

Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.)) (Diptera: Stratiomyidae) (BSF)

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Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF)



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ABSTRACT

Population growth and unprecedented economic growth and urbanization, especially in low- and middle-income countries, coupled with extreme weather patterns, the high-environmental footprint of agricultural practices, and disposal-oriented waste management practices, require significant changes in the ways we produce food, feed and fuel, and manage enormous amounts of organic wastes. Farming insects such as the black soldier fly (BSF) (Hermetia illucens) on diverse organic wastes provides an opportunity for producing nutrient-rich animal feed, fuel, organic fertilizer, and biobased products with concurrent valorization of wastes. Inclusion of BSF larvae/pupae in the diets of poultry, fish, and swine has shown promise as a potential substitute of conventional feed ingredients such as soybean meal and fish meal. Moreover, the bioactive compounds such as antimicrobial peptides, medium chain fatty acids, and chitin and its derivatives present in BSF larvae/pupae, could also add values to the animal diets. However, to realize the full potential of BSF-based biorefining, more research and development efforts are necessary for scaling up the production and processing of BSF biomass using more mechanized and automated systems. More studies are also needed to ensure the safety of the BSF biomass grown on various organic wastes for animal feed (also food) and legalizing the feed application of BSF biomass to wider categories of animals. This critical review presents the current status of the BSF technology, identifies the research gaps, highlights the challenges towards industrial scale production, and provides future perspectives.

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1. Introduction

With the rapid population growth, economic development, and urbanization, especially in low- and middle-income countries, meeting the demand for quality food and managing organic waste will require substantial resources. For example, the rapid increase in per capita income and urbanization is likely to result in structural changes in demand for food and other agricultural products (FAO, 2017; Surendra and Kuehnle, 2019). To meet the demand for these resources, agricultural production is projected to increase by 49% between 2013 and 2050 (FAO, 2017), while, from 2005/2007 to 2050, meat, egg, and dairy production are projected to increase by 76%, 64%, and 62%, respectively (Alexandratos and Bruinsma, 2012). Similarly, global food fish production in 2030 is projected to be 17.6% higher (30 million metric tons live weight equivalent) than that in 2016 (FAO, 2018). Since about six units of plant protein are required to produce one unit of high-quality animal protein (Pimentel and Pimentel, 2003), the projected increase in meat and dairy production will result in substantial increase in demand for protein-rich animal feeds (Surendra and Kuehnle, 2019). A significant amount of organic waste is generated worldwide and is not properly managed, especially in low- and middle-income countries (Kaza et al., 2018; Troschinetz and Mihelcic, 2009; Wilson et al., 2012). Annually, a third of all food produced (about 1.7 billion metric tons) is lost or wasted along the food chain, which has a significant environmental (3.3 billion metric tons of CO₂-equivalent greenhouse gas (GHG) emission per year) and economic (total social costs of \$ 1.2 trillion per year) footprints (FAO, 2014). Moreover, high moisture and nutrient contents, biological instability due to biodegradation and enzymatic activity, and the potential presence of pathogens make the organic waste management more challenging in comparison to other waste streams (Russ and Meyer-Pittroff, 2004; Varelas, 2019; Yin et al., 2014). Currently, landfilling and haphazard land disposal dominate the waste disposal methods, especially in low- and middle-income countries, resulting in substantial environmental (ground- and surface-water pollution and GHG emissions) (Bogner et al., 2008; Reddy and Nandini, 2011), public health (breeding ground for disease vectors) (Lohri et al., 2017; Reddy and Nandini, 2011), economic (transportation cost), and social (not-in-my-backyard) issues.

Overall, environmental, economic, public health, and food security issues associated with the ever-increasing and non-sustainable way of using limited resources for food and feed production, coupled with the generation of large amounts of organic wastes, requires a more economically-viable, environmentally-friendly, and technically-feasible approach to food and feed production and organic waste management. In this context, insect farming on organic wastes provides an avenue for wastes bioremediation

with concurrent generation of nutrient-rich feed (i.e., insect biomass) and organic fertilizer (Surendra and Kuehnle, 2019). Farming insects on different organic wastes such as food waste, human feces, and animal manure has been reported to reduce the organic waste by 25-72% (dry matter basis) (Diener et al., 2011; Henry et al., 2015; Lalander et al., 2015, 2019, Nyakeri et al., 2017b, 2019, Rehman et al., 2017a, 2017b, 2019; Somroo et al., 2019; Xiao et al., 2018b; Zhou et al., 2013) and nutrients such as nitrogen and phosphorus by 22-57% and 35-70%, respectively (Lalander et al., 2015; Myers et al., 2008; Rehman et al., 2017; Zhou et al., 2013). Many insects belonging to the orders Lepidoptera, Diptera, Hymenoptera, Coleoptera, Trichoptera, Hemiptera, and Odonata exhibit antifungal activity and/or antimicrobial peptides (Elhag et al., 2017; Henry et al., 2015); hence, insect farming on organic wastes significantly reduces pathogens (Erickson et al., 2004; Henry et al., 2015; Lalander et al., 2015; Liu et al., 2008) and odors (Beskin et al., 2018).

Several insects, such as mealworms (Tenebrio molitor, Alphitobius diaperinus, and Zophobas morio), locusts (e.g., Locusta migratoria and Schistocerca gregaria), crickets (e.g., Acheta domestica and Gryllodes sigillatus), house fly (Musca domestica), and the black soldier fly (Hermetia illucens) (BSF) have been identified as key players. However, the BSF, which is a detritivorous insect has received significant attention due to its ability to grow on diverse organic wastes, such as livestock manure (Beskin et al., 2018; Chen et al., 2019; Julita et al., 2018; Lalander et al., 2015; Li et al., 2011; Liu et al., 2008; Mazza et al., 2020; Moula et al., 2018; Myers et al., 2008; Newton et al., 2005; Oonincx et al., 2015; Rehman et al., 2017; Sheppard, 1983; Sheppard et al., 1994; Xiao et al., 2018b; Yu et al., 2011; Zhou et al., 2013), human feces (Banks et al., 2014; Lalander et al., 2013, 2015; Nyakeri et al., 2017b), organic fraction of municipal solid waste (Cai et al., 2018; Diener et al., 2011; Sarpong et al., 2019), food wastes (Nguyen et al., 2015; Nyakeri et al., 2017b; Surendra et al., 2016; Zheng et al., 2012), agri-residues (Lim et al., 2019; Mohd-Noor et al., 2017; Palma et al., 2018; Supriyatna et al., 2016), compost leachate (Green and Popa, 2012; Popa and Green, 2012), landfill leachate (Grossule and Lavagnolo, 2019), insect farm waste (Jucker et al., 2020), fish offal (St-Hilaire et al., 2007a), and vertebrate remains (decomposing swine carcasses) (Tomberlin et al., 2005); thereby producing protein- and fat-rich larval, prepupal, or pupal biomass (referred to as BSF biomass hereafter unless stated otherwise) for animal feed and biofuel applications. Moreover, the BSF is not known to vector pathogens or to be a nuisance to companion animals or humans (Diener, 2010; Furman et al., 1959).

The BSF has been studied for organic waste valorization and animal feed production for over seven decades. However, the potential of BSF technology for bioconversion of organic wastes into value-added products has not been well examined. There are



Fig. 1. A schematic of life cycle of the BSF.

only a few commercial-scale organic waste bioconversion facilities using the BSF, and these are sparsely distributed globally. Thus, the overarching goal of this review is to critically evaluate the current state-of-the-art of bioconversion of organic wastes via BSF farming with emphasis on conversion efficiency, biomass yield, factors affecting bioconversion, nutrient composition, and BSF biomass as an animal feed. This review also highlights the technical challenge associated with insect farming on organic wastes for animal feed applications. The environmental and economic aspects of bioconversion of organic wastes into BSF biomass are also briefly discussed. The review concludes with recommendations for further research and development, and commercialization of the technology.

2. Black soldier fly life cycle

The BSF has a historic distribution throughout the Nearctic region of the world. However, with globalization and technological advancements, this species can now be found in most temperate and tropic regions (Oliveira et al., 2015; Roháček and Hora, 2013; Sheppard et al., 1994). The BSF is wasp-like in appearance with unique color patterns of white and black over its body (Tomberlin et al., 2002). However, the BSF may also appear like other species of Hermetia and can easily be confused; adult specimens require verification by a taxonomic expert prior to commercialization. The life cycle of the BSF (Fig. 1) is like other holometabolous arthropods (May 1961). Adults live for approximately two weeks when only provided water. Usually males emerge two days prior to females and mating occurs two days later. The BSF is an eurygamous (mating on flight) insect, and thus requires broad areas for its nuptial flight (Caruso et al., 2013). They typically mate once, and females will lay one clutch of eggs during their life, usually two days after mating. Resulting eggs hatch in about four days with larvae needing about two weeks to complete development (Tomberlin et al., 2002). The prepupae, the later larval stage prior to pupation, empty their digestive track and disperse away from the food source in search of a dry and protected place to pupate (Sheppard et al., 1994). Pupae also require about two weeks to develop.

3. Bioconversion of organic wastes via the black soldier fly farming

The BSF larvae are voracious consumers of organic wastes, a detailed discussion of which is provided in a recent review by Gold et al. (2018). Studies have demonstrated that BSF larvae can consume a wide variety of organic resources ranging from food waste, agri-industry co-products, animal waste to meat-based products. Bonelli et al. (2019) described the morphological features and physiological functions of the midgut of the BSF larvae that enables BSF larvae to digest these diverse organic resources. In summary, the midgut of the BSF larvae has three different regions with distinct luminal pH: the acidic (pH = 6.0) anterior midgut, strongly acidic (pH = 2.0) middle midgut, and alkaline (pH = 8.5) posterior midgut. Such variation in luminal pH along the midgut plays crucial roles in enzyme activity, nutrient solubility, detoxification of compounds ingested with substrate, and shaping gut microbiota. The ingested polysaccharides and some lipids are digested in the anterior midgut by soluble amylases and lipases, respectively, while remaining lipids and protein are mainly digested in the posterior midgut by the actions of lipases and endo- and exo-peptidases, respectively. The alkaline pH in the posterior midgut also enables BSF larvae to grow in substrates rich in tannins and other secondary metabolites that bind to proteins (i.e.,

enzymes) at low pH ranges. The high activity of lysozyme and strongly acidic pH in the middle midgut is believed to be responsible for inactivation of pathogens ingested with substrates (Bonelli et al., 2019). In all instances, the composition of the substrates must be considered, which may have a major impact on BSF development, survival, nutritional composition, and the substrate bioconversion rate. For example, depending on the substrate type, development can take between two weeks to several months and the resulting larvae can have protein contents ranging from 10 to 40% of body weight (Oonincx et al., 2015). The same variability also occurs with both fat content and composition of resulting BSF larvae reared on different substrates.

Due to such variability, further research is needed to understand the effect of substrate pretreatment prior to feeding on nutritional composition of BSF biomass. For example, particle size and microbial community can be manipulated to enhance BSF larvae development. More specifically, large particle size (i.e., whole vegetables or meat products) are challenging for the BSF to digest (Brits, 2017). Size reduction of the substrates prior to introducing it to the BSF larvae allows larvae to access nutrients more effectively. Also pretreating the substrate with chemicals (Isibika et al., 2019), bacteria (Isibika et al., 2019; Rehman et al., 2019; Somroo et al., 2019) or fungi (Gao et al., 2019; Isibika et al., 2019) may facilitate bioconversion. Similarly, selected microbes are also known to enhance (i.e., accelerate) BSF larvae development (Rehman et al., 2019; Yu et al., 2011).

3.1. Conversion efficiency of organic resources

The organic (substrate) reduction and bioconversion rate of organic resources into BSF biomass are summarized in Table 1. Studies have reported variation in substrate reduction and bioconversion rate, not only with substrate type (Gold et al., 2020), but also with BSF strain, feeding rate, and larval density among others. Thus, Bosch et al. (2020) have discussed and proposed procedures for standardizing the bioconversion studies using BSF larvae. For example, the BSF larvae of the Wuhan (China) strain not only weighed more than the Guanghzhou (China) and Texas (USA) strains (14.4% and 37.0%, respectively), but also processed comparatively more substrate (Zhou et al., 2013a) (Table 1). Thus, selecting and maintaining the appropriate strain is critical in achieving improved substrate conversion and BSF biomass yield. In general, BSF colonies developed from local strains would result in better substrate bioconversion and minimize the risk of colony collapse (Zhou et al., 2013a). An extensive study on any variation in nutrient composition of different strains of the BSF larvae reared on a similar substrate is currently lacking.

Other factors that significantly affect substrate conversion include larval density, feeding rate, and feeding frequency (Banks et al., 2014; Diener et al., 2011, 2009; Parra Paz et al., 2015). To some extent, increasing the feeding rate results in faster growth and higher BSF biomass production, but adversely affects the waste reduction and biomass conversion rate (Banks et al., 2014; Diener et al., 2009). Conversely, a combination of high larval density and high feeding rate negatively affects the waste reduction and relative growth rate of the larvae (Parra Paz et al., 2015). Since the bioconversion of organic waste is mainly governed by the nutrient profile and composition of the organic waste, the feeding rate and larval density that result in a good balance between waste reduction and bioconversion rate vary with waste type. Moreover, the larval density and feeding rate vary with the goal of the bioconversion process. If the primary goal is organic waste valorization, a lower feeding rate and higher larval density are recommended; if the primary goal is BSF biomass production, a relatively higher feeding rate is preferred.

Studies have successfully explored co-treatment of organic wastes with BSF larvae and synergistic microorganisms to improve bioconversion (Abduh et al., 2017; Isibika et al., 2019; Mazza et al., 2020; Rehman et al., 2019; Somroo et al., 2019; Xiao et al., 2018b; Yu et al., 2011; Zheng et al., 2012). For example, Xiao et al. (2018b) observed improvement in BSF larvae weight, bioconversion rate, and substrate reduction by 16%, 13%, and 13%, respectively, while co-treating chicken manure with Bacillus subtilis and BSF larvae compared to treatment with BSF larvae alone. Positive effects on substrate conversion, larval weight gain, substrate reduction, and nutrient accumulation in BSF biomass have also been reported when chicken manure was co-treated with microbes isolated from eggs and larval gut of BSF (Mazza et al., 2020). Similarly, Somroo et al. (2019) reported improvement in BSF larval yield, substrate reduction, and bioconversion rate by 37%, 14%, and 38%, respectively, while co-treating sovbean curd residue with Lactobacillus buchneri compared to mono-treatment with BSF larvae alone. Fermentation with fungi or supplementing exogenous microbes was also reported to improve the bioconversion and feed conversion ratio of lignocellulose-rich substrate such as rice straw (Zheng et al., 2012a), corn stover (Gao et al., 2019), banana peel (Isibika et al., 2019), and mixture of dairy manure and chicken manure (Rehman et al., 2019). The improved performance during cotreatment is attributed to the exo-enzymes, small molecules (e.g., simple sugars, peptides, and short-chain fatty acids), and nutrients produced by the synergistic microorganisms that promote the growth and substrate consumption rate of the BSF larvae (Isibika et al., 2019; Xiao et al., 2018b). Thus, inoculating the substrate with synergistic microorganisms along with the BSF larvae improves the bioconversion of organic wastes into BSF biomass. Since temporal and spatial variations exist in the composition of organic wastes, identifying synergistic mixed microbial cultures effective over a wide range of wastes would likely to further improve the bioconversion of organic wastes into BSF biomass. However, the added microorganisms should not have any negative effect on the nutrient value of the BSF biomass including pathogenicity and generation of allergens or toxins among others.

3.2. Bioconversion approaches

Approaches adopted for mass production of BSF biomass vary globally. Broadly, the approach for BSF biomass production could be categorized into two groups; (i) systems based on natural oviposition and (ii) systems based on adult rearing and egg production.

Historically, the process relied on natural populations colonizing the organic waste and self-harvesting of BSF biomass (Sheppard et al., 1994). The BSF biomass production system based on natural oviposition is discussed in detail elsewhere (Hem et al., 2008; Nyakeri et al., 2017a; Park, 2016; Rana et al., 2015; Sheppard et al., 1994). This system is applicable for small-scale farmers or home gardeners in regions with natural population of BSF (Kenis et al., 2018). Concerning abiotic factors, low- and middle-income countries in the tropical regions have favorable climate conditions for such system (i.e., BSF technology) to solve problems associated with a poorly-developed sanitation infrastructure (da Silva and Hesselberg, 2020). However, this approach is inefficient when considering the amount of organic wastes to be reduced and to reach the desired production levels for meeting the livestock, poultry, and aquaculture feeds demand. Moreover, such an approach is highly dependent on natural conditions to allow for the BSF to be active and colonize in organic wastes. In such case, colonization is unpredictable, resulting in lower production of BSF biomass, as well as spoilage of wastes, which may emanate odors. Furthermore, other pest species such as the house flies are likely to proliferate as well.

Table 1

Substrate reduction, bioconversion, and feed conversion ratio of different organic wastes into BSF biomass.

Substrate	Substrate reduction	Bioconversion	Feed	Reference
	(% of dry matter) ^A	(%) ^B	conversion ratio ^C	
Human feces	25.2 - 54.6 ^a	1.6 – 22.9 ^a	2.0 - 33.9 ^a	(Banks et al., 2014)
Human feces	39.1 - 48.6	11.3 – 22.7	NA	(Gold et al., 2020; Lalander et al., 2019)
Mixture of food waste and human feces (19:1)	68.4 - 68.8	18.9 – 19.0	NA	(Dortmans, 2015)
Corn stover	39.9	1.4	28.6	(Wang et al., 2017)
Fermented maize stover (mean \pm SE, n = 3)	48.4 ± 0.0	5.0 ^b	10.3 ^b	(Gao et al., 2019)
Fruit and vegetable waste	46.7 - 60.0	4.1 - 10.8	9.3 – 12.5	(Giannetto et al., 2019; Lalander et al., 2019)
Food waste (Mean \pm SD, n = 3)	55.3 ± 4.1	13.9 ± 0.3	NA	(Lalander et al., 2019)
Canteen waste (Mean \pm SD, n > 3)	37.9 ± 3.8	15.3 ± 2.1	NA	(Gold et al., 2020)
Vegetable canteen waste (Mean \pm SD, n > 3)	58.4 ± 1.4	22.7 ± 1.1	NA	(Gold et al., 2020)
Kitchen waste (Mean \pm SE, n = 3)	56.0 ± 1.4	18.2 ± 0.2	NA	(Cai et al., 2017)
Municipal organic waste	68.0	11.8	5.8 ^b	(Diener et al., 2011)
Wheat-mill by-products (Mean \pm SD, n > 3)	56.4 ± 1.2	14.9 ± 0.3	NA	(Gold et al., 2020)
Poultry slaughterhouse waste (Mean \pm SD, n > 3)	30.7 ± 4.7	13.4 ± 0.5	NA	(Gold et al., 2020)
Abattoir waste (Mean \pm SD, n = 3)	46.3 ± 2.9	15.2 ± 1.6	NA	(Lalander et al., 2019)
Mixture of abattoir waste and fruit and vegetable waste (1:1) (Mean ± SD, n = 3)	61.1 ± 10.7	14.2 ± 1.9	NA	(Lalander et al., 2019)
Mushroom root waste (Mean, $n = 3$)	37.3 - 42.3	5.4 - 5.6	NA	(Cai et al., 2017)
Mixture of mushroom root waste and kitchen waste	40.1 - 47.9	10.2 – 15.3	NA	(Cai et al., 2017)
Primary sludge (Mean \pm SD, n = 3)	63.3 ± 1.9	2.3 ± 0.1	NA	(Lalander et al., 2019)
Undigested sludge (Mean \pm SD, n = 3)	49.2 ± 3.7	2.2 ± 0.2	NA	(Lalander et al., 2019)
Digested sludge (Mean \pm SD, n = 3)	13.2 ± 0.8	0.2 ± 0.0	NA	(Lalander et al., 2019)
Mixture of pig manure, dog food, and human feces (4:4:2) (Mean ± SD, n = 3)	55.1 ± 0.3	11.8 ± 0.3	NA	(Lalander et al., 2015)
Soybean curd residue	49.0 – 72.4	5.0 - 11.7	6.2 – 9.8	(Rehman et al., 2017; Somroo et al., 2019)
Soybean curd residues + Bacteria (<i>Lactobacillus buchneri</i>) (Mean ± SE, n = 3)	55.7 ± 0.9	6.9 ± 0.3	8.0 ± 0.3	(Somroo et al., 2019)
Fresh dairy manure	53.1	12.2 ^c	4.4 ^c	(Li et al., 2011b)
Cattle manure	12.7 – 43.2	3.8 - 6.3	4.2 - 10.3	(Gold et al., 2020; Rehman et al., 2017a, 2017b)
Poultry manure	35.8 - 60.0	7.1 – 10.2	5.6	(Lalander et al., 2019; Rehman et al., 2017; Xiao et al., 2018b)
Mixtures of dairy manure and soybean curd residues	32.4 - 68.3	9.7 - 15.2	3.4 - 4.4	(Rehman et al., 2017)
Mixtures of dairy manure and chicken manure	44.3 - 53.4	5.88 - 7.9	6.6 - 7.5	(Rehman et al., 2017)
Chicken manure + Bacillus subtilis (Mean ± SE, n = 3)	40.5 ± 0.8	11.5 ± 0.2	NA	(Xiao et al., 2018b)
Swine manure (Mean \pm SE, n = 3)	53.4 ± 0.3^{1}	NA	NA	(Zhou et al., 2013a)
	28.8 ± 0.2^{II} 49.7 ± 0.4 ^{III}			
Chicken manure (Mean \pm SE, n = 3)	61.7 ± 0.2^{I}	NA	NA	(Zhou et al., 2013a)
	31.8 ± 0.3 ^{II}			
	56.8 ± 0.4^{III}			
Dairy manure (Mean \pm SE, n = 3)	57.8 ± 0.7^{I}	NA	NA	(Zhou et al., 2013a)
	53.2 ± 0.5^{II}			
	25.8 ± 0.2^{III}			
Mixture of dairy manure and chicken manure (2:3) (Mean ± SE, n = 3)	42.0 ± 0.5	6.8 ± 0.0	6.1 ± 0.1	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure $(2:3)$ + <i>Paenibacillus</i> <i>polymyxa</i> strain KMZ (Mean ± SE, n = 3)	41.8 ± 0.5	8.1 ± 0.3	5.2 ± 0.1	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure (2:3) + Paenibacillus polymyxa strain ZRO2 (Mean ± SE, n = 3)	47.3 ± 0.5	9.5 ± 0.2	5.0 ± 0.2	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure (2:3) + Bacillus strains SMO1 (Mean ± SE, n = 3)	45.5 ± 0.6	8.9 ± 0.3	5.1 ± 0.2	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure (2:3) + Bacillus strains SMO2 (Mean + SF, $n = 3$)	42.2 ± 0.2	9.1 ± 0.3	4.6 ± 0.2	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure (2:3) + Bacillus strains MRO2 (Mean + SF, $n = 3$)	48.8 ± 0.7	10.8 ± 0.0	4.49 ± 0.1	(Rehman et al., 2019)
Mixture of dairy manure and chicken manure (2:3) + Bacillus strains MRO4 (Mean ± SE, n = 3)	34.5 ± 1.0	9.0 ± 0.1	4.2 ± 0.2	(Rehman et al., 2019)

^A Substrate reduction (% of dry matter) = [(Weight of substrate added – Weight of residue)/Weight of substrate added] × 100%; ^BBioconversion (%) = (Weight of larval biomass/Weight of substrate added) × 100%; ^CFeed conversion ratio = Weight of ingested substrate/Weight gained; NA: Not available; SD: Standard deviation; SE: Standard error; ^aWet weight basis; ^bCalculation based on the yield data; ^cWeight of inoculum (initial weight of larvae prior to feeding dairy manure) was neglected; ^lBSF, Wuhan strain, China; ^{II}BSF, Guangzhou strain, China; ^{III}BSF, Texas strain, USA.

In case of the system based on adult rearing and egg production, a BSF colony needs to be maintained to ensure a regular supply of BSF eggs and simultaneously facilitating the waste bioconversion (Sheppard et al., 2002). This approach allows eggs to be produced in-house for bioconversion of wastes by the resulting larvae. Thus, adult rearing and egg production is a critical component of this type of BSF biomass production system (Kenis et al., 2018). The details of maintaining the colony to ensure a regular supply of eggs can be found elsewhere (Bertinetti et al., 2019; Caruso et al., 2013; Cortes Ortiz et al., 2016; Dortmans et al., 2017; Hoc et al., 2019; Sheppard et al., 2002). More recent BSF biomass production systems rely on maintaining a BSF colony. Such system is commonly



Fig. 2. A schematic of a BSF-based biorefinery for producing value-added products with concurrent valorization of organic bioresources.

used as it allows for somewhat predictable input (organic waste) and output (BSF biomass production). However, the major limitation of this approach is the space requirement for a commercialscale production. This is because given the size of an adult cage [1.2 m (H) \times 1.2 m (W) \times 2.4 m (L)], and its egg production capability (i.e., approximately 10 g/day) (Tomberlin, unpublished data), the number of cages needed to feed an industrial BSF complex could result in a massive spatial footprint, which may limit such facilities to locate outside of urban areas. For example, as a rule of thumb, a facility treating one ton of organic waste per day will require about 50 m² area for maintaining the BSF colony (i.e., BSF nursery) (Dortmans et al., 2017). Furthermore, such large-scale facilities are susceptible to environmental variability (e.g., temperature, humidity), which can impact egg production and hatching. One approach to overcome the footprint requirement is to develop vertical system where cages can be stacked on top of one another.

3.3. Black soldier fly-based biorefinery

Like a petroleum refinery that produces different fuels and products from crude petroleum, BSF farming could also serve as a model biorefinery for valorizing various organic resources into feed, fuel, fertilizer, and other bio-based products. A BSF-based biorefinery (Fig. 2), a practical adoption of a circular economy approach, could potentially increase the economic viability of the BSF technology and minimize the environmental footprint of the aforementioned BSF-derived products.

As previously stated, BSF can convert diverse organic resources into protein- and fat-rich biomass (Banks et al., 2014; Diener et al., 2011; Gao et al., 2019; Lalander et al., 2019; Rehman et al., 2009, 2017; Somroo et al., 2019; Surendra et al., 2016; Wang et al., 2017; Xiao et al., 2018b; Zhou et al., 2013), which could replace a significant amount of conventional proteins in animal diets, without significantly affecting the performance of the animal (see sections 4 and 5 for details). Biodiesel from BSF fat (Feng et al., 2018, 2020; Ishak and Kamari, 2019; Kamarulzaman et al., 2019; Leong et al., 2016; Li et al., 2011a, 2011b, 2015, Nguyen et al., 2018, 2020; Pang et al., 2019; Su et al., 2019; Surendra et al., 2016; Wang et al., 2017; Zheng et al., 2012; Rehman et al., 2018) could be one of the major products of a BSF-based biorefinery. Since BSF biomass is rich in fat (21 to 40% of dry matter), this fat could be extracted and converted into biodiesel via transesterification. Studies have explored different techniques in extracting fat from BSF biomass (Feng et al., 2018, 2020; Mai et al., 2019; Matthäus et al., 2019; Ravi et al., 2019; Smets et al., 2020; Soetemans et al., 2019; Su et al., 2019). In general, BSF fat is rich in medium chain saturated fatty acids and low in polyunsaturated fatty acids, which could produce biodiesel with low viscosity and high oxidative stability (Surendra et al., 2016; Zheng et al., 2012a). Moreover, studies have shown that properties of the biodiesel produced from BSF fat are within the international standard for biodiesel including the American Society for Test and Materials (ASTM) D6751 (Ishak and Kamari, 2019; Su et al., 2019) and the European standard EN 14,214 (Ishak and Kamari, 2019; Li et al., 2011; Nguyen et al., 2018; Su et al., 2019). In biodiesel production, the feedstock accounts for majority of the production cost (up to 75%) (Canakci and Sanli, 2008). Thus, a large-scale production of fat-rich BSF biomass from low-value organic resources could substantially reduce the production cost of biodiesel.

The residual substrate following harvest of BSF biomass could be used as an organic fertilizer (Bloukounon-Goubalan et al., 2019; Cai et al., 2017; Choi et al., 2009; Liu et al., 2019; Rosmiati et al., 2017). The nutrient profile of the residue is mainly governed by substrate fed to BSF larvae. Studies have reported increased

Table 2

Proximate composition of BSF biomass reared on different organic wastes (Unit: % of dry matter; *: % of lyophilized prepupae matter).

Substrate	Growth	Crude	Crude fat	Carbohyd-	Crude	Ash	Gross energy	Chitin	Reference
	stage	protein		rate	nder		(KCdI/Kg)		
Chicken feed	Prepupae	41.2	33.6	NA	NA	10.0	NA	6.2	(Spranghers et al., 2017)
Biogas digestate	Prepupae	42.2	21.8	NA	NA	19.7	NA	5.6	(Spranghers et al., 2017)
Vegetable waste	Prepupae	39.9	37.1	NA	NA	9.6	NA	5.7	(Spranghers et al., 2017)
Restaurant waste	Prepupae	43.1	38.6	NA	NA	2.7	NA	6.7	(Spranghers et al., 2017)
Cafeteria waste (Mean ± SD, n = 3)	Prepupae	43.7 ± 0.6	31.8 ± 0.3	12.3 ± 0.3	10.1 ± 0.2	6.0 ± 0.0	5751.7 ± 52.4	NA	(Surendra et al., 2016)
Vegetable and fruit waste (7:3)	Larvae	41.9	26.3	NA	NA	13.0	NA	6.2	(Meneguz et al., 2018)
Vegetable and fruit waste (17:3)	Larvae	36.7 ± 2.7	33.0 ± 0.2	NA	NA	5.0 ± 0.0	NA	NA	(Giannetto et al., 2019)
$(Mean \pm SD, n = 3)$									
Vegetable and fruit waste (17:3)	Prepupae	39.9 ± 1.1	30.8 ± 0.2	NA	NA	5.7 ± 0.1	NA	NA	(Giannetto et al., 2019)
$(Mean \pm SD, n = 3)$									
Fruit waste	Larvae	30.7 –	40.7 -	NA	NA	7.2 – 7.9	27201.7	5.6	(Chun et al., 2019;
		39.8	47.4						Meneguz et al., 2018)
Fruit waste	Prepupae	43.8	46.8	NA	NA	5.7	27825.2	NA	(Chun et al., 2019)
Winery by-product	Larvae	34.4	32.2	NA	NA	14.6	NA	5.3	(Meneguz et al., 2018)
Brewery by-product	Larvae	53.0	29.9	NA	NA	7.3	NA	1.4	(Meneguz et al., 2018)
Brown algae (Ascophyllum nodosum)	Larvae	41.3 ± 1.1	8.1 ± 0.9	NA	NA	15.8 ± 0.7	NA	NA	(Liland et al., 2017)
$(Mean \pm SD, n = 4)$									
Food waste	Prepupae	42.0 -	35.0 -	NA	NA	3.1 - 4.6	NA	NA	(Salomone et al., 2017;
		43.7	37.2						Wang et al., 2020)
Pig manure [*] (Mean ± SE, n = 3)	Prepupae	42.8 ± 4.4	36.5 ± 3.9	NA	NA	3.7 ± 0.4	NA	NA	(Wang et al., 2020)
Poultry manure [*] (Mean \pm SE, n = 3)	Prepupae	41.7 ± 4.0	36.2 ± 3.5	NA	NA	3.8 ± 0.4	NA	NA	(Wang et al., 2020)
Cow manure [*] (Mean \pm SE, n = 3)	Prepupae	41.2 ± 2.1	35.7 ± 2.9	NA	NA	4.9 ± 0.5	NA	NA	(Wang et al., 2020)
Fermented maize stover	Prepupae	41.8	30.5	NA	NA	8.2	NA	NA	(Gao et al., 2019)
Cricket waste	Larvae	37.1	38.7	NA	NA	9.4	NA	NA	(Jucker et al., 2020)
Locust waste	Larvae	49.2	25.8	NA	NA	13.2	NA	NA	(Jucker et al., 2020)

NA: Not available; SD: Standard deviation; SE: Standard error.

concentration of total phosphorus (Lalander et al., 2015) and ammonium nitrogen (Green and Popa, 2012; Lalander et al., 2015) in the residue following BSF larval composting, while the pH of the residue usually is within the optimum range for plant growth (pH = 7.0 – 8.0) (Choi et al., 2009; Lalander et al., 2015). Moreover, studies have reported positive effects of chitin or its derivatives on plant (i.e., triggers growth and induces defense) (Sharif et al., 2018; Sharp, 2013). Since the residue contains larvae or pupae exuviae and dead imago, rich sources of chitin (Purkayastha and Sarkar, 2019), the residue could also trigger plant growth and induce plant defense. However, currently the studies exploring such aspects of residue is lacking. Additionally, the insect compost as a growth media could replace a substantial amount of peat and synthetic fertilizer in soilless agriculture (Setti et al., 2019). As discussed in the section 7, the bioconversion of organic wastes using BSF larvae reduces pathogen levels and degrades pesticides and pharmaceuticals, thereby preventing the spread of such contaminants into the environment. Additionally, the BSF bioaccumulate heavy metals such as cadmium (Cd) (Biancarosa et al., 2018; Purschke et al., 2017; Tschirner and Simon, 2015; van der Fels-Klerx et al., 2016a), lead (Pb) (Purschke et al., 2017; Tschirner and Simon. 2015: van der Fels-Klerx et al., 2016a), and arsenic (As) (Biancarosa et al., 2018, 2019), thereby improving the value of the residual compost as an organic fertilizer. Moreover, the residual biomass could be used as a substrate for producing and bio-based products following biochemical biofuel (e.g., anaerobic digestion (Bulak et al., 2020) and/or composting) and thermochemical (e.g., pyrolysis, hydrothermal carbonization) conversion processes. The downstream applications of the insect compost, however, depend on its composition, which is governed by the characteristics of the original organic waste fed to BSF larvae.

In addition to producing feed, fuel, and fertilizer, the BSF-based biorefinery has huge potential in producing other high-value products. For example, chitin, a polymer of glucosamine, is a major by-product of BSF biomass processing as it constitutes up to 7% of BSF biomass on dry matter basis (Meneguz et al., 2018; Spranghers et al., 2017). Chitin and its derivative, chitosan, have many industrial applications such as a binder (in dyes), edible films, industrial

membranes, biodegradable surgical thread, and a fining agent (in winemaking). Moreover, based on the physicochemical characteristics of the BSF-derived chitin, studies have suggested its potential applications in tissue engineering, textile industry, and as an adsorbent in water and wastewater treatment (Purkayastha and Sarkar, 2019; Wasko et al., 2016). Moreover, economicallyimportant bioproducts such as protein hydrolysates (Firmansyah and Abduh, 2019), bioplastic (Barbi et al., 2019), and natural pigments (e.g., melanin and ommochromes) (Ushakova et al., 2019) could also be derived from BSF biomass. Studies have also explored BSF biomass as a potential source of industrially important enzymes (e.g., trypsin, chymotrypsin, ligninase, and cellulase) (Bonelli et al., 2019; Kim et al., 2011; Lee et al., 2014; Muller et al., 2017; Park et al., 2012) and antimicrobial peptides. The successful development of a BSF-based biorefinery, however, will require a large-scale production of BSF biomass, which in turn will require mechanization and automation of rearing, harvesting, and processing.

4. Nutrient composition of black soldier fly biomass

The nutrient profile of BSF biomass is summarized in Tables 2 to 5. BSF biomass is rich in protein, fat and minerals, and has comparatively low fiber content. As shown in Table 2, crude protein and fat contents of BSF biomass vary from 30 to 52% and 21 to 40% of dry matter, respectively.

The crude protein content in BSF biomass compares well with the most common plant protein source: soybean meal, while the gross energy value of BSF biomass is usually higher than that of common energy sources in animal diets such as corn meal and wheat meal (Surendra et al., 2016). The crude protein content of defatted BSF biomass is higher than that of soybean meal and is comparable to fish meal (Surendra et al., 2016). The extracted fat from BSF biomass has other potential applications such as a feedstock for biodiesel (as discussed in section 3.3) or biobased lubricant (Alipour et al., 2019).

Table 3 Amino acids profile of the BSF biomass reared on different organic wastes (Unit: *: % of dry matter; **: % of crude protein; ***: % of lyophilized prepupa matter).

Essential amino acids												
Substrate	Growth stage	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionin	e Phenylalanine	Threonine	Tryptophan	Valine	References
Chicken feed*	Prepupae	2.03	1.36	1.72	2.86	2.34	0.76	1.70	1.64	0.67	2.41	(Spranghers et al., 2017)
Digestate*	Prepupae	2.03	1.35	1.84	2.95	2.57	0.87	1.87	1.68	0.62	2.49	(Spranghers et al., 2017)
Vegetable waste*	Prepupae	2.00	1.24	1.73	2.80	2.26	0.76	1.63	1.54	0.58	2.48	(Spranghers et al., 2017)
Restaurant waste*	Prepupae	1.99	1.38	1.91	3.06	2.30	0.71	1.64	1.62	0.54	2.82	(Spranghers et al., 2017)
Cafeteria waste* (Mean ± SD, n = 3)	Prepupae	2.22 ± 0.08	1.69 ± 0.04	1.51 ± 0.06	2.34 ± 0.05	2.19 ± 0.07	0.88 ± 0.02	1.50 ± 0.07	1.48 ± 0.07	NA	2.42 ± 0.06	(Surendra et al., 2016)
Abattoir waste**	Larvae	4.96	3.53	4.67	6.84	6.40	1.77	3.59	3.70	1.67	6.32	(Lalander et al., 2019)
Poultry manure**	Larvae	4.92	3.09	4.10	6.61	7.07	2.05	4.20	3.83	1.62	5.97	(Lalander et al., 2019)
Poultry manure ^{***} (Mean ± SE, n = 3)	Prepupae	2.06 ± 0.21	1.39 ± 0.11	1.77 ± 0.18	2.81 ± 0.25	2.41 ± 0.23	0.79 ± 0.05	1.83 ± 0.16	1.93 ± 0.17	0.75 ± 0.06	1.84 ± 0.18	(Wang et al., 2020)
Human feces**	Larvae	5.10	3.30	4.67	7.00	6.17	1.85	4.42	3.76	1.69	6.59	(Lalander et al., 2019)
Food waste**	Larvae	4.89	2.91	4.13	6.79	8.25	1.84	4.03	3.86	1.38	5.84	(Lalander et al., 2019)
Food waste ^{***} (Mean \pm SE, n = 3)	Prepupae	2.09 ± 0.20	1.42 ± 0.11	1.84 ± 0.16	2.86 ± 0.24	2.40 ± 0.21	0.80 ± 0.06	1.81 ± 0.18	1.91 ± 0.15	0.78 ± 0.07	1.97 ± 0.18	(Wang et al., 2020)
Pig manure ^{***} (Mean \pm SE, n = 3)	Prepupae	2.11 ± 0.17	1.39 ± 0.10	1.83 ± 0.14	2.81 ± 0.22	2.46 ± 0.19	0.81 ± 0.06	1.85 ± 0.15	1.95 ± 0.10	0.80 ± 0.06	1.95 ± 0.16	(Wang et al., 2020)
Cow manure ^{***} (Mean \pm SE, n = 3)	Prepupae	2.01 ± 0.16	1.30 ± 0.12	1.75 ± 0.15	2.74 ± 0.21	2.34 ± 0.22	0.75 ± 0.04	1.72 ± 0.15	1.84 ± 0.13	0.71 ± 0.05	1.92 ± 0.21	(Wang et al., 2020)
Fruit and vegetable waste**	Larvae	4.54	2.63	4.30	6.67	5.14	1.53	3.45	3.46	1.40	5.95	(Lalander et al., 2019)
Primary sludge**	Larvae	4.94	2.97	4.50	6.55	5.94	1.81	3.76	3.60	1.65	6.16	(Lalander et al., 2019)
Undigested sludge**	Larvae	4.94	3.13	4.47	7.56	6.42	1.83	3.99	3.70	1.71	6.08	(Lalander et al., 2019)
Digested sludge**	Larvae	4.49	1.98	3.90	6.25	6.17	1.81	3.73	3.31	1.79	5.28	(Lalander et al., 2019)
Household organic waste*	Larvae	1.94	1.32	1.57	2.59	2.22	0.58	1.51	1.42	0.53	2.25	(Kawasaki et al., 2019)
Household organic waste*	Prepupae	2.20	1.30	1.71	2.81	2.51	0.74	1.69	1.55	0.66	2.38	(Kawasaki et al., 2019)
Brown algae ^{**} (Mean \pm SD, n = 4)	Larvae	6.50	2.70	3.80	6.20	5.60	1.40	3.20	3.90	NA	5.50	(Liland et al., 2017)
Substrate	Growth s	tage Alan	ine As	spartic acid	Cysteine	Glutamic	acid Gly	cine Proli	ne Ser	ine 1	Tyrosine	References
Chicken feed*	Prepupae	2.52	3.	78	0.25	4.19	2.2	5 2.25	1.6	6 1	NA	(Spranghers et al., 2017)
Digestate*	Prepupae	2.43	3.	36	0.24	3.96	2.20	5 2.21	1.5	5 1	NA	(Spranghers et al., 2017)
Vegetable waste*	Prepupae	2.42	3.	59	0.21	4.13	2.22	2.14	1.5	1 0	NA	(Spranghers et al., 2017)
Restaurant waste*	Prepupae	2.78	3.	69	0.22	4.58	2.52	2 2.51	1.5	9 1	NA	(Spranghers et al., 2017)
Cafeteria waste* (Mean \pm SD, n = 3)	Prepupae	2.68	± 0.19 2.	64 ± 0.06	1.12 ± 0.05	2.85 ± 0.0	2 2.4	5±0.10 2.11	± 0.02 1.5	3 ± 0.01 2	2.37 ± 0.02	(Surendra et al., 2016)
Abattoir waste**	Larvae	5.72	9.	28	0.60	10.00	5.8	5.06	4.0	5 8	3.62	(Lalander et al., 2019)
Poultry manure ¹¹	Larvae	5.65	9.	26	0.55	9.66	5.4	5.20	3.8	4 t	0.21	(Lalander et al., 2019)
Fumal leces	Larvae	0.03	9.	00	0.67	10.59	5.9.	2 5./9	4.3	9	10.88	(Lalander et al., 2019)
FOUL WASLE Food waste*** (Moon + SE $n = 2$)	Dropupao	5.95	±0.21 2	00 ± 0.24	0.35 ± 0.02	9.04 4.19 ± 0.2	0 2.2	J.12 J±021 2.26	4.1 + 0.19 1.0) 2 ± 0 17 1	0.02	(Mang et al. 2019)
FOUL WASLE (Mean \pm SE, $\pi = 3$) Dig manuro ^{***} (Mean \pm SE, $\pi = 2$)	Propupae	2.44	±0.21 5.	70 ± 0.34 72 ± 0.26	0.20 ± 0.03	4.10 ± 0.2	9 2.2: o 2.2:	9 ± 0.21 2.20 5 ± 0.19 2.27	±0.16 1.9	2 ± 0.17 1 5 ± 0.14 1		(Wally et al., 2020)
Poultry manure ^{***} (Mean + SE n = 3)	Drepupae	2.31	± 0.19 3.	72 ± 0.30 61 + 0.20	0.28 ± 0.02 0.21 + 0.03	4.11 ± 0.3 4.07 ± 0.4	0 2.20 1 2.2	1 ± 0.10 2.27	± 0.14 1.7 ± 0.16 1.7	3 ± 0.14 1 2 ± 0.15 1		(Wang et al., 2020)
Cow manure ^{***} (Mean + SF n = 3)	Prepupac	2.55	+0.18 3	58 ± 0.32	0.21 ± 0.03 0.24 ± 0.01	4.07 ± 0.4 4.05 ± 0.3	7 2.2	7 ± 0.13 2.10 7 ± 0.20 2.17	± 0.10 1.7 ± 0.22 1.6	1 ± 0.13	NA NA	(Wang et al., 2020)
Fruit and vegetable waste**	Larvae	5 48	2 0.10 J. 8	06	0.50	9.54	, 2.1 5.2,	1 5.20	- 0.22 1.0 3.8	6	5 50	(Lalander et al. 2019)
Primary sludge**	Larvae	5 57	8	85	0.66	10.04	5.6) 5.20	4.0	6 6	5.02	(Lalander et al., 2019)
Undigested sludge**	Larvae	5.66	8	90	0.67	10.38	5.5	5.35	42	5 6	6.56	(Lalander et al., 2019)
Digested sludge**	Larvae	4.82	8.	26	0.65	9.57	4.8	3 4.65	3.6	6 (5.26	(Lalander et al., 2019)
Household organic waste*	Larvae	2.45	3.	43	0.28	3.99	1.9) 2.16	1.6	2 2	2.30	(Kawasaki et al., 2019)
Household organic waste*	Prepupae	2.28	3.	74	0.28	4.30	2.1	2.14	1.7	0 2	2.63	(Kawasaki et al., 2019)
Brown algae**	Larvae	6.40	8.	30	NA	11.90	4.3	5.20	4.3	0 4	1.20	(Liland et al., 2017)

NA: Not available; SD: Standard deviation; SE: Standard error.

Ta	ble	4	
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Fatty	acids	profile of	f the o	of the	BSF	biomass	reared	on	different	organic	wastes	(Unit:	% w	/w o	f fattv	/ acid	methy	l ester')
												· · · · · ·							

	C 1	<i>a</i> .			D 1	D1 . 1 .	c	01.1		** 1 *	654	N // IEA	DUEA	D.C.
Substrate	Growth stage	Capric C10:0	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmitol-eic (C16:1n-7)	Stearic (C18:0)	Oleic (C18:1n-9)	Linoleic (C18:2n-6)	Linolenic (C18:3n-3)	SFA	MUFA	PUFA	Keterence
Chicken feed	Prepupae	1.4	57.4	7.3	9.7	NA	1.4	7.5	NA	0.7	77.4	10.0	12.6	(Spranghers et al., 2017)
Digestate	Prepupae	1.2	43.7	6.9	10.1	NA	1.8	7.9	NA	0.8	64.8	19.1	9.6	(Spranghers et al., 2017)
Vegetable waste	Prepupae	1.6	60.9	9.5	8.7	NA	1.1	5.7	NA	1.4	82.8	9.5	7.0	(Spranghers et al., 2017)
Restaurant waste	Prepupae	2.0	57.6	7.1	10.3	NA	1.0	8.0	NA	1.1	78.3	12.0	9.4	(Spranghers et al., 2017)
Cafeteria waste (Mean ± SD, n = 3)	Prepupae	NA	44.9 ± 1.5	8.3 ± 0.6	13.5 ± 0.7	2.4 ± 0.2	2.1 ± 0.2	12.0 ± 0.7	9.9 ± 0.5	0.1 ± 0.0	69.9 ± 1.4	14.9 ± 0.8	12.5 ± 0.4	(Surendra et al., 2016)
Vegetable and fruit waste (7:3)	Larvae	NA	52.1	10.4	13.9	NA	2.6	8.5	7.0	1.7	78.9	12.3	8.8	(Meneguz et al., 2018)
Vegetable and fruit waste (17:3) (Mean \pm SD, n = 3)	Larvae	0.5	28.1	3.8	5.8	1.6	0.7	4.3	1.3	10.3	73.5	14.9	11.6	(Giannetto et al., 2019)
Vegetable and fruit waste (17:3) (Mean \pm SD, n = 3)	Prepupae	1.2	61.9	9.1	7.9	2.4	1.1	5.3	2.4	4.7	84.5	8.4	7.1	(Giannetto et al., 2019)
Fruit waste	Larvae	NA	57.4	9.6	13.1	NA	1.8	9.3	4.1	0.7	81.9	13.3	4.8	(Meneguz et al., 2018)
Winery by-product	Larvae	NA	34.7	6.6	18.9	NA	2.8	12.5	17.6	0.4	63.0	19.0	18.0	(Meneguz et al., 2018)
Brewery by-product	Larvae	NA	32.4	6.7	20.4	NA	1.8	9.2	23.6	2.5	61.3	12.7	26.0	(Meneguz et al., 2018)
Dairy manure	Larvae	3.1	35.6	7.6	14.8	3.8	3.6	23.6	2.1	NA	NA	NA	NA	(Li et al., 2011b)
Soybean curd residue + Bacteria (<i>Lactobacillus buchneri</i> (Mean ± SE, n = 3)	Larvae	1.5 ± 0.1	39.6 ± 1.5	6.5 ± 0.2	13.1 ± 0.5	2.7 ± 0.1	2.1 ± 0.1	11.5 ± 0.4	17.5 ± 0.4	1.4 ± 0.1	NA	NA	NA	(Somroo et al., 2019)
Soybean curd residue (Mean ± SE, n = 3)	Larvae	1.7 ± 0.1	36.9 ± 1.2	5.9 ± 0.2	13.2 ± 0.4	3.2 ± 0.2	1.7 ± 0.1	12.1 ± 0.6	17.0 ± 0.5	1.2 ± 0.0	NA	NA	NA	(Somroo et al., 2019)
Brown algae (Ascophyllum nodosum) (Mean ± SD, n = 4)	Larvae	NA	23.9 ± 2.9	6.7 ± 0.5	16.6 ± 0.1	2.5 ± 0.1	4.1 ± 0.1	17.9 ± 1.1	18.6 ± 0.9	1.6 ± 0.1	52.5 ± 3.5	22.2 ± 1.3	24.2 ± 1.7	(Liland et al., 2017)
Food waste	Prepupae	1.4	41.1	0.4	12.2	3.2	2.4	14.1	13.8	NA	NA	NA	NA	(Salomone et al., 2017)
Food waste (Mean \pm SE, n = 3)	Prepupae	2.3 ± 0.2	56.2 ± 5.8	9.3 ± 0.8	10.3 ± 1.1	2.4 ± 0.2	1.9 ± 0.2	7.1 ± 0.7	10.3 ± 1.2	NA	NA	NA	NA	(Wang et al., 2020)
Pig manure (Mean ± SE, n = 3)	Prepupae	2.1 ± 0.2	56.4 ± 6.4	8.5 ± 0.7	11.0 ± 1.0	2.2 ± 0.2	1.5 ± 0.1	7.1 ± 0.7	10.2 ± 1.0	NA	NA	NA	NA	(Wang et al., 2020)
Chicken manure (Mean ± SE, n = 3)	Prepupae	1.6 ± 0.1	56.9 ± 4.8	8.2 ± 0.9	8.4 ± 0.8	1.9 ± 0.1	1.4 ± 0.1	7.0 ± 0.6	10.7 ± 1.0	NA	NA	NA	NA	(Wang et al., 2020)
Cow manure (Mean ± SE, n = 3)	Prepupae	1.7 ± 0.1	54.2 ± 4.0	7.0 ± 0.7	7.9 ± 0.6	2.1 ± 0.2	1.2 ± 0.1	6.3 ± 0.6	11.0 ± 0.9	NA	NA	NA	NA	(Wang et al., 2020)
Fermented maize straw (mean ± SE, n = 3)	Prepupae	0.8 ± 0.0	22.4 ± 0.4	4.4 ± 0.2	13.1 ± 0.3	1.2 ± 0.1	1.9 ± 0.1	23.3 ± 0.5	24.0 ± 0.3	1.3 ± 0.1	45.4 ± 1.1	24.9 ± 0.6	25.4 ± 0.3	(Gao et al., 2019)

NA: Not available; SD: Standard deviation; SE: Standard error; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

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Substrate	Growth stage	Ca	Cu	Fe	К	Mg	Mn	Na	Ρ	S	Zn	Reference
Chicken feed	Prepupae	28.70	0.01	0.35	6.16	2.65	0.22	0.67	4.99	0.20	0.16	(Spranghers et al., 2017)
Digestate	Prepupae	66.15	0.01	0.43	6.75	3.13	0.38	0.89	4.44	0.31	0.05	(Spranghers et al., 2017)
Vegetable waste	Prepupae	28.72	0.01	0.11	5.94	2.46	0.24	0.60	4.04	0.18	0.07	(Spranghers et al., 2017)
Restaurant waste	Prepupae	1.23	0.01	0.11	5.98	2.11	0.02	0.68	4.08	0.11	0.07	(Spranghers et al., 2017)
Brown algae (Ascophyllum nodosum)	Larvae	30.00	0.01	0.35	21.30	6.20	0.17	12.30	11.30	NA	0.15	(Liland et al., 2017)
Food waste* (Mean \pm SE, n = 3)	Prepupae	19.48 ± 1.65	0.01 ± 0.01	0.42 ± 0.04	6.17 ± 0.71	2.71 ± 0.24	0.13 ± 0.02	0.59 ± 0.04	3.62 ± 0.41	0.11 ± 0.01	0.10 ± 0.02	(Wang et al., 2020)
Pig manure [*] (Mean ± SE, n = 3)	Prepupae	23.71 ± 2.27	0.01 ± 0.01	0.36 ± 0.04	6.04 ± 0.65	2.35 ± 0.22	0.17 ± 0.02	0.51 ± 0.06	3.94 ± 0.33	0.16 ± 0.02	0.11 ± 0.02	(Wang et al., 2020)
Poultry manure* (Mean ± SE, n = 3)	Prepupae	21.94 ± 1.81	0.01 ± 0.01	0.28 ± 0.02	6.71 ± 0.52	2.82 ± 0.19	0.16 ± 0.01	0.47 ± 0.05	4.14 ± 0.40	0.29 ± 0.03	0.14 ± 0.02	(Wang et al., 2020)
Cow manure* (Mean ± SE, n = 3)	Prepupae	34.71 ± 2.65	0.01 ± 0.01	0.39 ± 0.05	7.53 ± 0.61	2.11 ± 0.16	0.20 ± 0.01	0.39 ± 0.02	4.76 ± 0.31	0.24 ± 0.03	0.18 ± 0.02	(Wang et al., 2020)
NA: Not available: SE: Standard error.												

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Chitin is another important component of BSF biomass (~7% of dry matter), and is often falsely counted as crude protein in BSF biomass because of nitrogen present in the chitin molecule; the crude protein content is usually determined by multiplying the total Kjeldahl nitrogen value by a factor of 6.25 (Finke, 2007; Janssen et al., 2017). Despite studies have reported that a high content of chitin and chitin derivative chitosan in the diet negatively affects nutrient utilization (Karlsen et al., 2017; Khempaka et al., 2011; Kroeckel et al., 2012; Marono et al., 2016; Razdan and Pettersson, 1994; Schiavone et al., 2017; Vidanarachchi et al.,

2010), these compounds have been reported to have antimicrobial and prebiotic properties (Komi et al., 2018), which are discussed in

section 6.1. Nutrient composition of the BSF biomass, however, is highly dependent on the substrate fed to the larvae (Barroso et al., 2017: Danieli et al., 2019: Ewald et al., 2020: Gold et al., 2020: Lalander et al., 2019: Liland et al., 2017: Meneguz et al., 2018: Spranghers et al., 2017), larval growing conditions (Palma et al., 2019), processing methods (Huang et al., 2018; Larouche et al., 2019; Leni et al., 2019; Tschirner and Simon, 2015), and life stage at harvest (Giannetto et al., 2019; Kawasaki et al., 2019; Liu et al., 2017; Smets et al., 2020). For example, the carbohydrate content in the substrate influences the lipid content in the BSF larvae (Gold et al., 2018; Li et al., 2015). When BSF larvae are fed low protein and high carbohydrate substrates, carbohydrate is mainly converted by larvae into lipids (Pimentel et al., 2017). Several studies have reported higher lipid content in BSF larvae grown on the substrates low in protein and rich in carbohydrate when compared to larvae grown on substrates with balanced protein and carbohydrate (Barragán-Fonseca, 2018; Danieli et al., 2019; Gold et al., 2018; Jucker et al., 2017; Spranghers et al., 2017). While some feedstocks rich in protein improve protein content (in BSF biomass), larval weight, bioconversion and feed conversion rates, and reduce development time (Gold et al., 2018; Nguyen et al., 2013; Oonincx et al., 2015), the amino acid profile in BSF biomass is not greatly influenced by the amino acid profile of the substrate (Spranghers et al., 2017). The lipid profile of the substrate, however, directly influences the lipid profile of the BSF biomass (Ewald et al., 2020; Gold et al., 2018; Liland et al., 2017; Somroo et al. 2019).

Abiotic factors such as larval-rearing temperature also affect the nutrient profile of the BSF biomass. The crude protein content in BSF biomass, for example, decreased when the rearing temperature was increased from 28 to 34 °C (Palma et al., 2019). Similarly, processing method also affects the nutrient quality of the BSF biomass (Huang et al., 2018; Larouche et al., 2019; Leni et al., 2019). For example, compared to blanching, slow killing of BSF prepupae by freezing activates enzymatic reactions including melanization with tyrosine consumption, energetic metabolism, and lipolysis, thereby impacting the digestibility and nutritional quality (loss of cysteine and lysine) of BSF biomass (Leni et al., 2019). Moreover, blanching was found to result in low lipid oxidation, reduction in moisture content, color stability, and significantly reduced the microorganisms in the insect biomass (Larouche et al., 2019). Similarly, compared to microwave drying, BSF larvae protein prepared by conventional drying (60 °C) had a significantly higher Digestible Indispensable Amino Acid Score (DIAAS) and better digestibility (Huang et al., 2018). A substantial increase in the crude protein and decrease in gross energy contents in BSF prepupae was reported after partial or complete removal of fat from BSF prepupae (Surendra et al., 2016).

In general, the protein derived from BSF biomass has a good balance of essential and non-essential amino acids (Table 3). Except for methionine and lysine, BSF biomass has both essential and non-essential amino acids comparable to that of fish meal (Belghit et al., 2019; Henry et al., 2015; Liland et al., 2017; Makkar et al., 2014; Surendra et al., 2016). The concentration of the amino acids such as methionine, lysine, tryptophan, threonine, cysteine, and valine, which are often limiting amino acids in poultry and pig diets, is comparable to soybean meal. Additionally, BSF larvae are a rich source of bioavailable arginine (Belghit et al., 2019), which is often a limiting amino acid in plant-based protein (Andersen et al., 2013).

Lauric (C12:0), palmitic (C16:0), and oleic (C18:1) acids are the major fatty acids present in the fat derived from BSF biomass, accounting for about 32-60%, 8-20%, and 5-12% of total fatty acids, respectively (Table 4). Moreover, saturated fatty acids accounts for about 61-82% of total fatty acids, while monounsaturated and polyunsaturated fatty acids contribute to 9–19% and 4–26% of total fatty acids, respectively (Table 4). Although nutritionally important omega-3 and omega-6 fatty acids are present in BSF biomass, the concentrations of these polyunsaturated fatty acids are lower than in fish meal. However, as mentioned earlier, since amount and fatty acid profile of fat in the BSF biomass are directly related to the amount and type of fatty acids present in the substrate fed to BSF larvae, nutritionally important fatty acids, such as omega-3 and omega-6 fatty acids, could be enriched in the BSF biomass by manipulating the composition of substrate fed to the BSF larvae. For example, studies have reported a substantial increase in omega-3 fatty acids content in the BSF larvae fed with diets containing microalgae (Truzzi et al., 2020), seaweed (Liland et al., 2017), and fish offal (St-Hilaire et al., 2007a).

Like other nutrients, the micronutrients present in the BSF biomass is also dependent on substrate fed. In general, BSF biomass accumulates calcium (Ca) and manganese (Mn), but not sodium (Na) or sulfur (S) (Spranghers et al., 2017). Thus, the Ca (the most abundant mineral in BSF biomass) content in BSF biomass (up to 6% of dry matter, Table 5) is comparatively higher than that in other insects and fish meal (Wang and Shelomi, 2017).

The variation in nutrient composition of BSF biomass is both an opportunity and a challenge for BSF-based animal feed applications. It provides an opportunity to tailor the nutrient profile of the BSF biomass by manipulating the composition of the substrates fed to BSF larvae, harvesting the BSF biomass at appropriate stage (larval, prepupal, pupal), and processing of the harvested biomass. Similarly, since the characteristics of substrates vary substantially, maintaining consistency in the nutrient profile of the BSF biomass, especially when BSF system is employed for treating diverse organic wastes, could be challenging.

5. Black soldier fly biomass as animal/aquatic feed

BSF biomass has been studied as a protein-rich alternative feed for several decades. Although studies have reported mixed results on performance of BSF meal (larval-, prepupal-, or pupal-meal) as an animal feed depending on factors such as animal types, feed formulations, and feeding duration, several studies have shown that BSF meal could substitute a significant amount of conventional sources of protein (e.g., soybean meal and fish meal) in animal diets. The performances of BSF meal as poultry, fish, and swine feed are critically examined in the following sections.

5.1. Poultry

BSF meal has been widely studied as a poultry feed (Borrelli et al., 2017; Brede et al., 2018; Cullere et al., 2019, 2016; Cutrignelli et al., 2018; Dabbou et al., 2018; Hale, 1973; Kawasaki et al., 2019; Kierończyk et al., 2019; Lee et al., 2018; Marono et al., 2017; Maurer et al., 2016; Mbhele et al., 2019; Mwaniki et al., 2018; 2019; Nery et al., 2018; Onsongo et al., 2018; Pieterse et al., 2019; Ruhnke et al., 2018; Schiavone et al., 2018; Cutrignelli et al., 2019; Ruhnke et al., 2018; Schiavone et al., 2018; Schiavone et al., 2019; Ruhnke et al., 2018; Schiavone et al., 2018; Schiavone et al., 2019; Ruhnke et al., 2018; Schiavone et al., 201

2016, 2018, 2019; Secci et al., 2018, 2020; Veldkamp and van Niekerk, 2019; Wallace et al., 2018, 2017; Zotte et al., 2019). Studies have shown mixed results on performance of BSF meal as a poultry feed depending on poultry type (e.g., broiler or layer), poultry growth stage (e.g., starter, growing, or finishing stage), feeding duration, feeding mode (e.g., caged-fed or free range), inclusion rate of BSF meal in the diet, BSF meal types (depending on feedstocks used for growing BSF larvae), and method of processing the BSF larvae (e.g., dried or fresh, whole, chopped, or milled, defatted or non-defatted).

For example, no significant difference was observed between the treatment and the control diets in terms of diet preference, apparent digestibility of major nutrients, productive performance, mortality, carcass traits, and sensory and meat quality characteristics while feeding chicken; Cobb 500 (Pieterse et al., 2019) and Ardennaise (Moula et al., 2018), and quail (*Coturnix coturnix japonica*) (Cullere et al., 2016) with diet containing up to 15% of BSF biomass. However, Dabbou et al. (2018) observed mixed results while partially defatted BSF larval meal was used as a soybean meal substitute (up to 15%) in the diets of broiler chickens (Ross 308). The diet containing BSF larval meal improved live weight and daily feed intake of chickens during the starter period (1–10 days), but negatively affected the feed conversion ratio during both the growing (10–24 days) and finishing (24–35 days) periods.

Similar to broiler chickens, the effects of including BSF meal in the diets of layers have been inconsistent (Cutrignelli et al., 2018; Marono et al., 2017; Maurer et al., 2016; Ruhnke et al., 2018). In general, replacement (up to 100%) of soybean meal with BSF meal for a short period (up to 6 weeks) did not affect the performance (e.g., body weight, feed intake, feed conversion ratio, laying percentage, and egg quality) of laying hens (Maurer et al., 2016; Ruhnke et al., 2018). However, feeding BSF meal or larvae as a soybean meal substitute for a longer period (over 12 weeks) negatively affected the performance (Cutrignelli et al., 2018; Marono et al., 2017; Ruhnke et al., 2018). The reduced performance of the hens fed with BSF meal was attributed to either reduced availability of the heat labile amino acids (i.e., lysine, arginine, threonine) due to the Maillard reaction because the BSF larvae were dried at 90 °C for 60 min prior to grinding (Ruhnke et al., 2018), lower protein digestibility due to presence of chitin in BSF meal (Cutrignelli et al., 2018), or to lower feed intake possibly due to the darker color of BSF meal than soybean meal (Cutrignelli et al., 2018; Marono et al., 2017). Blackening of the BSF meal is due to the formation of iron-polyphenol complexes upon grinding BSF larvae (Janssen et al., 2019).

The mixed results observed in different studies could partly be due to differences in nutrient quality of the BSF biomass used. In addition, since the nutrient requirement of poultry varies with growth stage and poultry type, such variation may have also contributed to inconsistency on the performance of BSF meal as a poultry feed. Inconsistencies among the results of different feeding trials are partly due to differences in experiments, as the diets (i.e., control and experimental) fed to poultry were not isoenergetic and isoproteic/isonitrogenous in all studies. In general, studies have shown that BSF meal could substitute a substantial portion of the soybean meal in poultry diets without negatively impacting, if not improving, the performance and quality of products.

5.2. Fish

BSF meal as fish feed has been tested for different species of fish for about four decades. Several studies have investigated different aspects (including nutrient digestibility, growth performance, and meat quality) of including BSF meal as a substitute for fish and soybean meal in the diets of fish species including channel catfish (*Ictalurus punctatus*) (Bondari and Sheppard, 1987), yellow catfish (Pelteobagrus fulvidraco) (Hu et al., 2017; Xiao et al., 2018a), African catfish (Clarias gariepinus) (Fawole et al., 2020), Nile tilapia (Oreochromis niloticus) (Devic et al., 2018; Muin et al., 2017; Toriz-Roldan et al., 2019), blue tilapia (Oreochromis aureus) (Bondari and Sheppard, 1987), rainbow trout (Oncorhynchus mykiss) (Borgogno et al., 2017; Bruni et al., 2018; Cardinaletti et al., 2019; Dumas et al., 2018; Huyben et al., 2019; Mancini et al., 2018; Renna et al., 2017; Rimoldi et al., 2019; Rumpold et al., 2018; Sealey et al., 2011; Secci et al., 2019; Stadtlander et al., 2017; St-Hilaire et al., 2007b; Terova et al., 2019), turbot (Psetta maxima) (Kroeckel et al., 2012), European seabass (Dicentrarchus labrax) (Magalhães et al., 2017), Japanese seabass (Lateolabrax japonicus) (Wang et al., 2019), Atlantic salmon (Salmo salar) (Belghit et al., 2018, 2019a, 2019b; Biancarosa et al., 2019; Bruni et al., 2020; Lock et al., 2016; Stenberg et al., 2019), zebrafish (Danio rerio) (Zarantoniello et al., 2019), Siberian sturgeon (Acipenser baerii) (Caimi et al., 2020), Jian carp (Cyprinus carpio var. Jian) (Li et al., 2016, 2017; Zhou et al., 2018), meagre (Argyrosomus regius) (Guerreiro et al., 2020), and climbing perch (Anabas testudineus) (Vongvichith et al., 2020).

Studies have shown that BSF meal could substitute up to 50% of fish meal in the diets of rainbow trout without significantly affecting survival, growth performance, condition factor (body weightto-body length ratio), somatic indices, and yield and physical quality of fillet (Bruni et al., 2018; Mancini et al., 2018; Renna et al., 2017). More importantly, compared to the fish fed control diets, higher microbial diversity was observed in the intestine of the fish fed diets containing BSF meal, which could contribute to immunity, feed digestibility, physiological function, and welfare of the fish (Bruni et al., 2018). However, a higher inclusion rate of BSF meal (i.e., 50% replacement of fish meal) increased saturated fatty acids, mainly lauric acid, and decreased the mono- and poly-unsaturated (omega-3 and omega-6) fatty acids content in the fillet, thereby worsening the lipid health indices of the muscle (Mancini et al., 2018; Renna et al., 2017). On the contrary, St-Hilaire et al. (2007b) reported an increase in moisture and decrease in lipid and gross energy contents in rainbow trout (whole-body proximate composition) fed diets containing BSF prepupal meal as fish meal substitute; these differences could have arisen from the diet formulation, as tested diets were non-isoproteic and non-isolipidic.

Similarly, the use of BSF meal as substitute for fish and/or soybean meal in the diets of freshwater and marine Atlantic salmon had no significant effects on feed intake, feed conversion ratio, or whole-body contents of protein, lipid, amino acids, and minerals (Belghit et al., 2018, 2019).

The lack of a difference in growth of rainbow trout and Atlantic salmon fed with diets containing BSF meal could be due to the external supplementation of methionine and lysine in the diets containing BSF meal to meet the dietary requirements of the respective fishes (Belghit et al., 2018, 2019; Dumas et al., 2018; Lock et al., 2016). However, fish species differ in their amino acid requirements and are likely to tolerate different levels of fish meal substitution without supplementation of essential amino acids, despite low levels of lysine in the BSF-based diet (Belghit et al., 2019). Growth performance of European seabass was not affected when fish meal was substituted with BSF meal (inclusion rate of 19.5%) without supplementation of amino acids (Magalhães et al., 2017).

Studies have reported mixed results regarding the effects of feeding BSF meal on product quality (slaughter quality) depending on fish type, inclusion level, BSF meal type (enriched vs nonenriched), and supplementation of amino acids (Belghit et al., 2019; Mancini et al., 2018; Renna et al., 2017). For example, Renna et al. (2017) found increased dry matter and ether extract contents in the dorsal fillets of rainbow trout fed diet containing 40% BSF larval meal compared to the fish fed diets containing 20% or 0% BSF meal. However, other studies observed no significant effect of dietary inclusion of BSF meal on whole body composition of carp and Atlantic salmon (Belghit et al., 2019). However, studies have reported effects on the fatty acid profile of the fish fed diets containing BSF meal (Belghit et al., 2019; Mancini et al., 2018). Such variation in the fatty acid profile is likely to impact the sensory attributes (aroma and flavor) of fish products (Borgogno et al., 2017). However, findings on the dietary effects of BSF meal on sensory parameters of fish products are inconsistent (Belghit et al., 2019; Borgogno et al., 2017; Lock et al., 2016; Sealey et al., 2011).

Overall, studies have reported successful inclusion of BSF meal (up to 40%) as a fish meal substitute in the diets of different species of fish without significantly affecting the performance and quality of products, thereby indicating the potential of BSF meal as an alternative protein source for different species of fish. However, higher chitin content (negatively affects digestibility of the nutrients) and lack of proper balance of limiting amino acids (especially methionine and lysine) and fatty acids (especially omega-3 and omega-6 fatty acids) could be the limiting factor for the higher inclusion of BSF meal in fish diet. Removing chitin from insect biomass before processing as fish feed or incorporating chitindigesting enzymes (chitinases) in fish diet could improve the digestibility of the fish feed containing higher proportion of BSF meal. Additionally, improving the nutrient profile of the BSF biomass, especially with regard to limiting amino acids (e.g., methionine and lysine) and fatty acids (e.g., omega-3 and omega-6 fatty acids) contents by enriching the BSF larvae with substrate such as fish and/or algae processing wastes could allow for higher inclusion rates of BSF meal in diets for some fish species.

5.3. Swine

Compared to poultry and fish, BSF meal as a diet for swine has been less studied. Moreover, there is lack of consistency in performance of BSF meal fed pigs depending on the inclusion rate, feeding duration, and growth stage of the animal (Altmann et al., 2019; Biasato et al., 2019; Chia et al., 2019; Nekrasov et al., 2015, 2018; Newton et al., 1977; Spranghers et al., 2018; Velten et al., 2017; Yu et al., 2019a). Feeding piglets with diets containing BSF meal (inclusion rate up to 10%, substitution of soybean meal up to 65%) had no significant effect on growth performance, feed intake, feed-to-gain ratio, nutrient digestibility, gut morphology, and histological features (Biasato et al., 2019; Spranghers et al., 2018). However, a higher inclusion level of BSF meal in piglet diets (33% inclusion rate or 75% substitution of soybean meal) resulted in negative effect on dry matter digestibility, feed intake, growth rate, and feed and protein conversion ratios (Newton et al., 1977; Velten et al., 2017).

There are limited studies examining the effects of feeding the diet containing BSF meal on carcass yield and quality in pigs. Although Yu et al. (2019a) reported improvement in average carcass trait and muscle chemical composition (when fed a diet containing 4% BSF meal), Altmann et al. (2019) reported no significant effect on meat quality parameters, including sensory attributes (except stronger overall odors and higher juiciness values) when BSF meal was used as a soybean meal substitute. Yu et al. (2019a) attributed the improvement in meat quality to upregulation of lipogenic genes and genes related to muscle fiber composition. The contradictory observations between the studies could be attributed to the inclusion rate of BSF meal in the pig diet, feeding duration, and growth stage of the pig.

Overall, substantial amount of soybean meal could be replaced with BSF meal in feeding pigs without negatively affecting the growth performance and meat quality. However, extensive studies are necessary to determine the upper inclusion level of BSF meal, and its effects on the carcass yield and quality including sensory attributes.

6. Nutraceutical value of the black soldier fly biomass as animal feed

Besides their nutritional value, BSF meal is rich in bioactive compounds including chitin, medium chain fatty acids (C6-12), and antimicrobial peptides. A recent study identified a novel polysaccharide from BSF larvae (dipterose-BSF) capable of activating the innate immunity of mammalian macrophages (Ali et al., 2019). Thus, the possible antimicrobial and prebiotic effects of chitin and chitin derivatives, lauric acid, antimicrobial peptides, and novel polysaccharide present in BSF meal indicate that BSF meal not only provides an alternative to conventional sources of protein and energy in animal diet, but also a valuable alternative to in-feed antibiotics and prebiotics, thereby adding value to BSF meal in the diets of monogastrics. Additionally, inclusion of BSF meal may further increase the shelf-life of the feeds (Henry et al., 2015; Zhao et al., 2010). The potential benefits of chitin and chitin derivatives, lauric acid, and antimicrobial peptides present in BSF on animal health are discussed in the following section.

6.1. Chitin

Chitin and chitosan have a stimulatory effect on innate immune system (Lee et al., 2008), antimicrobial properties, and bacteriostatic effect on diverse, harmful, gram-negative bacteria, and positive effects on growth of beneficial microbes (Karlsen et al., 2017; Zhou et al., 2013). Although BSF meal, which contains 6-7% chitin on dry matter basis, has not been extensively studied as a source of chitin and chitosan, or for its prebiotic or stimulatory effects on the immune system, supplementing chitin in the diets of broilers and fish has shown positive effects (e.g., inhibition of pathogens and enhancement of beneficial intestinal microbiota) (Karlsen et al., 2017; Khempaka et al., 2011). For example, positive modification of gut microbiota of rainbow trout fed diets containing BSF meal (increased occurrence of well-known probiotics, Carnobacterium spp.) was attributed to the presence of fermentable chitin in the BSF meal (Bruni et al., 2018; Rimoldi et al., 2019; Terova et al., 2019). Chitin has also been suggested to have positive effects on prolidase activity, an enzyme responsible for hydrolysis and adsorption of proline and hydroxyproline in the small intestine of rainbow trout (Dumas et al., 2018). Moreover, acidic chitinase present in the stomach and intestine of chickens is capable of degrading chitin to chitin-oligosaccharides (Tabata et al., 2017). Feeding the diet containing BSF meal to laying hens significantly modified the gut microbiota composition (increase in population of potential chitin degraders) and increased short chain fatty acid production, suggesting a potential prebiotic effect of BSF meal (Borrelli et al., 2017). Thus, the inclusion of chitin-rich BSF meal in animal feed could increase the shelf-life of feed and reduce the use of antibiotics and prebiotics in animal production (Rimoldi et al., 2019; Yu et al., 2019b). Additionally, since neither fish, birds, nor mammals are capable of synthesizing chitin (Gasco et al., 2018), incorporating BSF biomass (as a source of chitin) in the diets of these animals could potentially stimulate their immune system.

6.2. Lauric acid

As pointed out earlier, BSF biomass is rich in medium chain fatty acids, particularly lauric acid (30–60% of total fatty acids) (Table 4). Like commonly-used antimicrobial agents, medium chain fatty acids have antimicrobial effects on a wide variety of

pathogens, including *Mycobacterium smegmatis*, *Chlamydia trachomatis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, and *Helicobacter pylori* (Churchward et al., 2018; Decuypere and Dierick, 2003; Gasco et al., 2018). Moreover, the fat from BSF prepupae has substantial antimicrobial effects against lactobacilli and D-streptococci (Spranghers et al., 2018). More importantly, these fatty acids as antimicrobial agents neither have a significant human or animal toxicity nor induce problem of residues and cross-resistance induction (Decuypere and Dierick, 2003). Thus, the BSF meal rich in medium chain fatty acids, especially lauric acid, could be a valuable alternative not only to conventional protein and energy source in animal diets but also to in-feed antibiotics used for promoting growth, and preventing and curating gastrointestinal diseases (Decuypere and Dierick, 2003).

6.3. Antimicrobial peptides

Insects produce a variety of antimicrobial peptides (Tonk and Vilcinskas, 2017; Vogel et al., 2018; Yi et al., 2014), which have effects against several types of bacteria, in which risk of bacterial resistance is low (Chernysh et al., 2015). BSF genome encodes 50 antimicrobial peptides, the largest antimicrobial peptide family identified in any insects (Zhan et al., 2020). Furthermore, studies have reported the strong negative effects of antimicrobial peptides derived from BSF larvae on gram-negative bacteria (Choi et al., 2018; Park and Yoe, 2017) and gram-positive bacteria (Park et al., 2015). Although the effects of antimicrobial peptides present in BSF biomass on the performance of different animals and/or against pathogenic agents are not well studied, a substantial decrease in diarrhea in the weaned piglets fed with diet containing insect meal (e.g., yellow mealworm, giant mealworm, house fly larval meal) was ascribed to the antimicrobial peptides present in the insect meal (Ji et al., 2016). With growing global concern of increasing antibiotics resistant bacteria and genes in the environment, antimicrobial peptides present in insect biomass are of significant interest, especially such antimicrobial peptides could potentially act against fungi and viruses (Gasco et al., 2018).

7. Microbial and chemical safety of the black soldier fly biomass as animal feed

Organic wastes are often contaminated with diverse microorganisms and pharmaceuticals (human feces and animal manures), pesticides (fruit and vegetable wastes), mycotoxins (improperly stored milling and brewery side streams), heavy metals and other toxins including dioxins, polychlorinated biphenyls and polyaromatic hydrocarbons (from organic fraction of municipal solid waste) (Gold et al., 2018). Thus, the presence of microbial and chemical contaminants in BSF biomass needs to be closely monitored to ensure the feed safety, especially if the organic wastes contaminated with such contaminants are used as substrate in rearing BSF larvae (Purschke et al., 2017). However, compared to the nutritional composition, the risk (e.g., microbial and chemical contaminations) associated with the use of the BSF for bioconversion of organic wastes into animal feed has not been well studied, thereby limiting the use of the BSF biomass reared on a variety of organic wastes to animal feeds (Gold et al., 2018).

Though BSF treatment is reported to reduce pathogenic microbes such as *Salmonella* spp. (Erickson et al., 2004; Lalander et al., 2015) and *Enterococcus coli* (Erickson et al., 2004; Liu et al., 2008) present in the organic wastes such as human feces and animal manures, studies have reported presence of food pathogens such as *Salmonella* spp. and *Bacillus cereus* both in BSF biomass and residues (Wynants et al., 2019). Moreover, treating the waste with BSF larvae had no effects on oocysts or eggs of the parasites such as *Eimeria tenella*, *E. nieschulzi*, and *Ascaris suum* (Muller

et al., 2019). Thus, proper pretreatment is necessary before using BSF biomass as animal feed and residue as fertilizer, especially when applied for growing food crops.

The gut microbiota of insects is fairly resistant to mild treatments such as heat (harsh treatments would negatively affect the nutrient quality of the insect biomass). Killing BSF larvae or prepupae by blanching could be one option as it not only reduces the microbial load in the BSF biomass, but is also rapid and less costly, mitigates lipid oxidation, increases color stability, and enhances flavor and taste (Larouche et al., 2019). Additionally, since studies have reported differences in core microbiota in larval gut (Shelomi et al., 2020; Wynants et al., 2019; Zheng et al., 2013), both nutritional and microbial composition of the substrate are likely to influence the larval gut microbiota (Bruno et al., 2019; De Smet et al., 2018; Jeon et al., 2011). Thus, the gut microbiota of BSF could be positively modified via dietary supplementation (Rumpold and Schlüter, 2013).

Possible bioaccumulation of heavy metals, pesticides, and pharmaceuticals is of serious concern while rearing BSF on the diverse organic wastes contaminated with such chemicals. For example, when BSF larvae were reared on the experimental substrate spiked with heavy metals, Cd and Pb bioaccumulated in the BSF biomass, with the bioaccumulation factor (concentration in the organism divided by the concentration in the substrate) of 6.1-9.5 and 1.2–2.6, respectively, exceeding the statutory thresholds for animal feed (Purschke et al., 2017; Tschirner and Simon, 2015; van der Fels-Klerx et al., 2016b), while no bioaccumulation was observed for chromium (Cr), nickel (Ni), As, zinc (Zn), and mercury (Hg) (Diener et al., 2015; Purschke et al., 2017). Similarly, no bioaccumulation of mycotoxins (aflatoxins B1/B2/G2, deoxynivalenol, ochratoxin A, zearalenone) (Bosch et al., 2017; Camenzuli et al., 2018; Gulsunoglu et al., 2019; Lalander et al., 2016; Meijer et al., 2019; Purschke et al., 2017), pesticides (chlorpyrifos, chlorpyrifos-methyl, pirimiphos-methyl, azoxystrobin, propiconazole) (Bosch et al., 2017; Gulsunoglu et al., 2019; Lalander et al., 2016; Purschke et al., 2017), and pharmaceuticals (carbamazepine, roxithromycin, trimethoprim) (Lalander et al., 2016) have been observed in BSF biomass. The concentration of pesticides and pharmaceuticals decreased in the residual substrate, which was attributed to degradation of these compounds by microorganisms associated with BSF larvae. Hence, the crops contaminated with compounds such as mycotoxins could be biosanitized using BSF larvae, providing an outlet for valuable crops that would otherwise have to be destroyed when mycotoxin concentration exceeds the legal safety levels for animal feed application. Contrary to Purschke et al. (2017), Biancarosa et al. (2018) found accumulation of As along with Cd in the BSF larvae fed with seaweed (Ascophyllum nodosum)-enriched media. Since Cd is believed to be transported into the cells via Ca²⁺ channels (Martín-Folgar and Martínez-Guitarte, 2017), a substantial bioaccumulation of Cd in BSF biomass could be attributed to the high concentration of Ca in BSF biomass (Finke, 2013; Somroo et al., 2019). Thus, while rearing BSF larvae for animal feed application on the organic wastes rich in heavy metals, especially Cd, Pb, and As, one option for achieving product safety would be co-feeding of such organic wastes with the feedstocks which are fairly low in heavy metals. Additionally, when BSF technology is employed for treating organic wastes highly contaminated with such heavy metals which prohibits the feed application of BSF biomass, BSF protein and fat could be used for other applications including bioplastic, biodiesel, and biobased lubricant production.

8. Environmental aspects of bioconversion of organic wastes into black soldier fly biomass

Recently, several studies focused on the environmental impacts of bioconversion of organic wastes into BSF biomass. Most of these studies are based on data from small-scale bioconversion facilities (Bosch et al., 2019; Mertenat et al., 2019; Roffeis et al., 2020; Salomone et al., 2017; Smetana et al., 2016) and only few are from large-scale facilities (Smetana et al., 2019). The environmental impact of bioconversion of organic wastes into BSF biomass is not consistent across the different scales of production (data from small-scale or pilot-scale studies) and modes of life cycle assessment (LCA) (Attributional LCA or Consequential LCA). However, there is consensus among the studies that environmental impact is largely dependent on the types of organic wastes used in rearing the BSF larvae. In general, the environmental impact of producing BSF biomass is higher when the organic wastes which are already being used for food or feed applications are used to rear the BSF larvae, rather than rearing the larvae on animal manure and food waste (Bosch et al., 2019). For example, when organic wastes with food or feed value were used as substrate for producing BSF biomass, the environmental impacts (e.g., global warming potential, land use, and energy use) of BSF biomass were higher than that of soybean meal and fish meal. However, except for global warming potential, environmental impacts of rearing BSF larvae on animal manure and food waste were lower than that of fish meal and soybean meal (Bosch et al., 2019; Roffeis et al., 2020). But current feed safety regulations prohibit feeding farmed insects (which includes BSF as defined by the European Union) with organic wastes such as animal manure and catering wastes for food and feed applications in the European Union (European Commission, 2001, 2017; IPIFF, 2019). Additionally, studies have shown that energy input for processing, especially drying of BSF biomass is the major contributor to environmental impacts of BSF production (Feng et al., 2019; Smetana et al., 2019). Thus, improvement in efficiency of BSF biomass processing, which is most likely with the upscaling of the technology since most of the data for LCA were derived from the small-scale studies, or use of renewable sources of energy such as solar dryer and photovoltaic, the environmental impacts of the BSF biomass production is likely to improve.

Additionally, excessive fishing from natural water bodies for both food and feed applications has significant environmental issues, including the loss of biodiversity. Thus, although BSF meal as animal feed compared poorly in terms of land, water, and energy use as compared with fish meal, BSF meal may outperform fish meal in terms of other environmental and ecological impacts (Smetana et al., 2019). Furthermore, since studies have shown degradation of pesticides and pharmaceuticals while treating organic wastes using BSF larvae (Bosch et al., 2017; Lalander et al., 2016; Purschke et al., 2017), treating organic wastes with BSF larvae could minimize the spread of pharmaceuticals and pesticides into the environment (Lalander et al., 2016). Thus, environmental impact analyses should also account these aspects of bioremediation of the organic micro-pollutants by BSF biomass. Also, studies have shown that bioconversion of organic waste (e.g., segregated kitchen waste) into BSF biomass has lower direct GHGs emissions than composting (Mertenat et al., 2019). Thus, LCAs and techno-economic analysis (TEA) should also be conducted to compare the currently adopted technologies for treating organic wastes such as composting, direct feeding to animals, and anaerobic digestion with BSF farming for recovering resources from diverse organic wastes.

9. Economics of bioconversion of organic wastes into black soldier fly biomass

Currently, studies on the economics of bioconversion of organic wastes into BSF biomass for animal feed application are limited. Moreover, although many private companies such as Agriprotein (South Africa), EnviroFlight (USA), Bioflytech (Spain), Enterra Feed Corporation (Canada), Entobel (Vietnam), Entofood (Malaysia), Entomo Farm (France), Hexafly (Ireland), F4F (Chile), Hermetia GmbH (Germany), InnovaFeed (France), and Protix (The Netherlands) are involved in the BSF larvae production business, information on their operational processes and financial aspects (e.g., costs and benefits) are not publicly disclosed; possibly to maintain their competitive advantages (Joly and Nikiema, 2019; Zurbrügg et al., 2018).

Joly and Nikiema (2019) have summarized the economic and environmental performances of BSF technology, which indicate that the economic performance of BSF technology varies substantially with location of the operation, substrates used, production scale, and intended use of the products. Some studies on the economic performance of BSF biomass production for animal feed have shown that the use of current technology (mostly dominated by batch mode operation using flat tray system, which are not only labor intensive but also require substantial space), has a higher cost than conventional animal feed such as fish meal and sovbean meal (Caruso et al., 2013b; Pleissner and Smetana, 2019). For example, for a facility converting 53.6 tons of wet food waste into 3.64 tons of dried prepupae and 6.35 tons of dried fertilizer per day, the operation alone will cost \$ 5,850 (5,282 €) per day [i.e., about \$ 1,600 (1,451 €) per ton of dried prepupae] (Pleissner and Smetana, 2019). But, as a rule of thumb, for a profitable operation of organic waste to BSF biomass conversion facility, the production cost per ton of BSF biomass should not exceed \$907 (\$1,000 per metric ton of BSF biomass) (Drew and Pieterse, 2015).

Life cycle cost analysis based on small-scale production has shown that labor accounts for 30-65% of the total operating cost (Joly and Nikiema, 2019), and labor and substrate acquisition account for about 90% of total cost of BSF-derived animal feed production (Roffeis et al., 2018). However, substrates, such as animal manure, food wastes, and sewage sludge, have negative values as the generators of such wastes must pay discharge fee (i.e., tipping fees) (Badgett et al., 2019; Yazan et al., 2018). Additionally, national, and/or local governments may provide incentives (including fiscal) for minimizing waste generation and/or managing wastes properly (Matheson, 2019). Thus, substantial improvement in economics of BSF-based animal feed production are likely to come from onsite production of BSF biomass using negativevalue wastes, with mechanization and process control, scaling up of the production systems, and with incentive-based governments' policies. For example, modelling and verification of business plans from insect factories have suggested that to be profitable, the bioconversion facility has to reach a processing scale of converting approximately 110 tons (100 metric tons) a day of organic waste into 7.72 tons (7 metric tons) of insect meal (Drew and Pieterse, 2015). However, the availability and use of under-utilized organic resources for the production of the BSF biomass would be critical for further development of the insect-based feed industry (Smetana et al., 2019). Moreover, BSF meal is rich in lauric acid, chitin, and antimicrobial peptides, which are reported to have antimicrobial and prebiotic attributes. Thus, if BSF meal could successfully substitute the use of antibiotics, prebiotics, and the conventional feedstuffs which are not certified as organic products, the animal and fish products could be certified as organic product and sold at a premium price thereby improving the economics of BSF meal as animal and fish feed.

10. Challenges of bioconversion of organic wastes into black soldier fly biomass

Compared to composting and anaerobic digestion, insect farming on organic wastes for resource recovery is relatively new technology. Most studies in this field have been conducted at a laboratory/bench-scale. Such an approach does not produce reliable data for industrial-scale application as bench-scale results do not always scale up in a linear fashion (Miranda et al., 2019). Two primary hurdles preventing the expansion to industrial scale are; (i) proprietary information related to methods employed by companies that prevent the use of said methods by others and (ii) lack of readily available infrastructure/add-on technology to explore such efforts. In both instances, one can be used to solve the other; industry could partner with academia to conduct such research without revealing trade secrets. Recently, such partnerships have begun to address the issues of scalability of benchscale to industrial-scale. One example is a pilot-scale study looking at short-, middle-, and long-term developments (consider it the progression of the insect agriculture industry) using sensitivity analyses (Smetana et al, 2019).

Regulations currently limit the expansion and diversification of insects in the food and feed sectors. For instance, regulations in the European Union limit the production of BSF to vegetable-based pre-consumer food waste (including dairy and eggs) and resulting products can only be used as feed for fur-producing animals, pets, and aqua-cultured fish (European Commission, 2017). Similar regulations exist in the United States (McDougal, 2018) and Canada (GlobeNewswire, 2018); however, in North America, BSF biomass can be used as poultry feed as well. The European Commission is currently exploring the possibilities for proposing a new revision of the feed ban rules in order to authorize pig and insect proteins in poultry feed when reared on substrates of vegetative origin (IPIFF, 2020). These regulations are in stark contrast with most of the world (e.g., China and other Asian countries), where BSF biomass can be produced on a variety of organic wastes and used as feed for a host of animals. However, the specific regulations in these nations are not transparent to the authors and require local regulatory expertise to interpret.

Concerning manure, studies have shown that BSF larvae can be reared on different types of manures. In many countries, significant amount of manure is produced than that can be assimilated/ applied in the available farmland, resulting in air, water, and soil pollutions (Ogejo, 2018). The question is whether the manure fed BSF biomass can be safely used as feed. If not, when the contamination level would be too high, biodegradation could be the purpose and the resulting larvae can still be used for non-feed purposes (van Huis, 2019), such as bioplastics, biolubricants, or biodiesel production.

A major challenge is to produce BSF meal that is competitively priced to that of fish meal and soybean meal. Avoiding loss, reuse, recycle, and landfills are the proposed measures for mitigating substantial amount of food currently being lost or wasted. Among the reuse methods, food waste is currently being investigated to be directly used as an animal feed which will make BSF biomass production on such substrate less competitive – economically and environmentally (Mohd-Noor et al., 2017; Pinotti et al., 2019). Thus, finding under-utilized and cheap organic waste streams would be a major challenge in mass production of BSF biomass. Another challenge could be making the organic waste stream high in carbon but low in nitrogen suitable for BSF larvae. Several options include the use of selffermentation as proposed for coconut endosperm (Mohd-Noor et al., 2017), pre-fermentation with fungi of corn stover (Gao et al., 2019), or adding nitrogen to almond hull-based feedstocks (Palma et al., 2019).

Several studies have shown that BSF larvae possess antimicrobial capability and are able to reduce pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli* in the substrates (Wynants et al., 2019). So far, disease and pest problems have not been reported in the literature (Eilenberg et al., 2015). However, it cannot be ruled out the possible existence of disease and pest problems. Thus, disease and pests could pose potential risk in BSF farming on organic wastes especially when single strain of the BSF is used worldwide.

11. Future perspectives

The viability of the industry using BSF biomass as an animal feed will depend on whether it will be competitive to other feed sources such as fish meal and soybean meal, and/or if it, when further documented, can provide nutraceutical values. A reduced price could be achieved by using low-value waste streams (e.g., animal manure), process scale up, and automation with internet of things (IoT). A higher price for BSF biomass meal would be acceptable if there were additional benefits such as for health of humans or animals. Health promoting insect ingredients are chitin, fatty acids such as lauric acid, and proteins with antimicrobial properties such as phenoloxidases and enzymatic activities, such as amylase and trypsin (Muller et al., 2017; Nekrasov et al., 2018; Vogel et al., 2018). Besides, the use of insect ingredients in pharmaceuticals and cosmetics needs full attention (Rabani et al., 2019; Verheyen et al., 2018). Additionally, fatty acid profile of the BSF larval fat is very similar to palm kernel fat and coconut fat and could be an alternative to these fats for food applications (Matthäus et al., 2019). For example, a recent study has shown that BSF larval fat could replace up to 25% of butter in certain bakery products (Delicato et al., 2020), thereby indicating need for further investigation on possibility of using BSF biomass as food (Delicato et al., 2020; Wang and Shelomi, 2017).

Expansion of the waste streams used to produce BSF biomass could stabilize and expand the industry while offering greater protection to the environment. Resources such as post-consumer food wastes or animal manure are viable materials that can easily be used to produce BSF biomass. By allowing such resources to be used, the industry would be placed in a position where partnerships across industries (e.g., dairy, poultry, beef) or municipalities (e.g., urban areas producing large quantities of organic wastes) could be developed. Doing so would provide safe alternatives for recycling these organic resources while allowing appropriate levels of BSF biomass production. However, caution should be exercised while developing appropriate quality assurance plans to ensure the safety of the materials produced as a feed ingredient.

In addition to its applications as a fish meal and soybean meal substitute in the diets of livestock and aquatic animals, BSF biomass may find application as a substitute for high-price feed ingredients such as the bovine liver powder commonly used for mass rearing of mosquitos. The mass production of mosquitos is becoming more widespread due to its increased application in genetic control programs fighting against malaria and other diseases. However, the most commonly used feed ingredient, the bovine liver powder, is not only limited in supply but also is costly. A study examining the use of BSF larval meal as a feed for mass rearing two mosquito species, Aedes aegypti and A. albopictus, has shown that BSF larval meal could support the growth and development of mosquitos without negatively impacting the quality of produced mosquitos; rather there were improvements in several parameters (Mamai et al., 2019) thereby indicating the other possible applications of BSF biomass.

One less explored aspect is the use of BSF larvae in entomoremediation, which highlights the possibility of recovering metals such as Cd and Zn (Bulak et al., 2018). Nyakeri et al. (2019) also proposed bioremediation of fecal sludge from the environment using BSF. It has also been suggested to dispose of mycotoxincontaminated grain by using edible insects (Ochoa-Sanabria, 2019). Thus, in future, BSF technology may find wider applications in bioremediation of heavy metals, pesticides, and pharmaceuticals, and biosanitization of mycotoxins contaminated grains.

Studies on the genetic modification of BSF to improve their performance are currently limited. However, Zhan et al. (2020) have suggested that gene manipulation could generate mutant BSF lines with improved characteristics such as prolonged feeding duration consequently resulting in increased size BSF biomass. Furthermore, a recent study has provided insights on vital genes and regulators governing fat accumulation in BSF, thereby indicating potential of using genetic engineering approaches for manipulating BSF fat production for feed and fuel applications (Zhu et al., 2019).

With the advancement in breeding technology, there is possibility of exploiting the genetics of the BSF in improving both bioconversion efficiency and nutritional characteristics of BSF biomass (e.g., higher content of limiting essential amino acids and polyunsaturated fatty acids). Industries are collaborating with animalbreeding institutes to investigate the possibility of improving the BSF strains. An option is to select for strains that can handle certain types of waste streams. Another option is to exploit the strains that are present worldwide. A global population genetic survey of BSF based on polymorphic nuclear genetic markers compared four genetically distinct strains grown on three substrates (Sandrock et al., 2019). Both strains and substrate showed highly significant impacts on all life history traits and body compositional characteristics. The results imply that efficiency and sustainability of BSF production could be substantially increased by considering BSF genetics, and the interaction with the substrate.

The exponential increase in publications dealing with the BSF is spectacular; 384 journal papers were published in the last three years (2017–2019), compared to 137 papers published during the preceding 70 years (1946–2016) (Web of Science consulted on the 18th of January 2020). This academic interest is a sign of the prospects of this insect in bioconversion. The BSF industry is likely be on the fore-front of the most promising technologies for recovering resources from diverse organic wastes. Studies have mostly focused on increasing the conversion efficiency and scaling up of the technology; however, more data are required for evaluating the economic and environmental performance of the BSF technology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contribution:

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Jeffery K. Tomberlin: Involved in writing background, BSF life cycle, bioconversion of organic wastes via BSF farming, bioconversion technologies, challenges of bioconversion of organic wastes into BSF biomass and future perspectives sections, and assisted in reviewing the manuscript.

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Further reading

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