presence of grease covering the restaurant waste, leading to the difficulty for BSFL to digest and convert the greasy waste into its body weight. Hence, the BSFL consumed more time for growing and developing into prepupae (Barry, 2004; Spranghers et al., 2017). Conversely, the fruit and vegetable residue was generally free from grease, oil and fat, favoring the physiological growth of BSFL. Apart from that, the harvested prepupae initially given with fruit and vegetable residue possessed a significantly higher ash content than the restaurant waste, namely, 96 and 27 g/kg dry weight, respectively. In the case of larval protein content, the BSFL could separately garner to the tune of 431 and 399 g/kg dry weight when fed with restaurant waste and fruit and vegetable residue, respectively (Spranghers et al., 2017). The higher larval ash content was possibly due to the presence of more minerals in fruit and vegetable residue than restaurant waste. Indeed, the presence of remnant meat composition in the restaurant waste could be the best justification of higher larval protein content than when fed with fruit and vegetable residue. Nevertheless, the difference in terms of larval protein contents was merely between approximately 43% and 40% while employing the restaurant waste and fruit and vegetable residue, respectively. Indeed, the use of fruit and vegetable residue to grow BSFL would enhance the larval mineral content (evidenced by high ash content), making the harvested larval biomass more suitable to serve as an animal feed. Later, Lalander et al. (2019) studied the application of fruit and vegetable residue as a feeding substrate for BSFL and found that the prepupal weight of 218 mg/larva could be obtained when the rearing period was extended to 28 days. By using a conventional BSFL feeding rate of 100 mg/larva/day, Kinasih et al. (2018) revealed that the development time to reach prepupal stage was 25 days when fed with fruit and vegetable residue. Subsequently, by administering a fruit residue alone to BSFL, Leong et al. (2016) showed that a high growth rate could be attained since the fruit residue was consisting of high volatile solids, leading to the large larval size. However, the low protein in fruit residue had inevitably caused slow eclosion into prepupae. Concisely, the presence of high volatile solids and protein in larval diet, i.e., when employing a fruit and vegetable residue to grow BSFL, is the key parameter contributing to the high conversion efficiency into larval biomass and hastening the larval development into prepupae (Lalander et al., 2019). On the other hand, the utilization of restaurant waste to rear BSFL could be possibly improved if the excessive grease, fat and oil are skimmed prior to the BSFL feeding.

Next, the sludge from secondary wastewater treatment plants had also been exploited for BSFL development; whilst focusing on the sludge reduction via larval valorization. Nevertheless, the BSFL were found requiring a long development time of up to 39 days for the first prepupa to emerge. Worse still, the emerged sludge-fed prepupa was smaller in size (about 70 mg/larva) in comparison with using chicken feed, animal manure, restaurant waste or fruit and vegetable residue (Lalander et al., 2019). As a positive aspect, the employment of undigested sludge was found to reduce the larval development time to 30 days and double the larval size to 145 mg/larva. Leong et al. (2016) had associated the slow growth of BSFL while being fed with sewage sludge was due to the presence of inadequate volatile solids and protein contents in sludge. Consequentially, the small prepupal size would largely hinder the fertility of adult flies to reproduce, disrupting its lifecycle and later, its potential application to valorize sludge (Kinasih et al., 2018).

### *4.2 Blended substrate*

The employment of low-cost single substrates has undoubtedly encountered several disadvantages for the rearing of BSFL. For instance, these single substrates of organic wastes are generally destitute of essential nutrients such as protein to enhance the larval growth. Moreover, during the processes to generate the organic wastes, the recalcitrant components for larval digestion such as lignin, cellulose, and hemicellulose are concentrated; thereby, hindering the BSFL development upon feeding, whilst later producing a low-value larval biomass. In fact, the palatable nutritional compositions that can ease the BSFL digestion are proteins, non-fibre carbohydrates and modest amount of lipids (Barragan-Fonseca et al., 2018; Beniers and Graham, 2019; Casartelli et al., 2019; Lalander et al., 2019). The usual organic wastes administered for BSFL rearing are dairy manure, waste coconut endosperm and sludge since these wastes have been produced in humorous amounts as the by-products from industries and agricultural activities. However, the waste properties are improper for the BSFL development; and blending with other waste substrates is viewed as a potential option to fortify the diet for BSFL alimentation. Ideally, blending could improve the nutritional balance in terms of C/N ratios and buffer capacities (pH) that are essential for the enhancement of co-digestion efficiencies by BSFL (Anjum et al., 2016; Li et al., 2009). The conventional nutrient-rich substrate used for blending is soybean curd residue (SCR) in which it is a by-product derived from soy milk or tofu productions, the major surplus organic waste from soybean industries. Worse still, the disposal of SCR could increase the environmental impacts, particularly the release of greenhouse gasses (Li et al., 2013). Hence, using the SCR as a blending substrate for BSFL could enhance the efficiency of bioconversions due to a better nutritional balance in blended larval substrate (Table 2). Another potential substrate for blending is a palm kernel expeller which is the by-product generated from the palm oil extraction process. Although palm kernel expeller has a sufficient nutrient, i.e., containing a high level of crude protein (approximately 17%), the exploitations as a co-substrate for BSFL feeding have still not been documented as opposed to SCR that possesses a crude protein of more than 25% (Li et al., 2013).

The increasing demand for milk consumption has directly given rise to the excessive dairy manure generation from unplanned farming, which brings various environmental problems, namely, unpleasant odors, water pollutions, spreading of diseases, etc. (Aguirre-Villegas and Larson, 2017; Lim et al., 2016). To mitigate those issues, the BSFL have been employed to convert this organic waste. However, this larval valorization approach is very slow and not promising since the dairy manure consists of primarily lignin, cellulose and hemicellulose, which cannot be effectively digested by BSFL for growing, even the manure has a good buffer capacity (Li et al., 2016a; Mata-Alvarez et al., 2014; Wang et al., 2017a). Ur Rehman et al. (2017b) had shown that the 1000 BSFL fed with 1 kg of dairy manure required a long development time of 24 days for the first prepupal appearance. The blending of dairy manure with SCR for larval co-digestion was then compared since the SCR was generally rich in water insoluble nutrients such protein and fat that could significantly spur the BSFL growth. Besides, the low buffer capacity of SCR (pH~5.7) could also be offset by the better buffer capacity of dairy manure (pH~8.4) upon blending to suit the palatability of

BSFL (Li et al., 2016b; ur Rehman et al., 2017b). The results showed that the blended substrate between dairy manure and SCR could curtail the BSFL rearing period for emerging into prepupae and higher organic reduction rate could be measured as well with increasing of SCR proportions. The ratio of dairy manure to SCR at 1:4 had led to the shortest rearing time (21 days) for larval development into prepupae with the survival rate attained at 98.8% while reducing 75% of hemicellulose and 70% of cellulose. Other ratio with more dairy manure than SCR, for instance, 4:1, the BSFL could only reduce 45% of hemicellulose and 52% of cellulose after 22 days of rearing period (ur Rehman et al., 2017b), signifying the importance of substrates blending for an effective valorization of hemicellulose and cellulose. The plausible rationale was blending had balanced the nutrients requirement by BSFL for assimilating the blended substrate into its biomass at a more appropriate pH of feeding medium.

Apart from dairy manure, waste coconut endosperm has also been employed to feed BSFL since it is also an abundantly available organic waste derived from agriculture. The fresh coconut endosperm will lose its soluble components upon the coconut milk extraction, leaving behind a residue know as waste coconut endosperm. This organic waste mainly consists of lignocellulosic materials (30% of rough fibers) with low protein and fat contents, 5% and 9%, respectively. Thus, co-digestion of blended substrates is perceived as an alternative way to enhance the nutrients of waste coconut endosperm prior to feeding to the BSFL to perform bioconversion into valuable larval biomass. Again, SCR had been exploited to ameliorate the protein content of waste coconut endosperm via blending as reported by Lim et al. (2019). Initially, feeding the BSFL with a waste coconut endosperm alone had led to the lowest larval weight gained of only 32.5 mg/larva. The insufficient protein content in waste coconut endosperm would consequently accelerate the pupation process, resulting in small prepupae formation. At the optimum blended ratio of 3:2 between waste coconut endosperm and SCR, the feeding of this blended substrate had permitted the BSFL to attain the highest weight of 67.5 mg/larva, twice the weight of BSFL fed with only waste coconut endosperm. The BSFL could as well amass the highest larval body lipid and protein at 39.2 and 14.5 mg/larva, respectively, while feeding with the optimum blended ratio substrate. In comparison with BSFL fed with a single substrate of waste coconut endosperm, only 17.2 mg lipid/larva and 4.1 mg protein/larva could be measured from the harvested larval biomass. Nevertheless, a further increase in protein content in blended substrate by increasing the proportion of SCR over waste coconut endosperm had significantly decreased the harvested BSFL weight. As the SCR would degrade faster than waste coconut endosperm naturally, the formation of ammonium in the blended substrate from protein degradation would acidify the larval medium, debilitating its buffer capacity. Also, the ammonia gas exuded from ammonium would retard the BSFL development since it affected the larval digestion system, leading to a small larval weight gained (Lim et al., 2019; Tschirner and Simon, 2015).

Sewage sludge is a well know organic waste produced from biological wastewater treatment plants worldwide. The traditional disposal of sewage sludge into the landfill will incontrovertibly extend the carbon footprint while having an expensive cost to handle. Thus, exploiting the sewage sludge for bioconversion into BSFL biomass is an alternative approach for waste reduction. Popa and Green (2012) confirmed that the BSFL had a potential for executing biotransformation when fed with a municipal raw sewage sludge. Although the sewage sludge is laden with various heavy metals such as lead, nickel, etc., none of the metals had influenced the BSFL lifecycle conspicuously as confirmed by Diener et al. (2015). The heavy metals from the sludge may be accumulated in BSFL body but it might not contaminate the lipid that was extracted from harvested larvae, for instance (Cai et al., 2018). Since the sewage sludge is generally lacking protein and digestible carbon sources, a facile blending with nutrient-rich organic matters could plausibly promote the co-digestion of sewage sludge by BSFL. Leong et al. (2016) presented that the BSFL weight had initially shown a small increment when fed with sewage sludge; however, its weight decreased after 4 days, resulting in an overall negative growth rate, i.e., -0.2 ± 0.01 mg/larva/day. Correspondingly, Cai et al. (2018) had also reported a similar observation in which the BSFL recorded a negative growth weight of -1.25 mg/larva due to the presence of insufficient nutrients in sewage sludge; leading to a short period of pupation process with small attainable larvae. In addition, the authors had further investigated the blending of chicken manure and wheat bran into sewage sludge to enhance the efficiency of BSFL bioconversion process. The results showed that the larval weight gained was proportional to the increase of either chicken manure or wheat bran portion in the blended substrates. The gains were recorded at 1.25, 10, and 20 mg/larva when fed with blended sewage sludge to chicken manure ratios of 75:25%, 50:50% and 25:75%, respectively. In the case of wheat bran, the study was investigated only for one ratio, namely, 84:16% of sewage sludge to wheat bran, with a weight gained determined at 12.5 mg/larva. However, when feeding the BSFL with blended substrates consisting of sewage sludge, chicken manure and wheat bran at various ratios, e.g., 63:21:16%, 42:42:16% and 21:63:16%, respectively, those were found to be better than any two blended substrates. The weight gained was recorded to be at least 22.5 mg/larva for any three blended substrates. The highest value gained could reach 28.75 mg/larva for an optimum blended proportion of sewage sludge:chicken manure:wheat bran of 21:63:16%. The BSFL rearing period was also reduced to only 12-13 days while using the optimum three blended substrates as opposed to 30 days when fed with the sewage sludge alone. From the principal component analysis and Pearson's correlation analysis, the contents of carbohydrate, potassium and nitrogen presented in blended substrates were found to be the primarily factors that simultaneously affecting the BSFL weight gained. Recently, Norgren et al. (2019) had investigated the use of bio-sludge from wastewater treatment of pulp and paper industry (PPBS) as a BSFL feeding substrate. The general composition of bio-sludge consists of 1.5%–8.3% of crude protein, 0.3%–3.3% of fat and 17%–40% of lignin. The prepupal weight gained upon feeding with this PPBS substrate was found to merely 0.4 mg/larva. Nevertheless, the prepupal weight increased slightly when other substrates were blended into PPBS, namely, 0.6 mg/larva when blended with water as a free surface, 2.0 mg/larva when blended with composted leachate, 3.5 mg/larva when blended with leachate and water as a free surface and finally, 4.8 mg/larva when blended leachate as a free surface. Although adding some materials into PPBS could increase the prepupal weight, the results had shown that the BSFL final weight was still very low because PPBS mainly comprised lignocellulosic which was difficult to be digested by BSFL (Norgren et al., 2019). Cai et al. (2018) had vindicated that using multiple blended substrates as the feeds for BSFL could result in better larval growth, growth rate and bioconversion efficiency than any low-cost single substrate or blending of two different substrates due to the more balance diets could be obtained from more blended substrates. Gold et al. (2020) had inclusively studied the multiple blended substrates for BSFL feeding. The formulations were calculated based on the composition of single substrates and the achievement ratio of protein to non-fibre carbohydrate at around 1:1. The results demonstrated that even at low larval feeding rate of 25%, feeding with multiple substrates could still proffer decent outputs such as an average survival rate of 99% and average larval weight of 43.5 mg/larva; in comparison with single substrate achieving an average survival rate of 95% and average larval weight of 40.1 mg/larva. Gold et al. (2020) had compared the performances of BSFL in valorizing the blended mill by-product, human faeces, cow manure and vegetable waste at 23:16:11:50% (F1) against blended mill by-product, canteen waste and vegetable waste at 33:33:33% (F2). Owing to the presence of canteen waste, the BSFL could accumulate 22.3% of lipid with F2 as opposed to 19% with F1 and 19.6% of protein with F2 as opposed to 13.8% with F1. However, the larval fiber contents were slightly lower with F1 (38.5%) than F2 (39.8%). Indeed, employing F1 as a BSFL feed had presented the best results, namely, 99.8% of survival rate, 64.2 mg/larva of larval weight, 64.1% of waste reduction and 31.8% of biomass conversion rate in comparison with other blended formulations. It could be noted that even the multiple blended substrates comprising of lower lipid, protein and non-fibre carbohydrate contents than lesser type of blended substrates, the BSFL conversion efficiency and larval development were still better since the former were more nutritionally balance for BSFL.

### *4.3 Microbial fermented substrate*

Another method to fortify the nutritional compositions of BSFL feeding substrates prior to the administrations is through microbial modification, i.e., by executing fermentation in waste biomasses or low-cost organics. The fermentation processes completed by various microorganisms can be specifically categorized into two types based on the inoculation modes. The *in*-situ fermentation transpires when the microorganisms are introduced to execute the fermentation process simultaneously with the valorization of organic substrates by BSFL. However, when the organic substrates are fermented early by the microorganisms before feeding to the BSFL is considered as *ex-situ* fermentation. In this case, the fermentation process is still ongoing during the larval feeding period. The presence of complex organic materials such as lignocelluloses from plant-based products in BSFL feeding substrates is generally difficult to be ingested since the larvae need to enter the epidermis layer of the plants prior to ingesting. Thus, microbial fermentation is deemed necessary to break down the complex components via hydrolysis while releasing myriad nutritional byproducts to spur the palatability of BSFL (Table 2) (Mohd-Noor et al., 2017; Wong et al., 2020).

#### *4.3.1 In-situ fermentation*

Among the microorganisms, *Saccharomyces cerevisiae*, a single cell yeast, had been employed to carry out *in-situ* fermentation in waste coconut endosperm before administering for BSFL feeding (Wong et al., 2020). The BSFL growth rate and waste-to-biomass conversion were found increasing with the increment of yeast concentrations, i.e., the highest values were achieved at 42.5 mg/larva/day and 11.5%, respectively, when the *in-situ* fermentation was carried out at the 2.5 wt% of yeast concentration. In comparison with the absence of yeast, the control waste coconut endosperm could only attain the larval growth rate of merely 3.25 mg/larva/day. Accordingly, the presence of yeast was propound could break down the carbohydrate compounds especially monosaccharides in waste coconut endosperm, leading to the better digestibility and assimilation of nutrients into BSFL bodies

(Wiedmeier et al., 1987; Yoon et al., 2003). However, the best lipid yield from harvest BSFL biomass, i.e., 49.4%, was attained when fed with 1.0 wt% of yeast concentration in waste coconut endosperm instead of 2.5 wt%. The transesterification of larval lipid permitted the biodiesel to contain a significant mixture of C12:0, C14:0, C16:0, and C18:1 (Table 3), indicating high in saturated fatty acids in which directly associated to the high in oxidative stability of produced biodiesel (Wong et al., 2020).

The *Bacillus subtilis*: S15, S16 and S19, isolated bacterial species from BSFL gut that could digest protein and organic phosphorus, had been exploited to inoculate chicken manure prior to feeding to the BSFL (Yu et al., 2010). The BSFL fed with any *B. subtilis in-situ* fermented chicken manure had resulted in higher prepupal weight and shorter development time in comparison with non-inoculated poultry manure. Neverthesless, among the *B. subtilis* species, the chicken manure innoculated with S15 had eventually engendered the highest prepupal weight of 94.6 mg/prepupa and shortest development time of 7.67 days. Moreover, Xiao et al. (2018) had also reported that the weight incremental rate of BSFL and material reduction rate by BSFL were increased by 15.9% and 40.5%, respectively, when fed with chicken manure initially inoculated with *B. subtilis* as opposed to control chicken manure. The symbiotic bacteria of *B. subtilis* could aid the assimilation process of BSFL to digest the non-digestible content, whilst providing the essential nutrients for BSFL growth as derived from fermentation process. Also, the presence of symbiotic *B. subtilis* would protect the growing BSFL from the surrounding risks such as parasitoids or pathogens (Douglas, 2015; Laughton et al., 2011). Other studies had also evidenced that the novel bacterial species isolated from immature black soldier fly could decompost various organic materials, leading to the enhancement of BSFL development upon ingestion and more mature BSFL could be subsequently harvested (Ahmad et al., 2006; Bosch et al., 2014; Fitt and O'Brien, 1985; Xiao et al., 2018; Zheng et al., 2012b). Apart from that, besides treating sole chicken manure via *in-situ* fermentation, the *B. subtilis* had as well acted as an exogeneous bacteria to colonize chicken manure blended with dairy manure prior to the BSFL feeding (ur Rehman et al., 2019). The dairy manure is usually rich in fibers such as the mixture of lignin, hemicellulose and cellulose that could hinder the BSFL assimilation for growth. Thus, the co-conversion with *B. subtilis* could assist the BSFL digestion process by modifying the fibers in terms of structure and chemicals (Li et al., 2016a; Masih-Das and Tao, 2018; ur Rehman et al., 2019). The mixing of dairy manure and chicken manure at the ratio of 2:3 had exhibited the best performances when simultaneously treated with *Bacillus* $MRO_2$ strain as a lignocellulosic degrading bacteria, leading to 99.07% of survival rate, 25.94 mg/larva of larval weight, 19 days of development time and 67.8% of lipid and 71.2% of protein utilizations for larval growth; as compared with the larval feeds inoculated with other bacteria strains or control feed that had attained the similar parameters at ca. 94.57%, 16.35 mg/larva, 20.58 days, 47.7% and 53.9%, respectively. In addition, the treated co-conversion substrate with *B.* $MRO_2$ had also obtained high values of fiber reductions which were 72.96% for cellulose, 68.52% for hemicellulose and 32.86% for lignin. It was suggested that the exogenous bacteria could strengthen the gut microbiome of BSFL by modifying the ingested lignocelluloses to facilitate the BSFL digestion. Thereby, the reduction of animal manures went in tandem with the enhancement of BSFL development (ur Rehman et al., 2019).

The inoculation with *Lactobacillus buchneri* bacterial species in SCR had also been investigated by Somroo et al. (2019). The BSFL fed with *in-situ* fermented *L. buchneri* SCR had presented higher survival rate (98%), larval weight (34.7 mg/larva) and bioconversion rate (6.95%) and shorter development time (16.1 days) than the BSFL fed with fresh SCR, respectively recorded at 95.4%, 25 mg/larva, 5% and 17.7 days. It was rationalized that the co-digestion with *L. buchneri* played a significant role to support the BSFL adapting to the new surroundings and food sources. In this regard, the BSFL could benefit from the positive interactions in which more nutrients availability to enhance the BSFL growth, gut microbiota development and digestive enzyme production upon feeding with *in-situ* fermented SCR by *L. buchneri* (Engel and Moran, 2013; Kaltenpoth, 2009; Scott et al., 2008; Somroo et al., 2019; Teixeira et al., 2008; ur Rehman et al., 2017b; Yun et al., 2014). In accounting the nutritional contents of harvested BSFL biomass, the initial presence of *L. buchneri* had spurred the larval lipid and protein to 30% and 55.3%, respectively, as opposed to control SCR, without the prior *in-situ* fermentation, in which had recorded slightly lower contents, namely, 26.1% and 52.9%, respectively. Nevertheless, the addition of symbiotic *L. buchneri* had no effect on fatty acids composition in harvested BSFL and the main composition of fatty acids was consisting of saturated fatty acids which were C12:0, C16:0 and C14:0 (Table 3). Both the protein and fatty acids mixture derived from BSFL were later confirmed to be suitable serving as animal feed and biodiesel, respectively (Somroo et al., 2019).

Zheng et al. (2012a) had verified that the presence of mixed bacterial consortia, in the form of commercial product know as Rid-X, could aid the digestion of rice straw blended with restaurant waste in converting into BSFL biomass. The Rid-X was composed of natural bacteria that could degrade celluloses and hemicelluloses due to the presence of various enzymes exuded by bacteria such as cellulose, lipase, protease and amylase. The celluloses and hemicelluloses from *in-situ* fermented substrate could be reduced by 65% and 55%, respectively, upon being valorized by BSFL as compared with only 27% and 32%, respectively, while using a control substrate. The lipid yield from 2000 larvae/batch was increased with the increase of Rid-X concentrations added into the blended rice straw (20%) and restaurant waste (80%), namely, approximately 32 g of lipid yield at 0.05 wt% of Rid-X and was increased to about 38 g of lipid yield at 0.4 wt% of Rid-X. Therefore, it could be concluded that the presence of more exo-bacteria could ultimately fasten the *in-situ* fermentation process and release more nutrients into BSFL feeding substrate to promote larval growth.

#### *4.3.2 Ex-situ fermentation*

The *ex-situ* approach via self-fermentation had been adopted by Mohd-Noor et al. (2017) to improve the nutritional characteristics of lignocellulosic biomass of waste coconut endosperm before administering to rear BSFL. The self-fermentation was associating to the ability of indigenous microorganisms to execute an intrinsic fermentation in organic materials over the time. The results had confirmed that the four weeks of fermentation's time were needed in order to release a maximum nutrient content from waste coconut endosperm, i.e., the highest total dissolved organic carbon concentration, especially organic acids, was measured at 70 ppm. Accordingly, the self-fermentation had mature once reaching four weeks in which the polysaccharides from waste coconut endosperm were significantly transformed into organic acids, softening the fiber property that was essential for maintaining the BSFL gut health (Caruso et al., 2014; Upadhaya et al., 2016). Consequentially, the BSFL achieved the highest growth of 35 mg/larva of weight gained and 2 mg/larva/day of growth rate. Furthermore, the BSFL also had accumulated the highest yields of lipid and protein at 57.95% and 15%, respectively, when fed with the week-4 self-fermented waste coconut endosperm. While using the fresh waste coconut endosperm (control) to feed the BSFL, the larval growths were only attained at approximately 22.5 mg/larva of weight gained and 1.5 mg/larva/day of growth rate with lipid and protein yields were found to be 20.70% and 12%, respectively, from harvested larval biomass (Mohd-Noor et al., 2017). Nevertheless, by increasing the self-fermentation period beyond four weeks, the overwhelming growth of microorganisms had impoverished the essential nutrients meant for BSFL growth. The dissolved organic compounds were depleted significantly, and because of that, the BSFL had to compete with microorganisms for common growth nutrients. Also, the microorganisms were protected by the strong cell walls or membranes that would forestall valorization by BSFL digestion (Leong et al., 2016). Thus, it is crucial to control the *ex-situ* fermentation activities to ensure the generated nutritive byproducts from fermentation to spur the BSFL growth will not be depleted by the unnecessary extension of fermentation time.

The *S. cerevisiae* yeast was employed by Li et al. (2015) to execute *ex-situ* fermentation in lignocellulosic biomass of rice straw at 37 °C for 48 hours to improve its protein content prior to BSFL feeding in producing larval biodiesel. The results showed that 89.6% of protein in BSFL feeding substrate could be assimilated into larval biomass within 14 days. The protein-rich fermented rice straw could enrich the accumulation of larval body lipid. Essentially, in the presence of sufficient digestible proteins, the BSFL could synthesize and excrete cellulases enzyme to convert lignocellulosic biomass into its fats and oils. And so, 5.2 g of total lipid could be extracted from the 200 mature BSFL/batch while later producing 4.3 g of biodiesel. This vindicated that the BSFL fed with microbial treated rice straw that consisted mainly of lignocelluloses could be potentially exploited as a feedstock for producing biodiesel while tapping into *ex-situ* fermentation process (Li et al., 2015).

Next, Gao et al. (2019) had assessed the bioconversion performance of BSFL in assimilating *ex-situ* fermented maize straw initially inoculated with *Aspergillus oryzae* fungus at 27°C for 24 hours. The fermented maize straw was more palatable to BSFL since the lignocellulosic content had been hydrolyzed into a more digestible composition as opposed to the untreated maize straw (Binod et al., 2010; Gao et al., 2019; Ware et al., 2005; Zheng et al., 2012a). Nevertheless, in comparing with the commercial wheat bran used as a reference, the feeding with fermented maize straw had led to smaller harvested BSFL, i.e., 1.49 mg/larva against 2.22 mg/larva, while requiring longer rearing duration. The protein content was found to be lower in fermented maize straw than wheat bran, whilst the fermented maize straw was still consisting of higher cellulosic content than the wheat bran, derailing the growth of BSFL (Gao et al., 2019). The BSFL fed with fermented maize straw possessed a lower proportion of saturated fatty acids (45.41%) than the BSFL fed with wheat bran (62.28%). Moreover, the lipid of BSFL fed with fermented maize straw diet had high proportions of monounsaturated fatty acids (24.86%) and polyunsaturated fatty acids (25.37%) which are significant for human health (Calder and Grimble, 2002; Sahena et al., 2009). Moreover, the BSFL fed with fermented maize straw also had a protein content (41.8%) comparable with the conventional soybean meal or aquatic meal (42.1%). Indeed, the biomass from BSFL fed with *ex-situ* fermented maize straw had adequate crude fiber (30.6%) which was usually lacking in other animal feeds (Makkar et al., 2014).

The *ex-situ* fermentation of waste coconut endosperm completed by mixed-bacterial powder (Reckitt Benckiser, UPN:1920080310) for 28 days prior to feeding to BSFL had been studied by Wong et al. (2019). The waste-to-biomass conversion (WBC) and protein conversion by BSFL were increasing with increasing of mixed-bacterial powder concentrations, reaching the highest values of approximately 9% for WBC and 60% for protein conversion with the initial inoculation concentration of 0.5 wt%. The addition of mixed-bacterial powder exceeding 0.5 wt% during the *ex-situ* fermentation had resulted in the descent of WBC and protein conversion since more microorganisms were competing with BSFL for common nutrients. Moreover, the effect of *ex-situ* fermentation time using the optimum concentration of mixed-bacterial powder, i.e., 0.5 wt%, was also investigated by Wong et al. (2019). In this regard, the best time frame to attain the maximum WBC and protein conversion concurrently was found to be 14 days. By prolonging the time frame beyond 14 days, e.g., 21 and 28 days in a similar study, had brought about no significant impact on BSFL growth and organic waste reduction. From the *ex-situ* fermentation process, the organic acids were found being exuded from the fermented waste coconut endosperm in which were essential for the larval gut health and development (Upadhaya et al., 2016; Wong et al., 2019). Although the addition of mixed-bacterial powder could aid the digestion of fibers from waste coconut endosperm into organic acids, amino acids and vitamins, the significant influence of high amount of celluloses or polymer structures coupled by the low protein content in waste coconut endosperm, i.e., merely 5.83% of protein constituent, had limited the BSFL growth (Caruso et al., 2014; Wong et al., 2019). To epitomize, depending on the type of BSFL feeding substrates, blending method for co-conversion may be somehow more effective than fermentation. Also, the *ex-situ* fermentation approach is deemed time consuming as certain period of time has to be earmarked for the inoculated exo-microbes to complete the fermentation process.

## 5. Application and limitation of harvested BSFL biomass

In addition to valorizing the solid organic wastes, the harvested BSFL biomass contains valuable biochemical compounds such as lipid, protein, chitin and myriad essential organic minerals that can be potentially employed for alimentation of farmed animals. Also, the larval lipid could be a promising solution serving as the new and sustainable feedstock for biofuel industries, in which various methods to optimize the transesterification process of larval lipid have been currently investigated to maximize and tune the quality of BSFL-based biodiesel (Wong et al., 2019).

The continuous growth of global population and industries has resulted in the rising of fossil fuel consumptions and lately, has been unabatedly dethroned by renewable fuels such as biodiesel which is non-toxic and eco-friendly toward the environment (Singh et al., 2020). Initially, the biodiesel that is derived from edible crops has led to food shortages. To cushion the menace, biodiesel produced from non-edible crops such as exploiting the spent cooking oil has been proven feasible. However, the operations for converting the spent cooking oil into biodiesel require a high investment cost since large amounts of contaminated matters are present in spent cooking oil, leading to the complication of chemical processes (Mohd-Noor et al., 2017; Tan et al., 2015). Next, the oleaginous microorganisms have been considered as a third generation of biofuel feedstock to produce biodiesel. Alas, the lipid-rich oleaginous microorganisms are experiencing high buoyancy and resisting from settling, incurring a high harvesting cost since extensive time and intensive energy are needed to separate the microalgal biomass from its large cultivation volume (Gerardo et al., 2015; Mohd-Noor et al., 2017; Pinzi et al., 2014). To circumvent these setbacks, the utilization of lipid in the form of fat body from BSFL for the production of biodiesel is gaining more attentions among researchers as a new generation of biofuel feedstock (Cheng and Timilsina, 2011; Manzano-Agugliaro et al., 2012; Mohd-Noor et al., 2017; Payne et al., 2016). The BSFL lipid had been found to possess a higher amount of saturated fats (67%) in comparison with soybean oil (11%) and palm oil (37%) (Hasnol et al., 2020). As the general composition of biodiesel is fatty acid methyl esters (FAMEs) mixture, Ushakova et al. (2016) had reported that the FAME profile from BSFL was loaded with C12:0 at 38.43 wt%, followed by C16:1 at 15.71 wt%, C14:0 at 12.33 wt%, C18:1 at 8.81 wt% and C18:0 at 2.95 wt%. Corresponding with Leong et al. (2016) and Wong et al. (2019) studies had also presented that the major composition of FAMEs in biodiesel produced from BSFL biomass was lauric acid (C12:0). Surendra et al. (2016) had found the C12:0 in FAMEs of biodiesel derived from BSFL reached the peak of 44.9% as opposed to soybean and palm oil-based biodiesel, namely, negligible and 0.1%, respectively. The high level of C12:0 content derived from BSFL lipid indicates the high quality of biodiesel with low viscosity whilst being more stable (Hasnol et al., 2020). Moreover, Zheng et al. (2012a) had confirmed the suitable properties of biodiesel derive from BSFL fed with rice straw and restaurant waste which was in conformity with the requirements of EN 14214 standard. Also, the quality was as well comparable with the biodiesel produced from rapeseed oil (Li et al., 2011).

Apart from being used for biodiesel production, the BSFL lipid is a good fat source for fishmeal in aquaculture. Li et al. (2016c) had proven the lipid from BSFL could substitute the soybean oil as verified in terms of growth of juvenile Jian carp as well as its fatty acid and lipid accumulations during maturity (Wong et al., 2019). The results showed that C12:0 and

C14:0 contents in muscle of experimental fishes were higher when fed with BSFL lipid than soybean oil, *viz.*, 0.49% and 1.30%, respectively, with soybean oil and 4.37% and 2.65%, respectively, with 100% substitution of soybean oil by BSFL lipid in Jian carp diet. However, the growth rates of fishes were not significantly affected by either soybean oil or BSFL lipid, *viz.*, 3.36% and 3.28% of specific growth rates, respectively. This had confirmed the potentiality of BSFL lipid to be adulterated into fishmeal, serving as an alternative composition to soybean oil. In addition, as the high level of medium-chain fatty acids especially C12:0 content in BSFL lipid is similar to that of coconut oil, Kim et al. (2020) proved that the BSFL lipid could significantly increase the unsaturated fatty acids, i.e., linolenic acid, in which resulted in the abundant of omega-3 fatty acids presented in edible chicken meats in comparison with coconut oil employment. Furthermore, the medium-chain fatty acids in BSFL lipid could promote the antibacterial activity and spur the growth performance of broiler chickens when it was laden as a lipid source in the chicken feed (Kim et al., 2020; Li et al., 2016c; Schiavone et al., 2018; Ushakova et al., 2016).

Upon extracting the lipid from BSFL biomass, the residual biomass with concentrated protein source can be subsequently introduced into animal diet (Wong et al., 2019). However, the chitin content in BSFL biomass residues should be degraded by chitinase or removed prior to the utilization since the chitin may retard the growth performances and nutrients adsorption by aquatic farmed fishes (Lindsay, 1984; Makkar et al., 2014; Spranghers et al., 2017; Wong et al., 2019). Li et al. (2017) had studied the replacement of fishmeal protein by defatted BSFL biomass that contained merely 56.9% of crude protein. The results presented that the specific growth rates of Jian carp were not significantly different between using fishmeal and defatted BSFL biomass as the Jian carp feed. Lock et al. (2016) had also proven the potential of replacing fishmeal with BSFL meal and brought about no significant impact on the growth performances of Atlantic salmon. The nutritional compositions of Jian carp fed with either fishmeal or defatted BSFL meal were both fallen within 19-20% for whole body of Jian carp for crude protein, 74-75% for whole body of Jian carp for moister and about 16% for whole body of Jian carp for lipid, heralding no difference (Li et al., 2017). To top it off, the high catalase activity in dietary defatted BSFL biomass was found contributing into boosting the antioxidant property in Jian carp. Nevertheless, the optimum proportion for substitution of defatted BSFL biomass was recommended to be 50 wt%, since the further increase of defatted BSFL biomass, i.e. 75 wt%, could damage the histopathological intestine and contribute to dietary stress (Li et al., 2017). Katya et al. (2017) had also documented that the percentage of BSFL to replace fishmeal or soybean meal as a protein source for juvenile barramundi should be lesser than 50 wt% for no adverse effect on the whole body proximate and amino acid compositions of barramundi. The BSFL biomass had also been exploited as a protein source instead of soybean meal, i.e., the crude proteins in bird diet were 184.9 and 185.2 g/kg as-fed basis when using soybean meal and replacement of 25 g/kg of soybean meal with BSFL biomass, respectively, for poultry diet (jumbo quails rearing) (Mbhele et al., 2019). Above all, the utilization of BSFL meal for broiler rearing had been reported could decrease the possibility of metabolic skeletal disorders during the development of bird health (Pieterse et al., 2014). The optimum BSFL level to substitute soybean meal was identified at 54 g/kg, since the further inclusion of BSFL in feed could contribute to depress the overall feed intake and body weight increment of jumbo quails.

Besides larval lipid and protein, other biochemicals that can be extracted from BSFL biomass are minerals, vitamins, chitin, etc. Minerals especially calcium can improve the quality of farmed animal growths since calcium is an essential component for muscle mass, enzymatic activity, neuro-signaling, metabolic reaction, synthesis of proteins, maintenance of osmotic and acidic-alkaline equilibria as well as construction of membranes in animal cells (Shumo et al., 2019). The deficiency of calcium will overall result in skeletal, immune and cardiovascular system disorders, bone loss, growth retardation and abnormal posture (Hafeez et al., 2015). Nevertheless, the presence of excess calcium can become a limitation for exploiting BSFL as an animal feed since it will increase the farmed animals' stomach pH. Thereby, impeding the digestion of consumed feeds by farmed animals. Prolonging the retention of remnant feeds in the animal stomach may lead to the diarrhea and risk of bacterial infections especially piglets (Lawlor et al., 2005; Spranghers et al., 2017). The vitamins from BSFL biomass are also considered essential to be presented in farmed animals' meal since it can strengthen the immune system and assist in the digestion process to produce more energies for metabolism and growth (Shumo et al., 2019). Moreover, Borrelli et al. (2017) had reported that BSFL biomass could be employed as a potential prebiotic for laying hens. The chitin content from BSFL could tweak and eventually balance the microbial communities, leading to the reduction of antibiotics utilization in the poultry industries that usually associating to the adverse effects on human health. In addition, Marono et al. (2017) had also presented that the chitin in BSFL meal could reduce the amount of triglycerides and cholesterols, benefiting the health of laying hens. Chitin from BSFL also found enhancing the eggshell thickness and microbiota diversity values in poultries (Kawasaki et al., 2019). Even though the chitin from BSFL has a general positive effect on poultries, the monogastric animals cannot digest the BSFL chitin easily while subsequently, bring about a negative effect on protein assimilation (Bovera et al., 2016; Longvah et al., 2011; Sánchez-Muros et al., 2014). Also, Makkar et al. (2014) had revealed that the high level of ash in BSFL meal could threaten the growing animal since it would retard the ingestion process of animals especially monogastrics animals and derail growth. Table 4 summarizes the advantages and disadvantages of utilizing BSFL biomass.

## 6. Conclusions

Black soldier fly larva (BSFL) has a great potential in waste management since it can valorize various organic wastes and transform into its biomass. The difference in organic wastes to serve as the feeding substrates can overall affect the BSFL biomass content especially larval body protein and lipid. Nevertheless, the presence of excessive lignocellulose in the feeding substrates can also hinder the digestion process of BSFL, inhibiting its development. The lack of essential nutrients in the substrates is usually associated to the small larval size harvested. Hence, blending with other substrates and fermentation by microbes have been investigated recently to fortify the nutritional values of BSFL feeding substrates. Upon reaching maturity, the BSFL are harvest for its valuable biochemical content. The protein from BSFL biomass is usually processed into farmed animal feed to replace or substitute fish meal. Moreover, the high content of C12:0 in FAMEs mixture has given rise to a good quality of biodiesel produced from BSFL lipid. Besides protein and lipid sources, chitin and calcium from BSFL feedstock also can be used for the livestock alimentation. However, the presence of excess chitin and calcium need to be monitored constantly to preempt retardation of animal growths. To conclude, the employment of BSFL can sustainably valorize various organic wastes, whilst producing green valuable larval biomass to underpin biofuel and livestock industries that eventually benefiting the environment in a dual way. For future development to employ BSFL in valorizing organic wastes, other low-cost and nutrient-rich substrates such as palm kernel expeller could be explored for blending with recalcitrant organic wastes in spurring the palatability of BSFL. The correlation between nutritional constituents of larval feeding substrates and accumulated biochemicals from harvested BSFL biomasses could be as well statistically studied to optimize the performances of BSFL in bioconverting organic wastes into valuable resources. Last but not least, the plausible applications of fine biochemicals derived from BSFL biomass could be investigated in bringing high values to the new industries.

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