Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (Hermetia illucens)

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ABSTRACT

Global population growth has led to an urgent need for more efficient food production systems. Furthermore, as income levels increase, dietary preferences are shifting to more animal-based products. However, current feed protein sources deplete wild fish populations and contribute to rainforest deforestation. Capturing the resources in organic waste could help alleviate environmental impacts of food production. The larvae of the black soldier fly (Hermetia illucens) are ferocious feeders on decomposing organic material and could be used as protein source in animal feed. This study evaluated development of black soldier fly larvae on eight urban organic waste fractions and two control substrates. Principal component analysis was conducted to identify substrate properties that contributed to treatment efficiency and larval development. The main treatment factors found to be affected by substrate were waste-to-biomass conversion ratio, larval development time and final prepupal weight. The substrate properties with the greatest impact on biomass conversion ratio and larval development time were content of total volatile solids and protein content, while only total volatile solids content affected final prepupal weight. It was concluded that black soldier fly larvae are versatile in their feedstock preferences and can be used to treat a variety of organic waste streams, provided that the total volatile solids and nitrogen content are sufficiently high to support larval development. Abattoir waste, food waste, human faeces and a mixture of abattoir waste — fruits & vegetables are waste streams that are highly suitable for fly larvae treatment.

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1. Introduction

The world’s population will have exceeded 9 billion by 2050, with more than half of this global population growth occurring in Africa (United Nations, 2015). According to FAO estimates, food production will have to increase by 70% in order to feed the global population and this growth will be accompanied by increased income levels, and thus a shift in food consumption with a higher demand for meat, especially fish and poultry (van Huis et al., 2013). However, instead of consuming fish directly, around one-third of global fish catches are processed into fish oil and fishmeal for use in livestock and aquaculture feeds (Tveterås and Tveterås, 2010). For example, with a Fish-in-Fishout ratio of 1.9–2.9 for carnivorous fish aquaculture, as conservatively calculated by IFFO (International Fishmeal and Fish Oil Organisation) (Seafish, 2011), the pressure on wild fish is increasing with the growing aquaculture industry. From 1990 to 2014, worldwide aquaculture production grew by 7.8% annually (FAO, 2014). As a consequence, the price of fishmeal, today a major ingredient in feed, tripled from 2000 to 2015 and it will become increasingly unaffordable for fish and meat production. Already today, 60–70% of production costs in animal husbandry derive from feed purchasing (animal meal, fishmeal, soybean meal). Meeting these needs will require the development of new animal feed production systems (FAO, 2009), so alternative sources of protein of animal origin are therefore being explored.

Insect farming can be a viable new source of animal protein. Insects can be farmed in high densities with small space requirements and they have a high bioconversion ratio (Dominicz and de Boer, 2012). Furthermore, many insects can be reared on waste streams, which keeps the environmental footprint low and assists in recycling of refuse (Smetana et al., 2016). Consequently, the use of fly larvae in waste management has started to gain attention (Cíková et al., 2015; Pastor et al., 2015). One species that has gained more attention than others is the black soldier fly (BSF), Hermetia...
Eggs of the black soldier fly, *H. illucens* L. (Diptera: Stratiomyidae), whose polyphagous larvae (BSFL) are known to feed and develop on a wide range of feed sources, such as kitchen waste (Diener et al., 2011; Nguyen et al., 2015), dairy manure (Myers et al., 2008b), chicken manure (Zhou et al., 2013) and human faeces (Banks et al., 2014; Lalander et al., 2013). One reason why this species is of particular interest is because the fly does not feed and thus is not a vector in disease transmission (Sheppard et al., 2002). Another reason is that, when the larvae stop feeding in the final larval stage, they are higher in fat than other fly larvae (Cicková et al., 2015). This makes BSFL of particular interest for use as a protein and fat source in animal feed (Wang and Shelomi, 2017) and for the production of biodiesel (Surendra et al., 2016).

However, the economic feasibility of a BSF system depends, among other factors, on the larval biomass produced from a certain amount of waste, in other words, the waste-to-biomass conversion ratio. This varies with the nature of input materials and may range from as little as 3% for biogas digestate to 23% on a wet-weight basis when working with fresh human excreta (Banks et al., 2014; Lalander et al., 2015; Newton et al., 2005; Spranghers et al., 2016).

There are many factors that affect the growth of insects. Assuming unlimited access to a selected material, development of insect larvae depends, in the first instance, on the presence of essential nutrients (Table 1).

Shortages, or even lack, of essential nutrients result in reduced growth and lowered survival rates (Cohen et al., 2004). However, even when the nutrients are present, they have to be bioavailable to the animal. Bioavailability is greatly dependent on the species of the animal in question and the type of food in which the nutrient is present. Unfortunately, almost nothing is known of the bioavailability of nutrients in insects. The majority of fly larvae, just like larvae of many species from other insect groups (e.g. beetles, true bugs, hymenoptera), feed on materials that are originally solids and converted into a liquid slurry before ingestion (Cohen et al., 2004). Such solid-to-liquid feeding or extraoral digestion is necessary as they lack biting and chewing mouthparts.

The main objectives of this study were to investigate the effect of different substrates on fly larvae composting in terms of larval growth (biomass conversion ratio, final larval weight), larval development time, waste reduction and amino acid profile of the larvae, and to identify the substrate parameters that influence the fly larvae composting process.

### 2. Material and methods

#### 2.1. Animals

Eggs of the black soldier fly, *H. illucens* L. (Diptera: Stratiomyidae), were obtained from a laboratory colony located at the Research Institute of Organic Agriculture (FiBL, Switzerland). Newly hatched larvae used for the experiments were reared on chicken feed (Granngården Hönssfoder Start, metabolisable energy content = 11.2 MJ kg⁻¹, 80% moisture) for around 10 days. They were then transferred to one of the 11 substrates listed below.

#### 2.2. Substrates

**Poultry feed**: Dry poultry feed (Granngården Hönssfoder Bas, ME = 10.9 MJ kg⁻¹) was dissolved in water to 40% dry matter (DM).

**Dog food**: Dry dog food (Purina Pro Plan puppy, ME = 16.0 MJ kg⁻¹) was dissolved in water to 40% DM.

**Food waste**: Food waste was collected from the local restaurant at Ultuna campus (Swedish University of Agricultural Sciences), Uppsala, and minced in a grinder (Palmia) with grinder plates Ø 5 mm.

**Fruit & vegetables**: Lettuce (50%), apples (30%) and potatoes (20%) were minced in a food processor (Moulinex, Masterchef 3000).

**Abattoir waste**: Abattoir waste was collected from a sheep farm in mid-west Sweden and was chopped with a knife and mixed manually in order to represent the content of abattoir waste in low and middle-income countries. It comprised 46% stomach contents, 16% blood (cattle blood bought in a retail outlet), 12% manure, 16% meat and 8% organs (lungs, heart).

**Abattoir-fruit & veg. waste**: Abattoir waste was mixed with fruit and vegetables in a 1:1 ratio based on wet weight.

**Poultry manure**: Fresh poultry manure (laying hens) was collected from the Swedish University of Agricultural Sciences experimental farm at Funbo-Lövsta, Uppsala.

**Human faeces**: Human faeces were collected fresh in plastic bags and stored at −20 °C.

**Primary sludge**: Dewatered primary wastewater sludge was sent under cooling from Hammarby sjöstadssverken in Stockholm.

**Undigested sludge**: Sewage sludge was retrieved before the digestion step (activated sludge) at Uppsala municipal wastewater treatment plant (Kungsängens reningsverk, Uppsala). The DM content was very low at the time of collection and the sludge was dewatered through a cloth, achieving a DM content of around 8%.

**Digested sludge**: Dewatered, anaerobically digested wastewater sludge was collected at Uppsala municipal sewage treatment plant (Kungsängens reningsverk, Uppsala).

All substrates were divided into feeding portions, bagged in freezer bags and stored at −20 °C until use.

#### 2.3. Experimental set-up

The experiments were conducted in triplicate in plastic containers (Smartstore classic 2, 21 cm × 17 cm × 11 cm) with netted lids, kept at 28 °C. In each box, 200 larvae (>0.2 cm in size, 10 ± 2 d old) were placed, giving a larval density of 0.6 larvae cm⁻². The substrates were applied every second or third day, with a feeding rate of 40 mg DM larva⁻¹ d⁻¹. The substrate portions were thawed and brought to room temperature before feeding. When 50% of the larvae had transformed into prepupae, feeding was stopped but the experiment continued until all larvae had either turned into prepupa or had died. The survival rate was determined at the end of the experiment by enumerating all emerging prepupae and dividing this number by the total number of larvae added at the start of the experiment.

#### 2.4. Sampling and analysis

All substrates were weighed when applied, as was the total remaining material at the end of the experiment. On all feeding occasions, the combined weight of 10 larvae was recorded. These
larvae were collected, washed in water, dried on a piece of paper and weighed, after which they were placed back on the substrate. All emerged prepupae were counted and weighed within one day of emerging. When no prepupae emerged on two subsequent analysis occasions, the remaining prepupae and larvae found in the material were picked out, counted and weighed. All dead larvae were counted, but were not taken into account when calculating the substrate-to-biomass conversion ratio (BCR).

Samples of all substrates and of the compost residue at the end of the experiments were taken for analysis of dry matter (DM) and total volatile solids (VS). Samples were dried at 80 °C for 48 h. After drying, the material was combusted in a muffle oven at 550 °C for 4 h for determination of VS. The pH was analysed five days after the start of the experiment, once a week during the duration of the experiment and at the end of the experiment. For these pH measurements, a radiometer electrode at room temperature was used: 10 g of sample were diluted with 50 mL deionised water and left to settle for 1 h at room temperature prior to analysis. At the end of the experiment, the prepupae were dried at 50 °C for 48 h and sent to Eurofins Food & Agro Testing Sweden AB (Swedac accredited lab) for amino acid profiling. The Swedish standard method (ISO 13903:2005) was followed for amino acid profiling. For analysis of total nitrogen, the samples were boiled in concentrated sulphuric acid according to the method described in Lander et al. (2015). Following acid boiling, the samples were neutralised to pH > 3 using 10 M NaOH, diluted 50-fold in deionised water and then digested using Spectroquant® Crack-Set 20 (1.14963.0001). The nitrate concentration in the digested diluted sample was determined at 340 nm using Spectroquant® nitrate test with concentration range 0.4–25 mg L⁻¹ (1.09713.0002).

### 2.5. Calculations

The percentage material reduction on a dry matter basis (Mat. red. DM) was calculated as:

\[
\text{Mat. red. DM} = \left(1 - \frac{\text{sub in DM}}{\text{mat. out DM}}\right) \times 100, \tag{1}
\]

where sub in DM and mat. out DM was the dry matter of the substrate fed and of the residue after the experiment, respectively.

The percentage waste-to-biomass conversion ratio on a dry matter basis (BCRDM) was calculated as:

\[
\text{BCR}_\text{DM} = \frac{\text{pp DM}}{\text{sub in DM}} \times 100, \tag{2}
\]

where pp DM and sub in DM was the total dry matter in the prepupae (pp) and the substrate (sub in), respectively.

The percentage protein conversion ratio on a dry matter basis (PrCRRDM) was calculated as:

\[
\text{PrCRR}_\text{DM} = \frac{\text{pp DM} \times \%\text{Pr}_{pp}}{\text{sub in DM} \times \%\text{Pr}_{\text{sub in}}} \times 100, \tag{3}
\]

where pp DM and sub in DM was the total dry matter and %Prpp and %Prsub in was the percentage crude protein (% of DM) in the prepupae (pp) and the substrate (sub in), respectively.

Values for carbohydrate content of similar substrates were taken from the literature. The total protein content (% of DM) was used to verify the similarity between the substrates tested in this study and those described in the literature. No reliable comparable literature value was found for the digested sludge. Based on the literature values of protein and carbohydrate concentration, the carbohydrate to protein ratio (CHO/Pr) was calculated.

The carbon to nitrogen ratio (C/N) was calculated by dividing the percentage of organic carbon by the percentage of total nitrogen, on a dry matter basis. The percentage of organic carbon was estimated by dividing the percentage of VS by 1.8 (Haug, 1980).

### 2.6. Statistical analysis

The Brown-Forsythe-Levene test was performed to verify equal variance between the data on the different substrates. Analysis of variance (ANOVA) with 95% confidence interval was performed to identify statistically significant differences between substrates. When a significant difference was found, Tukey post-hoc test with 95% confidence interval was performed. Principal component analysis (PCA) was performed to find the variables that mostly contributed to the data variance, while generalised linear regression was used to evaluate the variables selected in the PCA. All statistical analyses and graphical illustrations were carried out using RStudio (RStudio Team, 2016).

### 3. Results

The dry matter content of the different substrates varied between 41% for poultry feed and 8% for undigested sludge, while the VS content on a dry matter basis varied between 93% for dog food and 63% for undigested sludge (Table 2). Dog food had the highest protein concentration (40% of DM) and fruit & vegetables and the lowest (13% of DM). The pH after five days of BSF composting ranged between 4.3 (dog food, fruit & vegetables) and 8.9 (poultry manure). Fruit & vegetables had the highest CHO/P ratio and slaughterhouse waste the lowest. Poultry feed had the highest C/N ratio, while abattoir waste had the lowest.

The highest waste-to-biomass conversion ratio (BCR) was achieved with the abattoir waste (15% DM) and the highest material reduction occurred for poultry manure, of which 85% was reduced on a DM basis (Table 3). Undigested sludge had the lowest conversion ratio (2% DM) and material reduction (13% DM). Poultry feed had the highest protein conversion ratio (PrCR), while digested sludge had the lowest. The larvae developed rapidly on abattoir waste, abattoir waste-fruit & veg., dog food, poultry feed, food waste, human faeces and poultry manure (Fig. 1). By day 14 of the experiment, the first prepupae had emerged from these substrates and by day 19, 50% or more of the prepupae had emerged (Table 3). The prepupae in these substrates were quite large in all cases (>210 mg larva⁻¹) except those reared on poultry manure (165 mg larva⁻¹). The largest prepupae were those reared on abattoir waste-fruit & vegetables, which weighed just over 250 mg larva⁻¹ on average. Larval development was slowest on the digested sludge, where it took 30 days for the first prepupa to emerge and around 50 days for 50% of the prepupae to emerge. The prepupae that emerged from the different sludges were smaller than those on the other substrates; around 140 mg larva⁻¹ for those reared on undigested and primary sludge and 70 mg larva⁻¹ for those on digested sludge. Larval development was also slow on the fruit & vegetables substrate, 28 days for the first prepupa to emerge and around 28 days for 50% of the prepupae to emerge. However, in that case the average prepupal weight was quite high (220 mg larva⁻¹).

Principal component analysis was conducted in order to distinguish the most important parameters affecting BSF composting (Fig. S1). The BSF composting variables found to be affected by substrate properties were waste-to-biomass conversion ratio (% DM), larval development (time for first prepupa and for 50% of prepupae to emerge) and prepupal final weight (mg prepupa⁻¹). The survival rate did not vary greatly between the different substrates. The variables found to contribute most to BCR and larval development were VS and protein feeding rates. Volatile solids
feeding rate (VS$_r$) was found to be the most important parameter, contributing to 60% of the variance in BCR (Table 3). When the protein feeding rate (Pr$_r$) was included in the linear model, close to 80% of the variance in BCR was explained (Fig. 2). The model including both VS and protein feeding rate took the form $y_{VS+Pr} = a \times V_{S} + b \times Pr$, where a and b are model-derived constants representing the estimated slope in the correlations of VS$_r$ and Pr$_r$ with response variables. This model was not as strong in explaining the development time, but the degree of explanation increased greatly from the simpler model ($y_{VS}$), from 30% to 60% (Fig. 2c and d). For the preupal final weight, VS feeding rate was by far the strongest factor; including protein feeding in the model only increased the model strength from 0.87 to 0.9 (Table 4; Fig. 2f). The only substrate property investigated that was found to explain the variation in PrCR was VS feeding rate, while inclusion of protein feeding rate did not improve the model. The average crude protein content of the prepupae in this study was 41.2% (Table 5) and it did not vary greatly between the prepupae reared on the different substrates. The highest protein content was found for the prepupae reared on abattoir waste (44%) and the lowest for those reared on human faeces (39%). The combined amino acid component of the dry matter comprised 36 ± 0.6% (Table S1). The differences in amino acid profiles in the prepupae reared on different substrates were not large, although some were significant (Table 5).

### 4. Discussion

This study investigated how different substrates affect BSFL composting in terms of larval growth (biomass conversion ratio, final larval weight), larval development time and waste reduction. It has already been established in other studies that larval density, feeding rate and feeding frequency have a great impact on the efficiency of the process (Banks et al., 2014; Parra Paz et al., 2015), so those parameters were not analysed in this study. The same larval density (0.6 larvae cm$^{-2}$), feeding rate (40 mg DM day$^{-1}$ larva$^{-1}$)
and feeding frequency (every 2nd or 3rd day) were employed for all 11 substrates tested, in order to investigate the impact of the substrate itself. These variables are by no mean the most optimised, but were those used in this study. Dog food and poultry feed were included in the study, since these substrates have been used as model substrates in previous studies.

4.1. Substrate properties affecting the process

The two main factors found to contribute to the BSFL composting process were VS content of the substrate and nitrogen feeding rate (mg larva−1) (Table 4). In contrast, the C/N ratio was not found to directly correlate to the response variables (Fig. S1). Rehman et al. (2017b) stressed the importance of a good balance between VS and nitrogen in BSFL substrates. They found that BCR in BSFL composting was higher on soybean curd residue than on dairy manure, while mixtures of the two substrates yielded even higher BCR, which those authors attributed to better nutrient balance (C/N ratio) in the composite substrate. In this study, human faeces and undigested sludge had the same C/N ratio (8.5), but the BCR for human faeces was around 11%, while that of undigested sludge was just over 2% (Table 3). This is probably because the VS content of the undigested sludge was too low, even though the ratio of nitrogen to carbon was within a range similar to other substrates with higher BCR (dog food and abattoir - fruits & veg.). As the feeding rate was regulated by dry matter content in this study, the VS and protein feeding rates varied for the different substrates. To capture the variations in the amount of VS and protein received by the larvae reared on the different substrates, daily VS and protein feeding rates were calculated. The model including VS and protein feeding rates (yVE,P) was able to capture the variations in BCR for the different substrates (Fig. 2b). However, that model did not describe the variations in larval development time as well, e.g larvae reared on the fruit & vegetables substrate, which had a high VS and low protein content, had a longer development time than estimated by the model for that VS and protein feeding rate. The BCR did not correlate with the protein conversion ratio (PrCR): although there was no significant difference in the PrCR of fruit & vegetables, abattoir waste, poultry manure and human faeces, the BCR of these waste fractions varied between 4% (fruit & veg.) and 15% (abattoir waste). Interestingly, the PrCR for the mixture of abattoir waste and fruit & veg. (48%) was higher than that of the pure abattoir waste fraction (31%), although the BCR of the two substrates was almost the same (14–15%). The nutrient balance was better in the mixture of abattoir waste-fruit & veg., as the carbon added by the fruit & vegetable fraction balanced the high nitrogen content of the abattoir waste, enabling the larvae to utilise the available nutrients to a higher degree.

4.2. Factor affecting larval size and development

The larvae grew largest on abattoir waste (including that mixed with 50% fruit & vegetables) and were smallest on undigested sludge (Table 3). On all substrates, the larval weight gain appeared to be linear. The correlation between VS feeding rate and the final weight of the prepupae was very strong (Table 4; Fig. S2), and is likely the strongest factor controlling the final prepupal weight. The development of BSFL on cattle manure has been shown to be slower and the larvae considerably smaller (Myers et al., 2008a) than found for poultry feed (Diener et al., 2009). As observed in the present study, Nguyen et al. (2013) also found that the development of BSFL on fruit & vegetable waste was quite slow. However, the larvae fed fruit & vegetables in this study were considerably larger than in their study. The energy and protein content of the fruit & vegetables substrate in their study was lower than that of manure, yet the larvae fed fruit & vegetables grew larger and developed more quickly. This could be because, although the energy content was higher in manure, it consisted to a large extent of lignin, which the larvae could not degrade. Rehman et al. (2017a) found considerably lower BCR for dairy manure than for chicken manure even though the dairy manure had higher total organic carbon, because the proportion of lignin, cellulose and hemicellulose was higher. Another reason why the larvae fed the fruit & vegetables substrate were smaller for Nguyen et al. (2013) could be because they were handled to a greater extent (daily), which according to those authors can cause stress and thereby hamper growth.

The larvae reared on fruit & vegetables waste were quite large, but their development was slow compared with values reported in other studies on similar substrates. For example, in Spranghers et al. (2016) the required development time for the first prepupae to emerge was 19 days from the first feeding and in Meneguz et al. (2018) it was 20 days, compared with 28 days in this study. Fruit and vegetables are high in VS but low in protein, so the slow development could be due to a larger amount of substrate being required in order to attain a sufficient amount of protein for development. Interestingly, Meneguz et al. (2018) found a larger difference in development time between winery by-product waste and brewery waste (22 d and 8 d, respectively), where the winery by-product waste had similar properties to the fruit & vegetable waste in this study (8.3% DM, 90.5% VS and 12% protein on a DM basis), while the brewery waste had a considerably higher VS and protein content (23.2% DM, 96% VS and 20.1% protein on a DM basis).

It was also observed in the present study that, when a substrate was high in protein, the development was faster even when the VS content was not high. However, the larvae did not grow as large, as in the case of poultry manure. It appears that the larvae accumulated enough protein to continue their development, while consuming less energy, but this resulted in smaller larvae.
Spranghers et al. (2016) found that rearing BSFL on digested vegetable waste resulted in almost 40% lower larval biomass yield compared with rearing them on undigested vegetable waste, while the development time was not affected by consuming digested waste. In the present study, the larvae reared on digested sludge were the smallest (70 mg larva\(^{-1}\)) and had the longest development time, with BCR reduced by >90% compared with undigested sludge (Table 3). During anaerobic digestion, easily available carbon is reduced to methane, while nitrogen largely remains in the digestate (Zhang et al., 2014). The digested sewage sludge in this study had a low VS as well as protein feeding rate, which supports our suggestion that the VS content influences the size of the larvae, while the VS and protein content together affect the development time of the larvae.

### 4.3. Other factors that could have an impact on larval growth

Volatile solids and protein content seemed to explain the variations in BCR and larval development to a great extent. However, other factors are also likely to contribute. The fat content was not analysed in this study, but is likely to have an impact, since BSF

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**Table 4**

Model strengths (\(R^2\)) and significance level of models describing the correlation between volatile solids (VS\(_r\)) and protein (Pr\(_r\)) feeding rate, alone and in combination, on biomass and protein conversion ratio (BCR and PrCR, respectively), prepupal emergence rate and prepupal weight.

<table>
<thead>
<tr>
<th>Model strengths ((R^2)) for response variables (y)</th>
<th>BCR(_{final})</th>
<th>PrCR(_{final})</th>
<th>Prepupal weight</th>
<th>Prepupal emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS feeding rate (VS(<em>r)) (y</em>{VS} = a \times VS_r)</td>
<td>0.58***</td>
<td>0.55***</td>
<td>0.87***</td>
<td>0.54***</td>
</tr>
<tr>
<td>Protein feeding rate (Pr(<em>r)) (y</em>{Pr} = b \times Pr_r)</td>
<td>0.51***</td>
<td>0.15*</td>
<td>0.31***</td>
<td>0.33***</td>
</tr>
<tr>
<td>VS and protein feeding rate (y_{VS,Pr} = a \times VS_r + b \times Pr_r)</td>
<td>0.77***</td>
<td>0.56***</td>
<td>0.90***</td>
<td>0.63***</td>
</tr>
</tbody>
</table>

Significance level of model coefficients: \(p < 0.001^{***}\), \(p < 0.5^*\)
larvae need to accumulate energy in the form of fat for the adult phase (Sheppard et al., 1994). A substrate too rich in fat could also be detrimental, e.g. Memon (2010) found that blowfly larvae reared on a high fat diet lived for a shorter time and did not survive to adulthood. In the present study, no factors affecting the survival rate were identified and quite a high survival rate was found for all substrates (Table 3). On the other hand, emergence to flies was not monitored. Moreover, it is difficult to compare BSF with other insects, even other flies, as they do not feed as adults.

4.4. Material reduction

In this study, no particular substrate property was found to contribute to the material reduction. One factor that was not taken into account in this study, but which could contribute, was the amount of easily available carbon relative to the amount of slowly digestible carbon compounds, such as long-chain fibre compounds e.g. cellulose, lignocellulose and lignin (Pérez et al., 2002). Agricultural wastes, such as cattle manure, contain high proportions of cellulose and lignin and generally a lower proportion of easily available carbon. Li et al. (2011) found that 50% of cellulose and nearly 30% of hemicellulose was reduced after 21 days of BSF composting, while the relative lignin content increased because lignin was not degraded. In a later study, Li et al. (2015) found a small reduction in lignin content after BSF composting of anerobically digested corncrib, but pointed out that bacteria could be responsible for the lignin degradation, rather than the larvae themselves. The residence time in their fly larvae composting system was not reported. Lignin degradation is quite complex and is performed by either fungi or a consortium of bacteria (Brown and Chang, 2014). A material with high carbon content that comprises a great proportion of lignin could be expected to be broken down less well in BSF composting, which is generally a quite fast process.

4.5. Protein content and amino acid profile of the prepuce

In the concept of waste management with black soldier flies, the larvae or prepuce could be used either as animal feed (Wang and Sholomi, 2017) or for production of biodiesel (Leong et al., 2016). For use as feed, the protein content and the amino acid profile of the larvae/prepuce are important. In this study, the importance of waste substrate on the protein content and amino acid profile of the larvae was investigated. The results showed that protein content did not vary greatly on a DM basis, ranging between 44% (abattoir waste) and 39% (food waste and human faeces) (Table 5), while the combined amino acid component of the DM comprised 36±0.6% (Table S1). That is well in line with data presented by Liland et al. (2017), who showed that the deviation in gross protein and actual protein is related to the nitrogen content in the chitin compounds in the larvae, giving a misleading gross protein value. No factor was found to correlate with the protein content of the larvae (Fig. S1). Despite the protein content of the larvae not varying by much, the size of the larvae/prepuce varied considerably (70–250 mg larva⁻¹), as discussed above (Table 3).

There were some differences in the amino acid profile of the larvae/prepuce reared in different substrates (Table 5). The content of the non-essential amino acids tyrosine and lysine varied by 40–50% between the different substrates, while there was no significant difference in the essential amino acids arginine, histidine and threonine. The variation in the other amino acids was smaller (±20%). On comparing the results of this study to average values found in other studies using swine and dairy manure as feedstocks (Kroeckel et al., 2012; St-Hilaire et al., 2007; Stamper, 2015; Zhang et al., 2007), some differences were found. In general, the literature values were higher than the average obtained for the substrates evaluated in this study (Table S1). The methionine content found here was 1.8% of the crude protein content, while the average of the above studies was 2.1% of the crude protein content. The prepuce grown on the poultry manure substrate had a methionine content of around 2% of the crude protein content, which is comparable to that found in other studies. However, the methionine content of the larvae reared on poultry manure only differed significantly from that of the larvae reared on abattoir waste-fruit & veg., poultry feed and fruit & vegetables. So although substrate type appears to have some effect on the amino acid profile of BSF larvae, this effect does not appear to be large, confirming findings in other
4.6. **Fly larvae treatment for different waste fractions**

Based on the findings of this study, the most suitable substrates in terms of biomass yield for BSFL composting are those that contain a large proportion of easily available carbon and a sufficiently high protein content to support larval development. Abattoir waste, abattoir – fruits & vegetables, food waste and human faeces are examples of substrates that provide good conditions for larval growth, and for which BSFL composting would be a good option. Substrates that contain a high proportion of easily available carbon, but a low content of nitrogen, do not support larval development and thus the efficiency of the process is reduced. Thus in this study, the BCR of the fruit & vegetables substrate was 4%, compared with 15% for abattoir waste. The total biomass, and hence the potential revenue from treating fruit & vegetables waste in a BSFL composting system, would thus be much smaller. As the development time for the larvae would be considerably longer, the efficiency would be even lower, resulting in quite expensive treatment for small revenue.

The BSFL composting process was not optimised in this study, and thus the efficiencies can be expected to increase for some of the more promising waste fractions, e.g. abattoir waste, food waste and human faeces (Table 3). One way of increasing the possible revenue in waste management operations is by mixing waste fractions that have a high protein content with fractions that are high in easily available carbon, so that the larvae to a higher degree can make use of available nutrients (as in the abattoir waste-fruit & veg. mixture in this study). The conversion into larval biomass of protein-poor fractions could be increased, and thus the efficiency of the process time decreased, by addition of a protein-rich substrate, while higher utilisation of the available nutrients could be expected on combining a substrate rich in easily available carbon with a protein-rich substrate.

For any waste substrate, the total revenue from potential products would have to be evaluated based on the current demand and market value at the production location (Lohri et al., 2017). For example, it might be more profitable to anaerobically digest the waste prior to BSFL treatment in locations where vehicle gas has a higher value than animal feed protein, whereas the opposite may be true in a different location (Lalander et al., 2018).

5. **Conclusions**

The main substrate properties affecting BSFL composting were found to be VS and protein content of the substrate. The BSFL were effectively reared on many waste streams, including food waste, human faeces and abattoir waste. However, the larvae did not grow particularly well on different sewage sludges, as their VS content was too low. Larval growth on fruit & vegetable waste was slower and biomass conversion ratio quite low compared with other substrates, but the prepupa grew large. The amino acid profile of the prepupa did not vary greatly, with only smaller variations in the amino acid profiles of the prepupa reared on different substrates. The protein content of larval biomass varied only slightly (39–44% of DM), while great variations were found in final larval weight. The larvae of BSF are robust and can feed on a variety of substrates, provided that the VS and protein contents are sufficiently high to support larval development. Abattoir waste, food waste, human faeces and a mixture of abattoir waste – fruits & vegetables are highly suitable substrates for BSFL composting, while pure fruit & vegetable waste and different sewage sludges are less suitable.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2018.10.017.

**References**


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