



# Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review

Moritz Gold<sup>a,b</sup>, Jeffery K. Tomberlin<sup>c</sup>, Stefan Diener<sup>d</sup>, Christian Zurbrügg<sup>b</sup>, Alexander Mathys<sup>a,\*</sup>

<sup>a</sup>ETH Zurich: Swiss Federal Institute of Technology Zurich, Institute of Food, Nutrition and Health, Sustainable Food Processing Laboratory, Schmelzbergstrasse 9, 8092 Zurich, Switzerland

<sup>b</sup>Eawag: Swiss Federal Institute of Aquatic Science and Technology, Sandec: Department Sanitation, Water and Solid Water for Development, Überlandstrasse 133, 8600 Dübendorf, Switzerland

<sup>c</sup>Texas A&M University, Department of Entomology, 370 Olsen Boulevard, College Station, TX 77843, USA

<sup>d</sup>Biovision Foundation, Heinrichstrasse 147, 8005 Zurich, Switzerland

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## ABSTRACT

Processing of biowaste with larvae of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), is an emerging waste treatment technology. Larvae grown on biowaste can be a relevant raw material for animal feed production and can therefore provide revenues for financially viable waste management systems. In addition, when produced on biowaste, insect-based feeds can be more sustainable than conventional feeds. Among others, the scalability of the technology will depend on the availability of large amounts of biowaste with a high process performance (e.g. bioconversion of organic matter to proteins and lipids) and microbial and chemical product safety. Currently, in contrast to other waste treatment technologies, such as composting or anaerobic digestion, the process performance is variable and the processes driving the decomposition of biowaste macronutrients, inactivation of microbes and fate of chemicals is poorly understood. This review presents the first summary of the most important processes involved in black soldier fly larvae (BSFL) treatment, based on the available knowledge concerning five well-studied fly species. This is a starting point to increase understanding regarding the processes of this technology, with the potential to increase its efficiency and uptake, and support the development of appropriate regulations. Based on this review, formulating different types of biowaste, e.g. to produce a diet with a similar protein content, a balanced amino acid profile and/or pre- and co-treatment of biowaste with beneficial microbes, has the potential to increase process performance. Following harvest, larvae require heat or other treatments for microbial inactivation and safety.

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\* Corresponding author.

E-mail address: [alexander.mathys@hest.ethz.ch](mailto:alexander.mathys@hest.ethz.ch) (A. Mathys).

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## 1. Black soldier fly (BSF) biowaste processing

Especially in low- and middle-income countries, indiscriminate disposal, poor-quality treatment, and uncontrolled landfilling of biowaste contributes to negative environmental and public health outcomes (Hoornweg and Bhada-Tata, 2012; Komakech et al., 2014). Poor biowaste management practices also waste energy and nutrients, that could be used to meet the increasing global resource demands (Alexander et al., 2017a; Diener et al., 2014). Black soldier fly (BSF) biowaste processing is a relatively new treatment technology that has received increased attention over the last decades (Čičková et al., 2015; De Smet et al., 2018; Zurbrügg et al., 2018; Makkar et al., 2014).

The BSF, *Hermetia illucens* L. (Diptera: Stratiomyidae), can today be found in many countries with year-round warm, tropical or subtropical climates (Dortmans et al., 2017; Rozkosny, 1983; Üstüner et al., 2003). The natural diet of BSF larvae (BSFL) include animal manures, human excreta, fruit and vegetable wastes, and carrion (Rozkosny, 1983; Schremmer, 1986). BSFL consume this biowaste, convert it into larval biomass, and leave behind a compost-like residue, with characteristics similar to immature compost (Zurbrügg et al., 2018; Xiao et al., 2018).

A typical BSF biowaste processing facility consists of waste pre-processing (e.g. particle size reduction, dewatering, removal of inorganics), biowaste treatment by BSFL, separation of BSFL from process residue, and lastly, refinement of the larvae and residue into marketable products (Dortmans et al., 2017). Refinement of the larvae may include killing, cleaning, sterilisation, drying and fractionation (i.e. separation of proteins, lipids and chitin), and of the residue, (vermi-)composting or anaerobic digestion (Zurbrügg et al., 2018). In addition, a nursery maintaining healthy adult and larval BSF ensures a reliable and consistent supply of offspring for biowaste treatment (Dortmans et al., 2017).

## 2. Potential of BSF biowaste processing for waste management

The interest in BSFL biowaste processing has been largely attributed to the production of larval biomass for animal feeds with a high market value. BSFL biomass contains 32–58% proteins and 15–39% lipids (dry weight, Tables 1 and 2), which are valuable for the production of animal feeds for livestock (e.g. poultry, swine, and fish) (Barragan-Fonseca et al., 2017; Makkar et al., 2014; Sánchez-Muros et al., 2014; Wang and Shelomi, 2017) and pets

(Bosch et al., 2014), and biofuels (Leong et al., 2016; Manzano-Agugliaro et al., 2012; Surendra et al., 2016; Zheng et al., 2012). BSFL following partial removal of lipids can have a protein content of 55–65% (dry weight) (Schiavone et al., 2017; Surendra et al., 2016) and an amino acid profile similar to that of feed constituents, with promising results in animal feeding experiments (Sánchez-Muros et al., 2014). Lauric acid, antimicrobial peptides, and chitin in BSFL have the potential to make larval biomass an even superior feed constituent (Gasco et al., 2018).

Due to the high market prices for conventional feed constituents (soya bean meal 44% proteins ~400 USD/tonne, fish meal 64–65% proteins ~1600 USD/tonne, dry weight, based on data cited by FAO (2016) for 2011–15), revenues from BSF biowaste processing products could be much higher than for other treatment technologies. For example, compost from biowaste commonly has a low market value (6–16 USD/tonne) (Danso et al., 2006; Diener et al., 2014).

These revenues could contribute to the implementation and sustainable operation of biowaste management, especially in low- and middle-income countries, where financial resources for implementation and operation are frequently scarce (Guerrero et al., 2013).

## 3. Current challenges for BSF biowaste processing

Even though the scientific output on the topic has been increasing in the past decade (35 publications 2009–2013, 173 publications 2014–September 2018) (Scopus, 2018), BSF biowaste processing is a relatively new treatment technology (see Lohri et al. (2017) for the evolution of research articles published on different biowaste treatment technologies). Current challenges for the efficient and sustainable implementation and operation of this emerging technology include:

- Precise, reliable, and efficient operation of the BSF nursery (e.g. egg yield per female fly, hatching rate) to maximise young larvae production.
- Low technology readiness levels/economy of scale of technologies and facilities. This influences their financial viability and the possibility for the products to enter into markets. New partnerships between insect companies and technology providers are being established to deliver BSF biowaste processing facilities at scale (AgriProtein, 2017; BITS, 2017).

- Missing benchmarks for products: Even though research and companies start demonstrating the benefits of products (e.g. insects-based feeds) from BSFL, these benefits are still waiting to penetrate into the associated industries and result in a large product demand. This is also related to the low scale of facilities discussed above.
- Incomplete or restrictive regulations: Several countries (e.g. EU, USA, Canada, Mexico, Australia, China, South Africa, Kenya, Uganda) have started allowing the use of BSFL for production of feeds under certain conditions (e.g. registration, processing, animal-specific) (EC, 2017; KEBS, 2017; Lahteenmaki-Uutela et al., 2017; UNBS, 2017).

Challenges that will be specifically discussed in this review include the variable BSFL treatment process performance and larvae and residue product safety.

### 3.1. Variability in BSFL larvae (BSFL) treatment performance

Table 1 summarises biowaste previously used in research on BSFL treatment and introduces the classification into different bio-

**Table 1**  
Description of biowaste and side streams used in BSFL treatment. This review defines wastes as discarded by-products or side streams of urban activities that are typically heterogeneous in their characteristics and have a low economic value. In contrast, side streams are more homogenous and therefore commonly have a higher value. Poultry feeds are also included as they are frequently used as an indicator for high process performance.

Biowaste/side-stream types	Description	References
Human manures	Human faeces from source separation toilets. Faecal sludge from onsite sanitation technologies.	(Banks et al., 2014; Diener et al., 2011a; Lalander et al., 2013; Nyakeri et al., 2017a)
Animal manures	Excreta of poultry, cows or swine. Depending on the management practices, this can include bedding material (e.g. straw, hay) and animal feed.	(Liu et al., 2008; Moon et al., 2001; Myers et al., 2008; Newton et al., 2005; Nguyen et al., 2013; Nyakeri et al., 2017b; Oonincx et al., 2015a; Rehman et al., 2017; Sheppard et al., 1994; Zhou et al., 2013)
Fruit wastes	Discarded fruits (e.g. apples, pears, oranges or coconut endosperm). Typically produced by food companies or fruit markets.	(Jucker et al., 2017; Mohd-Noor et al., 2017)
Vegetable wastes	Discarded vegetables, for example sugar beet pulp, banana peels, cowpea, soya bean curd residue, lettuce, beans, cabbage. Typically produced by food companies or vegetable markets.	(Jucker et al., 2017; Nyakeri et al., 2017b; Oonincx et al., 2015b; Rehman et al., 2017; Tinder et al., 2017)
Municipal organic solid wastes	Mixed waste of discarded fruits, vegetables, and food scraps. Produced by households, restaurants, markets, malls, companies, and public institutions.	(Diener et al., 2011a; Nguyen et al., 2013; Nyakeri et al., 2017a; Oonincx et al., 2015b; Spranghers et al., 2017)
Millings and brewery side streams	Side streams typically produced by the milling and brewery industry: Sorghum, dried distiller grains with solubles, wheat, bran, spilled grains and grinding dust.	(Nyakeri et al., 2017a, b; Tinder et al., 2017; Tschirner and Simon, 2015)
Poultry feeds	Feeds used for poultry. Frequently used as a control feed in BSFL feeding experiments.	(Diener et al., 2009; Gobbi et al., 2013; Liu et al., 2017; Nguyen et al., 2013; Oonincx et al., 2015b)

**Table 2**  
Process performance parameters in BSFL treatment for different biowaste/side-stream types (see Table 1). Previous results that were produced with poor biowaste preparation, feeding rates or larval densities, and experimental methods based on the current BSFL treatment process understanding were excluded from this table as they underestimate process performance (Oonincx et al. (2015a), Tschirner and Simon (2015), Banks et al. (2014) and Diener et al. (2009)). Larval dry mass is typical 60–70% (Diener et al., 2009; Spranghers et al., 2017).

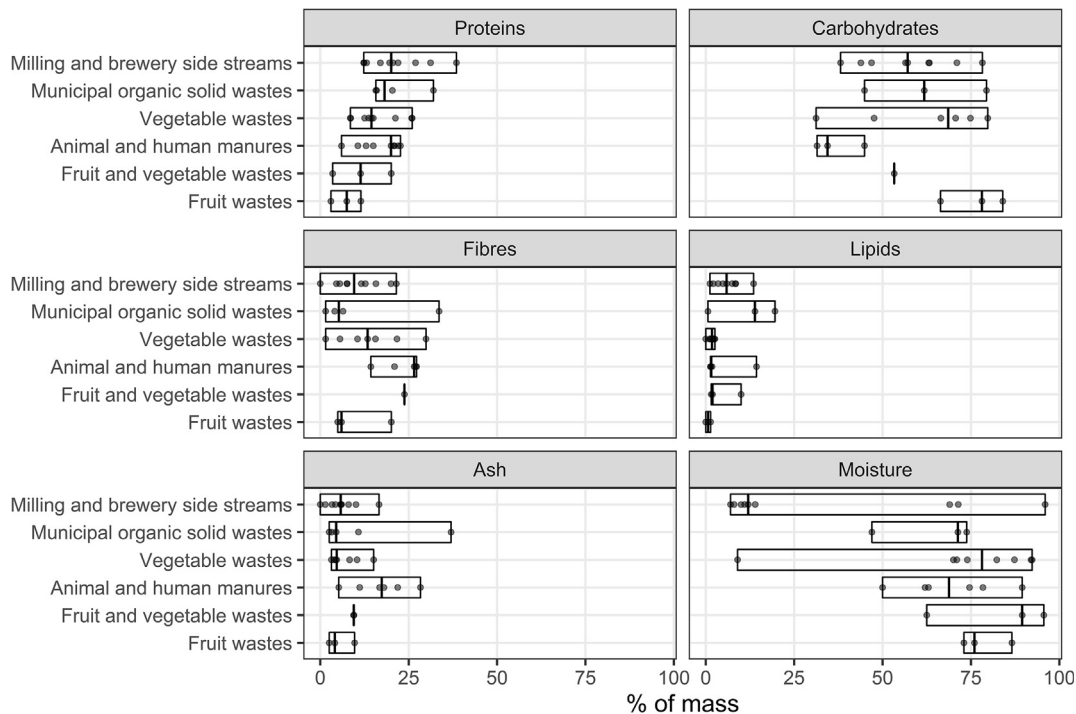
Biowaste		Prepupa larval weight mg wet wt.	Development time days	Waste reduction		Bioconversion rate % wet wt.	Proteins % dry wt.	Lipids % dry wt.
				% wet wt.	% dry wt.			
Manures	Poultry	150–255 <sup>a,b</sup>	–	50 <sup>b</sup>	32–62 <sup>a,c</sup>	3.7 <sup>b</sup>	34–35 <sup>a</sup>	–
	Swine	113–218 <sup>a,d</sup>	34 <sup>e</sup>	39 <sup>f</sup>	29–53 <sup>a,c</sup>	4.0 <sup>f</sup>	32–43 <sup>a,g</sup>	33 <sup>g</sup>
	Cow	74–147 <sup>a,d</sup>	24–31 <sup>d,h,i</sup>	63 <sup>h</sup>	26–58 <sup>a,c,d,h,i</sup>	6.3 <sup>h</sup>	34–35 <sup>a</sup>	–
	Human	70–299 <sup>i,v</sup>	27 <sup>j</sup>	46–55 <sup>k</sup>	55 <sup>j</sup>	–	45 <sup>v</sup>	18 <sup>v</sup>
Fruit wastes		55–174 <sup>l,v</sup>	15–52 <sup>l,p,v</sup>	–	32–36 <sup>p</sup>	–	35–58 <sup>p</sup>	15–38 <sup>p,v</sup>
Vegetable wastes		101–184 <sup>j</sup>	16–48 <sup>h,l,m,v</sup>	74 <sup>h</sup>	72 <sup>h</sup>	9.7 <sup>h</sup>	44 <sup>m</sup>	–
Fruit/vegetable wastes		123–154 <sup>e,l</sup>	29–37 <sup>e,l</sup>	–	–	–	39 <sup>w</sup>	33 <sup>w</sup>
Municipal organic solid wastes		101–220 <sup>e,q</sup>	16–37 <sup>e,o,q,r,v</sup>	–	60–68 <sup>j</sup>	11.8 <sup>q</sup>	36–46 <sup>o,r,v</sup>	25–39 <sup>o,r,v</sup>
Millings and brewery side streams		78–290 <sup>m,n,v</sup>	16–39 <sup>m,s,v</sup>	38–59 <sup>n</sup>	–	–	37–45 <sup>m,n,v</sup>	27–39 <sup>m,n,v</sup>
Poultry feeds		99–184 <sup>e,t</sup>	15–24 <sup>e,o,r,t,u</sup>	–	42 <sup>t</sup>	–	33–39 <sup>o,t</sup>	34 <sup>o</sup>

<sup>a</sup>(Zhou et al., 2013); <sup>b</sup>(Sheppard et al., 1994); <sup>c</sup>(Oonincx et al., 2015a); <sup>d</sup>(Myers et al., 2008); <sup>e</sup>(Nguyen et al., 2013); <sup>f</sup>(Newton et al., 2005); <sup>g</sup>(St-Hilaire et al., 2007a); <sup>h</sup>(Rehman et al., 2017); <sup>i</sup>(Li et al., 2011); <sup>j</sup>(Diener et al., 2011a); <sup>k</sup>(Banks et al., 2014); <sup>l</sup>(Jucker et al., 2017); <sup>m</sup>(Tinder et al., 2017); <sup>n</sup>(Tschirner and Simon, 2015); <sup>o</sup>(Spranghers et al., 2017); <sup>p</sup>(Mohd-Noor et al., 2017); <sup>q</sup>(Diener et al., 2011b); <sup>r</sup>(Oonincx et al., 2015b); <sup>s</sup>(Tomberlin et al., 2009); <sup>t</sup>(Diener et al., 2009); <sup>u</sup>(Gobbi et al., 2013); <sup>v</sup>(Nyakeri et al., 2017a); <sup>w</sup>(Nyakeri et al., 2017b).

waste types used in this review. Values in Table 2 demonstrate that one current challenge of BSFL biowaste processing is the precise, reliable, and efficient operation of BSFL treatment. Considering typical process and product parameters, BSFL process performance (e.g. bioconversion of organic matter to proteins and lipids) is currently variable for the same biowaste, and between different biowaste types. Even though scalability of data generated from these bench-scale experiments could also be an issue, as it is unknown if these results truly translate to industrial production levels, challenges with variable process performance are also being reported from BSFL biowaste processing operators. This impacts day-to-day operation (e.g. operation over or under the treatment capacity) and the sustainability and scalability of this technology.

### 3.2. Influence of BSFL treatment performance on sustainability

By providing BSFL biomass to global food and feed systems, BSFL biowaste processing has the potential to contribute to meeting the increasing nutritional demands of the growing global human population (FAO, 2009). But, feeds produced from BSFL do not necessarily have a lower environmental impact than conventional feed



**Fig. 1.** Macronutrient (in % of dry mass) and moisture (in % as received) in biowaste typically used in BSFL treatment. Bars are the range of all reported values. The vertical line within the bar is the median of all reported values as a metric for their variability. Carbohydrates were estimated by subtracting the sum of proteins, fibres, lipids and ash from 100%. See Table 1 for the descriptions of the biowaste types (Diener et al., 2009; Jucker et al., 2017; Li et al., 2011; Mohd-Noor et al., 2017; Nguyen et al., 2013; Rose et al., 2015; Egan, 2018; Spranghers et al., 2017; Tinder et al., 2017; Tschirner and Simon, 2015).

constituents such as soya bean and fish meal, which are currently frequently deemed unsustainable (Alexander et al., 2017b; Salomone et al., 2017; Smetana et al., 2016; Van Huis and Oonincx, 2017). Biowaste that is not already used as animal feed or for other resource recovery options has the largest potential for sustainable products. New methodologies to quantify the sustainability of waste management and feed production systems based on BSF biowaste processing are being developed (Chaudhary et al., 2018; Smetana et al., 2016).

### 3.3. Influence of BSFL treatment performance on technology scalability

Existing BSF biowaste processing facilities indicate that they are financially sustainable when processing several tonnes to several hundred tonnes of biowaste per day with a high process performance (AgriProtein, 2018; Diener et al., 2015a; Protix, 2018). As access to large and continuous amounts of biowaste is typically already subject to fierce competition, BSF biowaste processing facilities will rely on a mixture of biowaste for their long-term operation. Scale is also required for this technology to have an impact on global food systems. Hundreds of thousands of tonnes of BSFL would need to be produced per year, considering around 145 million tonnes soya bean (in 2007) (Hardy, 2010) and 15.8 million tonnes fish meal and oil (in 2014) (FAO, 2016) is being produced per year.

### 3.4. Reasons for the variable process performance

The biowaste macronutrients, proteins, carbohydrates, fibres and lipids are frequently thought to have the largest influence on process performance (Nguyen et al., 2013; Oonincx et al., 2015a, 2015b; Tinder et al., 2017). As shown in Fig. 1, biowaste and organic side streams previously used in BSFL treatment have a

variable composition in these nutrients which could explain the variability in process performance. Based on the median of all macronutrient results included in this review, human and animal manures, milling and brewery side streams and municipal organic solid wastes are higher in proteins in comparison to fruit and vegetable wastes which are higher in carbohydrates. Human and animal manures and fruit and vegetable wastes have a higher median fibre and ash content. Municipal organic solid wastes are highest in lipids.

Moisture content and pH that are not specifically considered in this review, also have a large influence on process performance (Cammack and Tomberlin, 2017; Ma et al., 2018). As shown in Fig. 1, biowaste moisture content is typically 70–80%, suitable for BSFL treatment (Dortmans et al., 2017). A moisture content in this range is important for larval development and BSFL and residue separation (Cheng et al., 2017).

Other reasons for the variability include the lack of standard operating procedures for conducting BSFL feeding experiments. Parameters including, but not limited to, different operating parameters such as biowaste descriptions, genetics (Zhou et al., 2013), feeding rate (Diener et al., 2009; Myers et al., 2008), feeding intervals (Banks et al., 2014), larval density, temperature (Harnden and Tomberlin, 2016; Tomberlin et al., 2009) and time of harvest (Liu et al., 2017) can vary significantly across studies.

### 3.5. BSF biowaste processing product safety

Biowaste include a high number and diversity of microbes. In addition, human and animal manures may contain pharmaceuticals, poorly stored milling and brewery side streams mycotoxins, fruit and vegetable wastes pesticides, and municipal organic solid wastes heavy metals and other toxins such as dioxins, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs).

Thus, transfer and accumulation of these contaminants to BSFL in the feed and food chain are of concern to this new industry (Van der Fels-Klerx et al., 2018).

Previous research identified that BSFL treatment can reduce numbers of microbes (Lalander et al., 2013). In addition, dioxins, PCBs and PAHs, and selected pesticides, pharmaceuticals and mycotoxins did not accumulate in BSFL in the few existing studies (Bosch et al., 2017; Charlton et al., 2015; Lalander et al., 2016; Purschke et al., 2017). In contrast, cadmium, lead, mercury, zinc, and arsenic are taken up by BSFL from biowaste and can exceed the maximum permissible levels of animal feed regulations (Biancarosa et al., 2017; Diener et al., 2015b; EC, 2002; Gao et al., 2017; Purschke et al., 2017; van Der Fels-Klerx et al., 2017). Due to the volume of biowaste reduced in BSFL treatment, heavy metals concentrate in the residue and could limit its application as soil conditioner or compost. Whereas microbes in and on the larvae can be a risk for animal feed safety, and in the residue for public and environmental health (Lalander et al., 2013), several studies have shown that inoculation of biowaste with microbes can increase the BSFL process performance (Xiao et al., 2018; Yu et al., 2011; Zheng et al., 2012).

A lack of understanding about the fate of potential contaminants (including parasites and viruses) in BSF biowaste processing currently limits the use of biowaste with the highest potential to produce sustainable animal feeds. For example, in the EU, use of

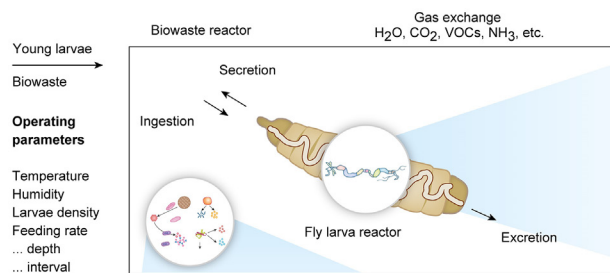
biowaste (except for pre-consumer vegetable and fruit wastes) are currently not permitted for BSFL production as animal feed (EC, 2017; EFSA, 2015; Makkar et al., 2014).

#### 4. Purpose of this review

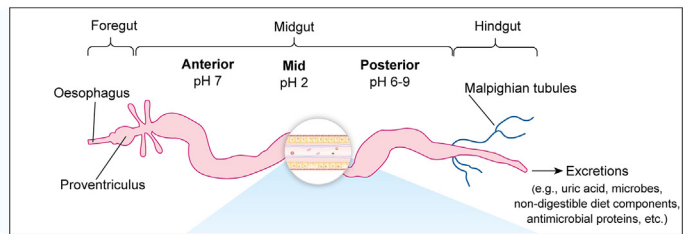
In contrast to other biowaste treatment technologies, process mechanisms in BSFL treatment are poorly understood. BSFL have a similar ecological niche and phylogenetic order (relative to well-studied insects from other orders) to larvae of well-studied fly species, this suggests that their digestion developed in a similar way from the same original digestive process (Terra and Ferreira, 2012, 1994; Terra and Regel, 1995). These include housefly larvae (HFL), *Musca domestica* (Diptera: Muscidae), green bottle fly larvae (GBFL), *Lucilia sericata* (Diptera: Calliphoridae), stable fly larvae (SFL), *Stomoxys calcitrans* (Diptera: Muscidae), and common fruit fly larvae (FFL) (also called vinegar fly), *Drosophila melanogaster* (Diptera: Drosophilidae).

Using available research on larvae of these well-studied fly species, this review summarises the current knowledge on the decomposition of biowaste macronutrients and chemicals and the inactivation of microbes by fly larvae and in BSFL treatment. It then uses this knowledge to discuss the influence of biowaste macronutrients, microbes, and chemicals on BSFL treatment process performance, and highlights future research directions.

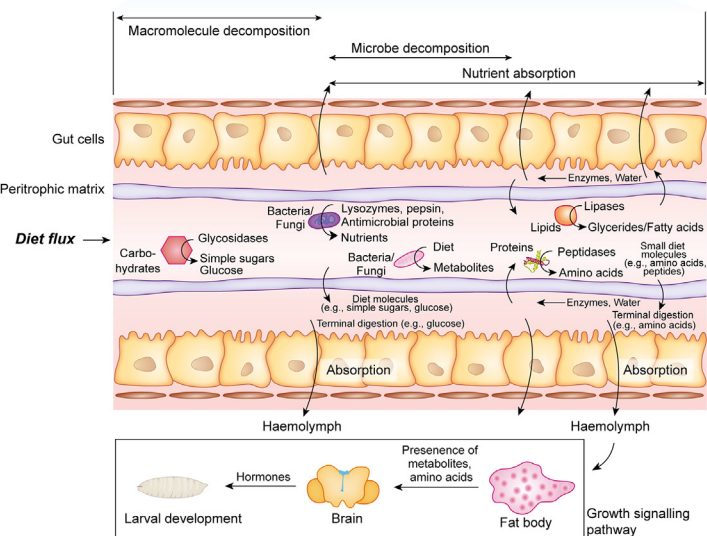
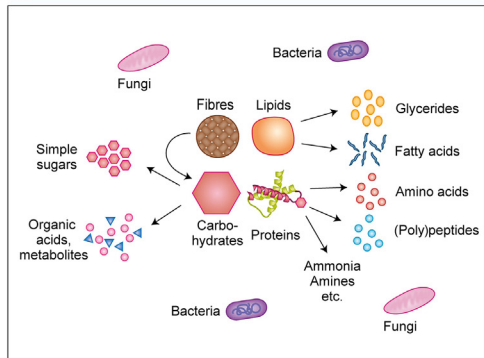
#### a) System description



#### b) Fly larva reactor



#### c) Biowaste reactor



**Fig. 2.** Schematic summary of important processes in BSFL treatment, developed based on well-studied fly larvae. (a) BSFL treatment conceptually divided into a larva (Section 7) and biowaste reactor (Section 8). (b) The larva midgut is most important for nutrient sensing, decomposition and absorption (Section 7.2). The midgut is compartmentalised longitudinally considering pH (Section 7.3) and enzymes (Section 7.4), and lined by the peritrophic matrix for the different phases of enzymatic hydrolysis of diet constituents. Size of macronutrients is reduced along the midgut, with nutrient absorption mostly taking place in the posterior midgut (Section 7.5). Some enzymes appear to be returned by water fluxes. The fat body signals larva development in the presence of amino acids via the IIS/TOR pathway (Section 7.6). (c) Enzymatic and microbial decomposition of biowaste macronutrients in the biowaste reactor. Adapted from: Beskin et al., 2018; De Smet et al., 2018; Espinoza-Fuentes and Terra, 1987; Lee and Brey, 2013; Lemaitre and Miguel-Aliaga, 2013; Wong et al., 2016

The goal of this review is to increase the understanding of BSFL treatment to lead to the enhancement of its performance and product safety, for uptake of heterogeneous biowaste. This could result in improved biowaste management, especially in low- and middle-income countries.

## 5. BSFL biowaste treatment

BSFL treatment usually takes place in boxes, bins or containers. For example, 40–80 cm × 60–200 cm × 17–30 cm used by Diener et al. (2011b) and Zurbrugg et al. (2018). At the beginning of BSFL treatment, young BSFL with an age of typically 4–9 days and a weight of approximately 1–2 mg, reared on a standard diet (often poultry feeds), are placed on a defined amount of biowaste (Jucker et al., 2017; Spranghers et al., 2017; Tschirner and Simon, 2015). Diener et al. (2009) proposed feeding rates of 100–125 mg per larva, per day (based on poultry feed, with a 60% moisture content). Biowaste is either provided once, or through periodic feeding (Banks et al., 2014; Dortmans et al., 2017).

During treatment, BSFL pass through six life stages between which, larvae moult (i.e. process of shedding their exoskeleton, allowing larvae to grow) (May, 1961). As summarised in Table 2, the last BSFL instar (prepupa) is reached after 15–52 days. At this life stage, BSFL are 70–299 mg (Table 2) and 6–20 mm (Nguyen et al., 2013; Rozkosny, 1983; Tinder et al., 2017). Full-scale operations tend to reduce the number of feedings to reduce operational costs and select their harvest time to maximise larval production and product quality (Liu et al., 2017).

## 6. System description of BSFL treatment used in this review

As shown in Fig. 2, conceptually, the container in which BSFL treatment takes place can be divided into two reactors. The fly larva as a reactor which includes all processes inside the BSFL, and the biowaste as a reactor which includes all processes in the biowaste outside BSFL. Processes in both reactors are influenced by operational parameters such as container dimensions, temperature, larval density, humidity, feeding rate, and feeding interval. As will be reviewed in the following sections, fly larvae and waste reactors are connected through ingestion, secretions, and excretions. In addition, both reactors consume and produce gases (Beskin et al., 2018; Oonincx et al., 2010). The overall system includes multiple fly larva reactors that operate in parallel within the biowaste reactor. For example, Dortmans et al. (2017) use four larvae per cm<sup>2</sup> of the biowaste reactor surface area.

## 7. Description of processes in the fly larva reactor

### 7.1. Well-studied fly larvae

Previous BSFL research focused on measuring the influence of biowaste macronutrients, microbes, and chemicals in controlled feeding experiments, but few did study the underlying physiological, microbial, and biochemical processes. Fortunately, such research is available for other fly larvae species.

Adult house flies are a recognised pest of humans, pets, and livestock; HFL typically feed on animal manures. Extensive research exists on digestion mechanisms (e.g. physiology, enzymes, nutrient absorption) of HFL (Espinoza-Fuentes and Terra, 1987; Lemos et al., 1993; Lemos and Terra, 1991a; Pimentel et al., 2018; Terra and Jordão, 1989; Terra and Regal, 1995; Zhang et al., 2017). GBFL have mostly been studied for their importance in maggot therapy, with research focusing on antimicrobial processes (Cazander et al., 2009; Lerch et al., 2003; Luther, 1951; Mumcuoglu et al., 2001; Sanei-Dehkordi et al.,

2016). GBFL typically feed on living animal or human flesh in some instances, depending on the species; however, they consume decaying animals, and manures. After their medical importance, GBFL have also been studied for their use in forensic entomology (Tarone et al., 2011). The stable fly has been studied as it is an important animal pest. SFL typically feed on animal manures (Albuquerque and Zurek, 2014; Scully et al., 2017) mixed with urine and straw, or other cellulose-based materials. It has mainly been studied for interactions between SFL and microbes (Rochon et al., 2004; Romero et al., 2006). FFL typically feed on decaying fruits. They are of importance as a model organism for genetics and more recently immunology, host-microbe interactions and metabolism (Jeon et al., 2011; Lee and Brey, 2013; Mirth and Piper, 2017; Rodrigues et al., 2015; Shin et al., 2011; Storelli et al., 2011; Wong et al., 2016; Yamada et al., 2015). The common fruit fly is one of the most well studied organisms in the world with decades of knowledge of its genomics and molecular biology which allows for unique research on metabolic pathways.

HFL were previously also studied for treatment of poultry, swine, and cow manure (Barnard et al., 1998; Calvert et al., 1970; Hussein et al., 2017; Koné et al., 2017; Nordentoft et al., 2017; Schuster et al., 2013; Wang et al., 2017a; Wang et al., 2016; Wu et al., 2017; Yang et al., 2015; Zhang et al., 2014, 2012) and GBFL for treatment of poultry slaughterhouse waste, fish waste and swine manure (Nuov et al., 1995; Yehuda et al., 2011). In contrast to the BSF, all of these fly species feed as adults which makes them vectors for the transmission of pathogens (Čičková et al., 2015).

### 7.2. General fly larvae digestion and gut physiology

Similar to other insects and mammals, fly larvae feed to obtain nutrients for their metabolic requirements (Cohen, 2005). The monomer of carbohydrates, glucose, is used by fly larvae as a building block for tissues and as fuel. Fly larvae are also surrounded by the carbohydrate chitin (Cohen, 2005). Amino acids, the building blocks of proteins are important molecules for the production of fly larvae tissue, hormones, and transport proteins (Cohen, 2005). Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are considered essential amino acids for insects (Cohen, 2005). Lipids serve as energy storage, provide structural constituents for cell membranes and organelles, and are important for the production of hormones (Carvalho et al., 2012; Cohen, 2005). Sterols, vitamins, and minerals are also indispensable for larval development, but due to the scarce information on their decomposition and importance for larval development they are not considered in this review (Cohen, 2005).

Fly larvae feed through mouthparts located at the anterior end of their elongate-oval shape. Little research exists on fly larvae mouthparts. The existing research concludes that fly larvae ingest liquids and solids without specifying the maximum particle size (Brooke and Fraenkel, 1958; Oliveira et al., 2015). Following ingestion, the food passes through the food pipe (oesophagus) (Mumcuoglu et al., 2001), the proventriculus (a valve with a potentially grinding function), into the midgut (Chapman, 2013). The midgut, shown in Fig. 2, is the longest and most important part of the larva digestive tract for diet sensing, decomposition and nutrient absorption, that doubles back and forth upon itself between the anterior and posterior end of the fly larvae (Chapman, 2013; De Smet et al., 2018; Luther, 1951). Kim et al. (2011a) extracted enzymes from the BSFL gut and salivary glands and the latter included only 10% of the total enzymatic activity. Along the midgut, through the combined action of the gut environment, enzymes, and microbes, the diet is broken down into smaller molecules for absorption through the gut cells into the

haemolymph (Chapman, 2013). The haemolymph is analogous to the blood in vertebrates and transports nutrients within the larval body (Chapman, 2013). Nutrients are stored in the larval fat body, which is important for the accumulation of lipids and controlling the larval metabolism (Chapman, 2013). From the midgut, the diet passes to the hindgut and malpighian tubules. The malpighian tubules, located at the midgut and hindgut junction are connected to the haemolymph and are important for maintaining a balance among nutrients, water, and ions within the larvae. They exchange nutrients, ions, nitrogenous substances (e.g. uric acid) and other metabolic wastes with the haemolymph and hindgut, that exceed the larval demands (Chapman, 2013; Murakami and Shiotsuki, 2001). These molecules and non-digestible diet constituents, enzymes, metabolites, microbes, antimicrobial proteins, or diet constituents not absorbed are then excreted from the hindgut (Chapman, 2013; Engel and Moran, 2013; Espinoza-Fuentes and Terra, 1987). Fly larvae are continuous feeders and in GBFL and HFL the diet has a residence time in the larvae of around 60–180 minutes (Dadd, 1970; Espinoza-Fuentes and Terra, 1987; Mumcuoglu et al., 2001; Terra and Ferreira, 2012).

### 7.3. Gut environment

Oxygen and pH are important properties of the gut environment as they influence the decomposition of diet constituents, the activity of enzymes and shape the number and diversity of gut microbes.

The physiology of fly larvae suggests that the midgut has both aerobic and anaerobic sections. Openings at the front and end of BSFL connect to their respiratory system and could supply oxygen (Rozkosny, 1983). Active transport mechanisms or diffusion through the small diameter of the midgut could allow for an oxygen supply into the gut (Chapman, 2013). This has also been indicated by the cultivation of aerobic, facultative aerobic and anaerobic bacteria from guts of all reviewed fly species (Jeon et al., 2011; Scully et al., 2017; Wang et al., 2017a; Zurek et al., 2000).

As shown in Fig. 2, based on research for HFL and FFL, the fly larvae midgut pH is buffered longitudinally in several sections: a neutral anterior midgut (pH 7), an acidic mid-midgut (pH 2), and a neutral to alkaline posterior midgut (pH 6.3–9.3) (Espinoza-Fuentes and Terra, 1987; Overend et al., 2016; Shanbhag and Tripathi, 2009). The variability in the posterior midgut is due to a higher alkalinity reported for FFL.

### 7.4. Gut enzymes

Enzymes are the most important driver for diet decomposition. They hydrolyse macronutrients into smaller molecules to be absorbed through the gut cells for the fly larva metabolism (Chapman, 2013). The decomposition of one macronutrient is likely due to the action of dozens of enzymes that sequentially reduce molecule size. In this way, carbohydrates are reduced to simple sugars, proteins into amino acids and lipids into glycerides and fatty acids (Carvalho et al., 2012; Kim et al., 2011a). For example, in the adult common fruit fly, around 350 enzymes are estimated to catalyse the decomposition of carbohydrates, proteins and lipids (Lemaitre and Miguel-Aliaga, 2013).

In HFL, most digestive enzymes are found in the posterior midgut, which suggests it is where diet decomposition and nutrient absorption primarily take place. Except during moults, enzymes are continuously released into the midgut through the release of secretory vessels from the gut cells (Espinoza-Fuentes and Terra, 1987). Excreted enzymes vary based on the diet (Section 7.6), gut environment (e.g. pH) and along the gut and its compartments (Espinoza-Fuentes and Terra, 1987). Important enzymes in fly

larvae that hydrolyse glycoside links in carbohydrates include amylase, maltase and glucosidase, peptide bonds in proteins pepsin, trypsin, aminopeptidase and serine, and ester bonds in lipids triacylglycerol lipase, phospholipase and phosphatases (Blahovec et al., 2006; Greenberg and Paretsky, 1955; Kim et al., 2011b; McDonald et al., 2011; Zhang et al., 2017). Fly larvae also have a number of lysozymes, chitinases and glucanases that may participate in the decomposition of microbes (Fujita, 2004; Lemaitre and Miguel-Aliaga, 2013). Cytochrome P450 and glutathione-S-transferase enzymes are important for the decomposition of mycotoxins or insecticides (Section 7.8) (Cochrane and Leblanc, 1986; Fusetto et al., 2017).

As shown in Fig. 2, the fly larva midgut is lined by a structure attached to the anterior midgut, known as the peritrophic matrix. The peritrophic matrix can be found in most insects and compartmentalises the midgut into sections for the different phases of enzymatic hydrolysis of diet molecules (Bolognesi et al., 2008; Terra, 2001). Espinoza-Fuentes and Terra (1987) reported that in HFL diet molecules and enzymes are transported forward by water fluxes from the anterior to posterior midgut, and within the posterior midgut.

### 7.5. Decomposition of diet macronutrients

Even though fly larvae may excrete enzymes such as amylase or maltase from the salivary glands through the mouth onto the diet, as reported for HFL, most diet decomposition takes place in the midgut (Terra et al., 1988). Following ingestion, the diet enters the endoperitrophic space (i.e. outside of the peritrophic matrix) where it has contact with enzymes catalysing the initial decomposition of macronutrients such as amylase and maltase (carbohydrates), pepsin and trypsin (proteins) and triacylglycerol lipase and phospholipase (lipids) (Pimentel et al., 2018; Terra and Ferreira, 2012). As shown in Fig. 2, according to results for HFL, with the flux of diet and water, diet constituents pass along the gut until their size has been sufficiently reduced by the enzymes to enable them to penetrate together with the enzymes through the peritrophic matrix into the ectoperitrophic space (i.e. inside of the peritrophic matrix). With the water flux in the posterior midgut, enzymes and diet molecules are then returned to the anterior section of the posterior midgut where decomposition takes place by enzymes such as maltase (carbohydrates) and aminopeptidase (proteins) (Espinoza-Fuentes and Terra, 1987). Enzymes are then returned through the peritrophic matrix into the endoperitrophic space to locate new substrates for hydrolysis (Espinoza-Fuentes and Terra, 1987). As shown in Fig. 2, in HFL, glucose produced by amylase from diet carbohydrates is already absorbed in the mid-midgut. In the posterior midgut, residual diet carbohydrates and carbohydrates originating from the decomposition of microbes (Section 7.7) are thought to be decomposed and absorbed (Pimentel et al., 2018). Results for HFL and FFL suggest that fly larvae do not have enzymes for the initial decomposition of celluloses and hemicelluloses, but microbes in the larval gut may decompose them into a form that can be metabolised by fly larvae (Espinoza-Fuentes and Terra, 1987; Lemaitre and Miguel-Aliaga, 2013; Terra and Ferreira, 2012). Through diffusion or active transport, monomers of macronutrients are then transported into the haemolymph.

### 7.6. Metabolic pathways and regulation

Based on studies of FFL, ingestion, decomposition and absorption of diet constituents, and larval development in fly larvae, is controlled by complex hormonal regulations. Different processes adjust larval development and the decomposition of diet constituents to the current nutritional demands, which change over

time as larvae grow (Almeida de Carvalho and Mirth, 2017; Colombani et al., 2003; Lajeunesse et al., 2010; Lemaitre and Miguel-Aliaga, 2013).

Nutrient sensing appears to start before ingestion. Shen and Cai (2001) demonstrated that feeding rate is regulated by a hormonal pathway and increases with sugars in the diet. However, Almeida de Carvalho and Mirth (2017) conclude that FFL regulate food intake to maintain protein intake at the cost of consuming too little or too much carbohydrates. As FFL aim to keep the development time as short as possible (Rodrigues et al., 2015), protein excess bears a metabolic cost due to the production of toxic nitrogenous wastes (e.g. ammonia, uric acid) and protein deficiency prolongs development time (Almeida de Carvalho and Mirth, 2017).

Following ingestion, the larva digestive tract appears to balance nutrient decomposition and absorption by regulating the amount of enzymes secreted into the gut, secreting less enzymes for nutrients in excess and more enzymes for nutrients in deficit (Clissold et al., 2010; Rodrigues et al., 2015). For example, for carbohydrates, Benkel and Hickey (1986) showed that the presence of glucose reduced amylase production (Brown et al., 1999; Wu et al., 2003). For lipids, Zinke et al. (2002) and Handke et al. (2013) showed an upregulation in genes for lipid synthesis in the larval body, on low-protein diets, which was also confirmed by Pimentel et al. (2017) for BSFL.

Ultimately, demonstrated by the extensive research on FFL, larval development (e.g. larval size and composition, growth rate, development time) appears to be regulated by the larval fat body in response to nutrition (Chapman, 2013; Koyama and Mirth, 2018). In FFL, illustrated in Fig. 2, in the presence of amino acids, the fat body secretes signals that lead to the release of insulin-like hormones from the brain that initiate complex metabolic processes that ultimately lead to larval growth (Arquier et al., 2008; Colombani et al., 2003; Géminard et al., 2009; Okamoto and Yamanaka, 2015). These processes called insulin/insulin-like growth factor signalling (IIS) pathway and the Target of rapamycin (TOR) pathway are suppressed during starvation or on low-protein diets and reduce growth rate and increase development time (Koyama and Mirth, 2018; Shin et al., 2011; Storelli et al., 2018, 2011).

### 7.7. Gut microbes

Fly larvae feed on diets with a high number and diversity of microbes. Microbes in the fly larvae gut have multiple functions that are important for larvae development (Douglas, 2010; Lemaitre and Miguel-Aliaga, 2013).

Several processes enable fly larvae to use microbes, such as bacteria and fungi as food (Brookes and Fraenkel, 1958; Chang and Wang, 1958; Cohen, 2005; Lam et al., 2009a; Schmidtman and Martin, 1992). For example, decomposition of carbohydrates such as starch in the anterior midgut, and absorbed in the mid-midgut, reduce the nutrients available for microbial metabolism (Section 7.5) (Espinoza-Fuentes and Terra, 1987). In addition, fly larvae excrete pepsin and lysozyme into the midgut and microbial cells are exposed to acidic pH levels that decompose them (Sections 7.3 and 7.4). Also, fly larvae excrete proteins with antimicrobial properties (e.g. antimicrobial peptides, reactive oxygen generating enzymes) (Choi et al., 2012; Elhag et al., 2017; Espinoza-Fuentes and Terra, 1987; Lemaitre and Miguel-Aliaga, 2013; Lemos et al., 1993; Lemos and Terra, 1991b; Nayduch and Joyner, 2013; Pöppel et al., 2015; Vogel et al., 2018). Lysis of bacteria and fungi in the midgut thereby releases nutrients that can be decomposed and absorbed in the posterior midgut.

Such decomposition of microbes via gut-based mechanisms including pH, enzymes and antimicrobial proteins can explain the selective inactivation of microbes, as reported for fly larvae.

Through the use of fluorescent bacteria, it was identified that there was complete inactivation of *Escherichia coli* and *Bacillus subtilis* through the gut passage of GBFL (Lerch et al., 2003; Mumcuoglu et al., 2001; Valachova et al., 2014). *E. coli* and *Salmonella enteritidis* numbers were also reduced in HFL and *Salmonella enterica* and ΦX174 virus in BSFL (Lalander et al., 2014; Nordentoft et al., 2017). Other species, for example, *Enterococcus faecalis* for BSFL and *E. coli* for SFL had low reductions in the larvae and residue (Lalander et al., 2013; Rochon et al., 2004). This demonstrates that results vary among fly larvae and inactivation is sometimes contradictory for the same fly species (Lalander et al., 2013; Rochon et al., 2004; Valachova et al., 2014). Variable results observed for GBFL in their medical applications, suggest that microbe inactivation by fly larvae depends on the specific microbe and strain, and varies with microbial dose, nutrient availability and the duration that larvae feed on the diet (Barnes et al., 2010). Similar mechanism likely apply to fungi. When exceeding a certain size and density, due to their inactivation mechanisms, fly larvae are able to “control” fungi in the diet for which they compete for nutrients (Lam et al., 2009b; Rohlfis et al., 2005), with certain fungi surviving the gut passage (Coluccio et al., 2008).

Microbes that survive the gut passage are candidates for microbes that contribute more to larval development, besides just being a nutrient source. This appears to be especially important in low-protein diets, or diets that lack other essential nutrients (e.g. methionine) (Rhinesmith-Carranza et al., 2018). That fly larvae cohabit in some form of symbiosis (Douglas, 2010) with microbes is indicated by several studies that were not able to grow FFL, HFL or SFL on diets with an absence of bacteria (Lysyk et al., 1999; Romero et al., 2006; Watson et al., 1993), whereas the addition of certain bacteria promoted their development. In contrast, it appears that fly larvae maintain their own gut microbes originating by transfer from the adult fly, larval diet and the environment (Jeon et al., 2011; Scully et al., 2017; Zurek and Nayduch, 2016).

In all reviewed species, gut bacteria are dominated by the phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Boccazzi et al., 2017; Scully et al., 2017; Shin et al., 2011; Singh et al., 2014; Storelli et al., 2011; Su et al., 2010; Wang et al., 2017a; Wong et al., 2014; Zhao et al., 2017; Zheng et al., 2013; Zurek et al., 2000). Dominating genera have a large variability between fly larvae and studies, likely due to the variability in diets. In BSFL, *Bacterioides*, *Dysgonomonas*, *Phascolarctobacterium* were abundant genera of bacteria (Zheng et al., 2013) and *Pichia*, *Trichosporon*, *Rhodotorula* and *Geotrichum* of fungi (Boccazzi et al., 2017).

The role of fly larval gut microbes on larval development is poorly understood. As shown in Fig. 2, one key function of gut microbes includes the provision of metabolic products through the decomposition of diet macronutrients. These are relevant for the larval metabolism and protection from pathogens (Lemaitre and Miguel-Aliaga, 2013; Wong et al., 2016). As fly larvae feed on diets high in carbohydrates (Fig. 1), gut microbes can metabolise starch, sugars and fibres into organic acids such as short chain fatty acids and/or simple alcohols (Cohen, 2005; Romero et al., 2006; Wong et al., 2016). Jeon et al. (2011) and Lee et al. (2014) identified that bacteria in the BSFL gut possess enzymes that can hydrolyse starch, cellulose, proteins and lipids, and thus contribute to biowaste decomposition. Zhao et al. (2017) analysed gut microbes in SFL and concluded that they likely contribute to the decomposition of fibres. In low-nutrient diets, Shin et al. (2011) identified that carbohydrate decomposition by *Acetobacter pomorum* in FFL produces metabolites that affected larval development by influencing growth signalling pathways (Section 7.6). Storelli et al. (2011) reported that in FFL, *Lactobacillus plantarum* increases amino acids extracted from low-protein diets which increases larval development. Erkosar et al. (2015) attributed this to the



activation of larval enzymes that decompose proteins. Other bacteria and fungi may likely still be discovered which produce metabolites that are involved in other pathways influencing larval development (Lee and Brey, 2013).

### 7.8. Decomposition of chemicals

In nature, fly larvae grow on diets with potential contaminants such as secondary plant metabolites, insecticides or mycotoxins. Thus, they have strategies that allow them to nevertheless thrive in the presence of contaminants. Pharmaceuticals, pesticides and mycotoxins used in feeding experiments so far have not had a detrimental effect on BSFL development (Lalander et al., 2016; Purschke et al., 2017). Similar to macronutrients, the decomposition of chemicals involves a number of complex metabolic processes, mostly taking place in the larval midgut, malpighian tubules and fat body. In general, detoxification of chemicals involves cytochrome P450 monooxygenases and glutathione-S-transferase enzymes, but also relies on the action of gut microbes (Chung et al., 2009; Cochrane and Leblanc, 1986; Fusetto et al., 2017). The decomposition of the insecticide imidacloprid in FFL has been well studied. The chemical is metabolised by larval enzymes into oxidative metabolites and by gut microbes into nitro-reduced metabolites, of which some are toxic and are excreted by the larvae (Hoi et al., 2014). Such detoxification pathways likely also apply for BSFL (Bosch et al., 2017; Charlton et al., 2015; Lalander et al., 2016; Purschke et al., 2017). However, results by Bosch et al. (2017) for the decomposition of the mycotoxin aflatoxin B1 by BSFL suggest that the exact decomposition pathways for chemicals may vary between fly species (Foerster, 1983).

### 7.9. Fate of heavy metals

Heavy metals such as copper, zinc or iron are essential for metabolic processes in fly larvae (Balamurugan et al., 2007). However, at high concentrations they have been shown to be detrimental to larval development (i.e. reduced larval biomass and an increased development time) (Diener et al., 2015b; Purschke et al., 2017). They are sequestered from the diet to cells or vesicles in the epithelium of the midgut, malpighian tubules or fat body (Sohal, 1974; Sohal et al., 1977). Heavy metals that are toxic in lower quantities such as cadmium, lead and mercury are also integrated into these structures by proteins called metallothioneins that bind cadmium and copper and store them within cell organelles called lysosomes (Maroni et al., 1986; Maroni and Watson, 1985). These pathways could also apply for BSFL which accumulate heavy metals (Section 3.5). Uptake of cadmium in insects is also mediated through calcium channels in the gut cells. As BSFL have more than ten times the level of calcium than HFL, they may sequester more cadmium than HFL, as reported by Wang et al. (2017b) (Braeckman et al., 1999; Finke, 2013).

## 8. Description of processes in the biowaste reactor

Several studies with BSFL and HFL operated biowaste treatments with fly larvae next to biowaste treatments without fly larvae. The results suggest that treatments without fly larvae had a considerable waste reduction (30% dry weight reduction), as well as some inactivation of pathogens (e.g.  $<2 \log_{10}$  *S. enterica*), pharmaceuticals and pesticides (Section 8.3) (Lalander et al., 2013). However, these were lower than when biowaste was treated with fly larvae (Lalander et al., 2016, 2013; Nordentoft et al., 2017; Wang et al., 2016). As biowaste often has a high number and diversity of microbes, such waste reduction and inactivation without fly larvae indicate a significant contribution of microbial processes.

### 8.1. Microbial decomposition of macronutrients

Microbial decomposition of biowaste constituents involves the metabolism of a diverse group of microbes that use products from each other's metabolism. Hydrolytic enzymes produced by certain microbes are similar in function to those found in fly larvae (Section 7.7) and can decompose carbohydrates, proteins, and lipids, but also have the potential to use a large number of other biowaste molecules or larval excretions (e.g. uric acid). More importantly, facultative and obligate anaerobic microbes found in biowaste, have cellulase enzymes that hydrolyse fibres (i.e. cellulose, hemicellulose, and lignin) that are typically difficult to decompose by fly larvae and most insects (Section 7.5) (Terra and Ferreira, 2012). For example, in swine manure treatment with HFL, Zhang et al. (2012) identified glucosidase, cellulase, protease and phosphatase enzyme activities in the residue which indicated that enzymes are present that can hydrolyse glycoside links in fibres.

Reduction of fibres in BSFL treatment varies between studies. BSFL treatment of fermented corncobs did not reduce cellulose and hemicellulose, and reduced lignin by 2% (Li et al., 2015). Treatment of cow manure reduced cellulose by 17% and hemicellulose by 5% in Li et al. (2011) and around 50% for both fibres in Rehman et al. (2017). These large differences in fibre reduction could be due to the variable chemical composition of different fibres, analyses methods, different BSFL treatment operations, and different compositions and quantities of microbes, nutrients, and environmental parameters influencing microbial ecology and metabolism. Temperature, oxygen and the presence of glucose and other readily metabolisable sugars can suppress the microbial fibre decomposition (Gikes et al., 1991; Linden and Shiang, 1991). Results by Zheng et al. (2012) for BSFL treatment of rice straw and restaurant waste, exemplify that the presence of sufficient amounts of bacteria can contribute to the hydrolysis of fibres. The inoculation of biowaste with an unknown mix of microbes (Rid-X<sup>®</sup>) increased reduction of cellulose by 37% and hemicellulose by 23%.

These results suggest that microbial number and diversity in different biowaste types have a large influence on the microbial contribution to the decomposition of biowaste constituents. Different types of biowaste such as human excreta, different types of animal manures, municipal organic solid wastes and fruit wastes have very different microbial compositions and quantities. Ryckeboer et al. (2003) summarised microbial communities in biowaste and concluded that species of the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* dominate. Even for the same type of biowaste, such as for different animal manures, different microbial compositions can be expected due to factors such as animal species, diet and manure storage duration (Albuquerque and Zurek, 2014).

Products of the microbial hydrolysis of biowaste (e.g. organic acids, sugars) are then available for microbial and/or larval metabolism. Larvae likely benefit from the action of microbes as they provide molecules for their metabolism, that without their action would not have been available (Chapman, 2013; Douglas, 2010). In addition, microbes can convert non-protein molecules into microbial biomass that can then be decomposed by the larva and thereby increase the overall pool of amino acids (Raubenheimer and Simpson, 1999). However, microbes may also compete with fly larvae for biowaste constituents. Albuquerque et al. (2014) reported that SFL development on horse manure was shorter for manure stored for one week compared to fresh manure, but development decreased on manure with further storage. This could be attributed to a complete decomposition of biowaste constituents by microbes, or conversion into a form that has a low value for fly larvae development (Karigar and Rao, 2011).

## 8.2. Microbial dynamics

Microbial number and diversity are different between biowaste types and change during fly larvae treatment (Scully et al., 2017). Results for HFL and FFL suggest that the overall diversity of bacteria and fungi decreases. Zhang et al. (2012) and (2014) concluded that the decomposition of microbes in the fly larvae gut, aeration of the biowaste by fly larvae, reduction in nutrient availability and increase in temperature and pH reduces the microbial diversity in full-scale treatment of swine manure with HFL and shifted it from anaerobic to aerobic microbes (Beskin et al., 2018; Wang et al., 2016). Stamps et al. (2012) reported that FFL reduce the fungal diversity. In addition, fly larvae transfer specific bacteria from their gut to the biowaste reactor. Zhao et al. (2017) concluded that HFL transfer bacteria of the phyla *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* from the gut, to biowaste. Thereby, fly larvae may promote microbes in biowaste that assist their development (Storelli et al., 2018; Vogel et al., 2018).

## 8.3. Microbial decomposition of chemicals

The microbial metabolism and the change in the physical and chemical environment in the biowaste reactor also decompose mycotoxins, pharmaceuticals and pesticides (Lalander et al., 2016; Li et al., 2013; Zhang et al., 2014). Lalander et al. (2016) observed reductions between 30 and 90% for three pharmaceuticals (carbamazepine, roxithromycin, trimethoprim) and >99% for two pesticides (azoxystrobin, propiconazole), in the absence of BSFL.

The processes leading to these reductions in biowaste are poorly understood, but likely similar to those of other biowaste treatment technologies, such as composting. Here, depending on the compound, reductions in the order of 50% to >99.99% were reported for composting of animal manures (Ho et al., 2013; Ramaswamy et al., 2010; Selvam et al., 2012). In composting, processing conditions (e.g. temperatures >75 °C) and the microbial metabolism and associated enzymes are thought to contribute to their decomposition (Karigar and Rao, 2011; Mondini et al., 2004). For example, bacteria and fungi typically found in biowaste of the genera *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Flavobacterium* and *Aspergillus* have been reported to be involved in the decomposition of mycotoxins (Dalié et al., 2010; Ji et al., 2016).

## 9. Influence of biowaste composition on process performance

Fig. 2 summarises the most relevant processes in BSFL treatment, driven by the microbial and larval metabolism that decompose biowaste macronutrients and chemicals, and inactive microbes. This conceptual understanding can be used to understand the influence of macronutrients on process performance (i.e. larval development time, weight and composition) and the influence and fate of microbes and chemicals. Solutions can then be developed to reduce the process performance variability of heterogeneous biowaste and ensure product safety.

### 9.1. Carbohydrates

Biowaste is mostly comprised of carbohydrates (Fig. 1). In BSFL treatment, carbohydrates are decomposed by various enzymatic processes in the larvae (Section 7.4) and biowaste (Section 8.1). As shown in Fig. 2, carbohydrates are ingested by fly larvae, or following hydrolysis by microbes resulting in the production of simple sugars, organic acids, and other metabolites. In the midgut, these compounds and certain microbes are decomposed into

monomers, used by microbes, or absorbed by the gut cells for use in the larval metabolism (Section 7.5).

Results of this review suggest that biowaste carbohydrates influence the BSFL lipid content (Section 7.6). On low-protein and high-carbohydrate diets, carbohydrates are converted by larvae into lipids and stored in the fat body (Handke et al., 2013; Pimentel et al., 2017). Thus, BSFL produced on low-protein and high-carbohydrate diets are typically higher in lipids in comparison to BSFL produced on diets more balanced in carbohydrates and proteins (Barragán-Fonseca, 2018). For example, Sprangers et al. (2017) produced BSFL prepupae with 37% lipids on vegetable waste with 45% carbohydrates in comparison to 21% lipids for digestate with 7% carbohydrates. Jucker et al. (2017) reported a higher BSFL lipid content for fruit waste than vegetable waste, which is lower in carbohydrates (Fig. 1). Tinder et al. (2017) reported a higher energy content for BSFL prepupae produced on biowaste higher in carbohydrates and lower in proteins. As lipids are higher in energy than proteins, this can indicate an increase in BSFL lipids.

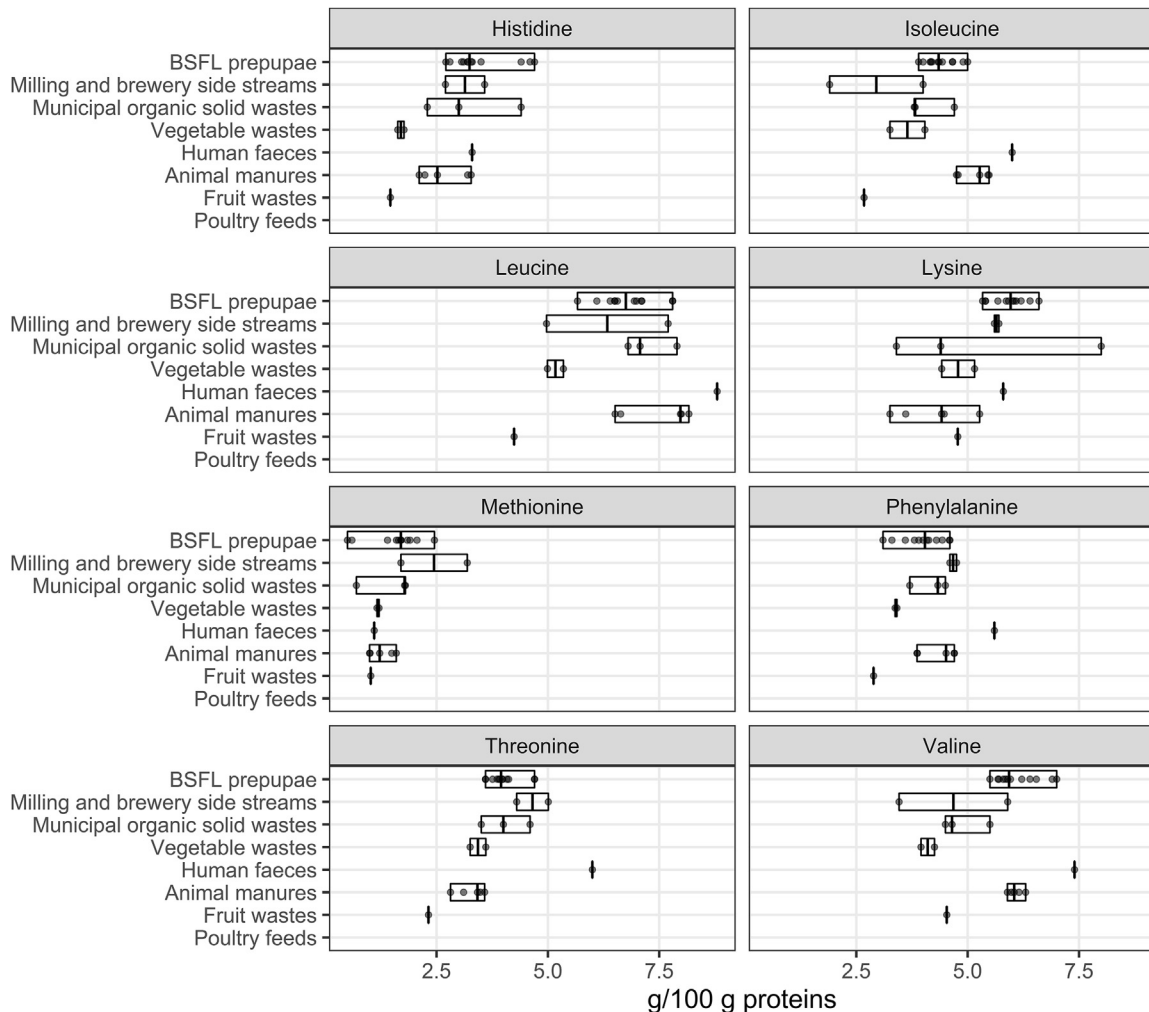
### 9.2. Fibres

It is likely that BSFL do not have enzymes for the decomposition of fibres (Section 7.5). However, microbes in the larval gut and biowaste can hydrolyse them and make the nutrients available for larval development (Sections 7.7 and 8.1). As shown in Fig. 2, carbohydrates, simple sugars and other metabolites result from the hydrolysis of fibres. As fibres are a large and chemically diverse group of biowaste constituents with variable decomposition processes and a continuum to carbohydrates, groups of fibres that can be decomposed by BSFL still have to be established. This area of research could be quite fruitful if methods were developed to either allow BSFL to consume cellulose degradation products after microbial decomposition, or if BSFL were found to contain cellulose degrading microbes that could be amplified to allow more efficient use of nutrients locked in the cellulose matrix.

Barragán-Fonseca (2018) demonstrated that one key driver for high BSFL treatment process performance is the diet protein and carbohydrate content. Because fibres are indigestible, large amounts of fibres can reduce the process performance by reducing the overall nutrient density for BSFL development. This could be one contribution to the lower dry weight waste reduction and longer development time of biowaste high in fibres, such as animal manures (Fig. 1, Table 2). Treatment of biowaste before BSFL treatment (i.e. pre-treatment) or inoculation at the beginning of BSFL treatment with microbes (i.e. co-conversion) that can degrade fibres, offer important future research possibilities to improve BSFL process performance (Zheng et al., 2012). In addition, future biowaste characterisation should consider the function of different carbohydrates (e.g. starch, sugars, available carbohydrates) for BSFL (Barragán-Fonseca, 2018).

### 9.3. Proteins

Proteins have been identified as the most important biowaste macronutrient influencing BSFL process performance (Nguyen et al., 2013; Oonincx et al., 2015a, 2015b; Tinder et al., 2017). Proteins in biowaste deliver amino acids for larval development (Section 7.2). As shown in Fig. 2, amino acids are ingested as proteins, (poly)peptides or amino acids by fly larvae (Chapman, 2013). Proteolytic processes of microbes in the biowaste before or during BSFL treatment could also convert biowaste proteins into non-protein nitrogen, such as ammonia and thereby reduce the pool of amino acids (Section 8.1). In the midgut, proteins and amino acids are decomposed by peptidases and absorbed by the gut cells (Section 7.2). In the presence of sufficient amounts of



**Fig. 3.** Essential amino acids (in g/100 g proteins) of BSFL prepupae and of biowaste typically used in BSFL treatment. Results for fruit and vegetable wastes were calculated based on Jucker et al. (2017) with data from the USDA Food and Nutrition Information Center (<https://www.nal.usda.gov>) (Attia and Abou-Gharbia, 2011; Bosch et al., 2014; Chen et al., 2017; De Marco et al., 2015; Liland et al., 2017; Myer et al., 2000; Spranghers et al., 2017; St-Hilaire et al., 2007a; Tschirner and Simon, 2015).

amino acids, insulin-like hormones that trigger larval development (Sections 7.6 and 7.7) are released. On low-protein diets, gut microbes can signal larval development by the production of metabolites from carbohydrates and increase the amount of amino acids that can be extracted from the diet (Section 7.7). These results were produced with mono-associated FFL on low-protein diets and should be validated by future research with BSFL.

This understanding suggests that when operating with a variety of biowaste, the quantity of proteins in the diet is a key parameter that drives larval development. Formulating diets with variable protein contents to a standard protein content could be a key driver for an efficient and reliable process performance (Barragán-Fonseca, 2018). Previous research identified that in general, BSFL grown on biowaste higher in proteins have a higher larval weight, bioconversion rate, feed conversion rate and larval protein content, and a lower developmental time and lipid content (Nguyen et al., 2013; Oonincx et al., 2015a, 2015b; Tinder et al., 2017). BSFL grown on biowaste lower in proteins have a higher developmental time, are smaller and have more lipids (if the biowaste includes high amounts of carbohydrates, Section 9.1) (Jucker et al., 2017).

Barragán-Fonseca (2018) and Cammack and Tomberlin (2017) worked with artificial diets and only varied the amount and ratio of proteins and carbohydrates. This research identified that both the quality, quantity and ratio of proteins to carbohydrates is important for process performance and that there this a trade-off

between development time, and larval weight and composition. The authors concluded that development time was shorter on balanced or protein-biased diets with protein:carbohydrate ratios in the order of 1:1–4:1. In contrast, larval weight was higher and fat content lower on carbohydrate-biased diets with protein:carbohydrate ratios of 1:2–1:4 (Barragán-Fonseca, 2018). Barragán-Fonseca (2018) demonstrated that diets high in proteins can also produce larvae with a high lipid content, and thereby reduce the larval protein content (in % dry mass).

Current biowaste characterisation before BSFL treatment does not reflect the biological function proteins have for BSFL. Biowaste proteins are frequently estimated through determination of biowaste nitrogen and multiplication with a conversion factor. This method overestimates the protein content of biowaste that include significant amounts of non-protein, nitrogenous constituents such as ammonia, nitrate and nitrite (Chen et al., 2017). For example, based on complete amino acid analysis, Chen et al. (2017) determined a conversion factor of nitrogen to proteins of 4.78–5.36 for different animal manures, compared to 6.25, which is the typically used factor. This could be one explanation for the lower process performance of animal manures in comparison to other biowaste (Table 2).

After the quantity of proteins, protein quality (including amino acid composition and digestibility) could be a key parameter driving larval development (Barragán-Fonseca, 2018). The comparative

slaughter technique (i.e. comparison of the amino acid composition of the diet to the amino acid composition of the animal following slaughtering) previously used to determine energy requirements of livestock, but also proposed for insects, can provide preliminary information on how well the amino acid requirements of BSFL are met by biowaste (Cohen, 2005; McDonald et al., 2011). Fig. 3 shows the composition of essential amino acids in biowaste proteins, next to BSFL prepupae. It shows the quality of proteins of different biowaste types in comparison to the essential requirements of BSFL. It shows that fruit and vegetable wastes are low in essential amino acids. In combination with their low protein content (Fig. 1), this could contribute to a lower developmental time of BSFL grown on fruit and vegetable wastes (Table 2) (Jucker et al., 2017). Millings and brewery side streams, municipal organic wastes and animal manures have a more similar amino acid composition to BSFL. Interestingly, poultry feed which has the lowest developmental time for BSFL (Table 2) has the most similar amino acid composition to BSFL. Thus, formulations of biowaste with different amino acids that complement each other could increase process performance beyond simply the mean performance of the different biowaste types (McDonald et al., 2011). The comparative slaughter technique is an oversimplification for BSFL treatment, as microbes in the biowaste and larval gut can be additional consumers or producers of amino acids (Sections 7.7 and 8.1).

The digestibility of proteins could be an additional future parameter to account for different protein qualities of biowaste. Protein quality in feeds for livestock is frequently determined *in vitro* using pepsin and hydrochloric acid (McDonald et al., 2011). However, corrective equations to account for shortcomings of this method have not yet been established for fly larvae. Digestibility of proteins can be both positively and negatively influenced by biowaste processing such as size reduction or heat treatment that also occur during biowaste production or management (Cohen, 2005; McDonald et al., 2011).

Amino acid composition results for BSFL in Fig. 3 suggest that biowaste proteins and amino acids have a smaller influence on the BSFL amino acid composition than biowaste fatty acids on BSFL fatty acids (Section 9.4). Spranghers et al. (2017) and Liland et al. (2017) determined the amino acids profile of BSFL produced on different diets. The standard deviation of BSFL essential amino acids between diets ranged from 2 to 21%. This is much lower than the influence of the diet on the BSFL fatty acid composition which can exceed 100% (Liland et al., 2017; Oonincx et al., 2015b; Spranghers et al., 2017).

#### 9.4. Lipids

Lipids are typically a minor constituent of biowaste (Table 2). As shown in Fig. 2, biowaste lipids are decomposed in the biowaste and/or larval gut into free fatty acids or mono- and diglycerides for absorption by the gut cells and use in the larval metabolism (Sections 7.4, 7.7 and 8.1).

Research for BSFL suggest that lipids do not likely limit larval development, unless provided in excess. When changing both proteins and lipids in different diets, Oonincx et al. (2015b) reported that proteins had a larger influence on larval development than lipids. In addition, BSFL on a low-lipid diet were higher in lipids, potentially as this diet was higher in carbohydrates (Section 9.1). Excess lipids such as in restaurant waste have been suspected to decrease larval development (Nguyen et al., 2013).

In contrast to carbohydrates and proteins that are decomposed into monomers for use in completely new body molecules, the composition of biowaste lipids directly influences the BSFL fatty acid composition. Carvalho et al. (2012) demonstrated that the diet lipid composition directly influenced the FFL fatty acid

composition. Plant-based diets that are high in long unsaturated fatty acids produced FFL phospholipids, that are longer and more unsaturated, in comparison to diets depleted in lipids. St-Hilaire et al. (2007b) and Liland et al. (2017) confirmed this influence of diet lipid composition for BSFL. BSFL content of omega-3 fatty acids increased when fish offal and seaweed that are high in these fatty acids were formulated into the diet.

#### 9.5. Microbes

Biowaste used in BSFL treatment typically have a high number and diversity of microbes. As presented in this review, and shown in Fig. 2, microbes in the biowaste and larval gut have multiple functions that are important for the BSFL process performance. In the biowaste, microbes are important for the hydrolysis of biowaste macronutrients, especially fibres that typically cannot be decomposed by BSFL (Section 8.1). Following ingestion, microbes are selectively inactivated by gut pH and by enzymes and antimicrobial proteins and used by larvae as food providing additional nutrients than those found in the diet (Sections 7.3 and 7.7). Microbes that survive these processes are potential candidates for gut microbes that can contribute to larval development (Section 7.7). Gut microbes are also excreted together with antimicrobial proteins into the biowaste (Sections 7.7 and 8.2). Over time, BSFL change the microbial diversity in the biowaste (Section 8.2).

BSFL are able to reduce microbes that can be feed or food pathogens. However, these antimicrobial processes in the larval gut and biowaste are yet poorly understood. They depend on several factors including the composition of microbes, nutrient availability and composition, and operating parameters such as temperature and pH. In BSFL, *E. coli* were reduced more at temperatures of 27 °C and 32 °C, than 23 °C, and in poultry manure compared to hog manure (Erickson et al., 2004; Liu et al., 2008). De Smet et al. (2018) suggest the lack of reduction in hog manure is due to the reduced stability of antimicrobial proteins produced by BSFL, due to the lower pH compared to poultry manure. Vogel et al. (2018) suggest that BSFL adjust their antimicrobial proteins based on their diet. Similar to FFL, research for BSFL suggest that different mechanisms exist for gram-negative and gram-positive bacteria, which could explain the survival of *E. faecalis* in BSFL, a common gram-positive gut bacteria (Lalander et al., 2016; Lemaitre and Miguel-Aliaga, 2013; Zdybicka-Barabas et al., 2017). These results suggest complex and dynamic processes for inactivation of food and feed pathogens, including spores that likely also survive the gut passage (Coluccio et al., 2008). Thus, heat or other treatments for microbial inactivation after BSFL harvest, are crucial to ensure food and feed safety (Schlüter et al., 2017; Van der Fels-Klerx et al., 2018).

Selected microbes in BSFL treatment have the potential to result in a large increase in process performance. Two studies with BSFL demonstrated that co-conversion of biowaste with *B. subtilis*, isolated from the larval gut, can increase the process performance. Addition of *B. subtilis* to poultry manure increased BSFL weight by 9–22%, bioconversion rate by 13% and waste reduction by 13% compared to BSFL without *B. subtilis* addition (Xiao et al., 2018; Yu et al., 2011). Authors of these studies concluded that *B. subtilis* decomposes biowaste constituents that cannot be decomposed by BSFL, such as fibres. On low-protein diets, *A. pomorum* and *L. plantarum* in FFL guts promoted the development of larvae mono-associated with these bacteria (Shin et al., 2011; Storelli et al., 2011). As these bacteria boosted larval development and are easy to cultivate, they are also two interesting candidates for BSFL treatment.

#### 9.6. Chemicals

Based on the results of this review, BSFL have mechanisms for the decomposition of biowaste chemicals that are supported by

microbes present in the biowaste and gut. Pharmaceuticals, pesticides and mycotoxins are decomposed into different forms by microbial action in the biowaste and enzymes in the larval gut (Sections 7.8 and 8.3). Reductions for these compounds are similar to those determined for composting (Section 8.3). However, more research is required as pharmaceuticals and pesticides include enormous and heterogeneous groups of compounds with different properties influencing their decomposition. Also, the influence of potential degradation products of chemicals on animal health has not been investigated. In addition, whether antibiotics, nowadays commonly used for livestock (McDonald et al., 2011), alter microbial numbers and diversity (which is important for many processes in BSFL treatment, Sections 7.7, 7.8 and 8.1) and thereby reduces the process performance, requires further research.

In contrast to pharmaceuticals, pesticides and mycotoxins, heavy metals are sequestered in the larval midgut from the biowaste, with transporters or proteins and stored in the larval body (Sections 7.8 and 8.3). Thus, protecting biowaste from high heavy metal contaminations is currently the only way to prevent heavy metals in BSFL and in the residue.

## 10. Summary and outlook

BSFL biowaste processing is an emerging biowaste treatment technology which can produce marketable high-value products that can contribute towards sustainable and financially viable resource recovery-based waste management systems. One challenge of the technology includes an efficient operation of BSFL treatment and safe larval and residue production with several biowaste streams that have variable characteristics.

Research on the processes in BSFL treatment are in its infancy. Knowledge for different well-studied fly larvae summarised in this review are consistent between fly species and to the current knowledge on BSFL. Thereby, this review is a starting point to increase the process understanding of this technology.

Numerous biowaste characteristics that are frequently intertwined and microbes in the biowaste and larval gut likely contribute to the process performance. This creates a challenge in predicting BSFL treatment process performance and product safety. Processes that drive process performance are dynamic, based on larval age/size, biowaste macronutrient composition and microbial numbers and diversity.

Especially protein and digestible carbohydrate quantity, quality and their ratio appear to be important for process performance. Future research should include biowaste characterisation that reflects the biological function of nutrients in the larvae. Following, formulating biowaste with a similar protein/carbohydrate content, balanced in amino acids and different types of carbohydrates has the potential to increase the efficiency and reliability of the process performance.

Recent research started exploring the manifold roles that microbes play in BSFL treatment. Pre- and co-treatment of biowaste with beneficial microbes has a large potential to increase process performance, for example to reliably decompose fibres that can comprise a significant fraction of biowaste. However, BSFL treatment operators are yet hesitant, considering that microbes could also be pathogenic for animals and need to be inactivated to ensure microbial product safety.

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