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Process efficiency in relation to enzyme pre-treatment duration in black soldier fly larvae composting

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ABSTRACT

Black soldier fly larvae (BSFL) composting is a treatment in which biodegradable food waste is converted into animal-feed protein and organic fertiliser. BSFL composting has greatest potential for mixed food waste, but under European Union regulations only plant-based waste is permitted as feed for larvae. Biomass conversion efficiency (BCE) in BSFL composting is lower for plant-based waste than for mixed food waste. One way of improving BCE for plant-based waste is to add enzymes to make the waste more available to the larvae, but enzyme pre-treatment is not commonly applied prior to BSFL composting. Therefore this study examined the impact of enzyme pre-treatment duration on process efficiency in BSFL composting of lettuce-cabbage waste pre-treated with enzymes for 0–4 days. The results showed that total solids (TS) in larvae decreased with longer enzyme pre-treatment. Direct addition of enzymes at the start of BSFL treatment (0 day pre-treatment) resulted in 22% higher BCE on a volatile solids (VS) basis compared with the control, while longer pre-treatment did not improve BCE further. Much of the VS was respired in the 0–day pre-treatment, resulting in lower mass of residues at the end of treatment. Longer pre-treatment increased microbial respiration, suggesting that the microbial community consumed more easily available carbohydrates during the pre-treatment step, which counteracted the purpose of enzyme pre-treatment, i.e. increasing BCE during BSFL composting.

1. Introduction

Fly larvae composting is a method that complies with the principle of circular economy, in that the waste or side-stream in one process is used as the resource in another (European Union, 2016). One fly species that has an impressive ability to convert food waste into its own biomass in its larval state is the black soldier fly (*Hermetia illucens* L. (Diptera: Stratiomyidae)) (Čičková et al., 2015; Tomberlin et al., 2015).

The protein content (~40% on a dry matter basis) and amino acid profile of black soldier fly larvae (BSFL), with considerably higher amounts of methionine (often a limiting essential amino acid) than in soy (Lalander et al., 2019), makes this product suitable for animal feed (Lalander et al., 2019; Sealey et al., 2011; Surendra et al., 2016). The other end-product from BSFL composting is larvae frass-compost, which is not a mature compost. The nitrogen and phosphorus content in larvae-frass compost is higher than that in organic compost (Chiam et al., 2021), and similar to that in commercial organic fertilisers such as SAFI (Beesigamukama et al., 2020) and NY 525–2011 (Cai et al., 2019) and in liquid inorganic fertilisers using biochar (Tan et al., 2021).

The highest process efficiency in terms of waste-to-biomass conversion in BSFL composting is achieved using food waste as a substrate (Gold et al., 2020; Lalander et al., 2015). Under European Union Regulation (EC) No 1774/2002, only plant-based biodegradable streams are permitted as feed for larvae intended for use as a feedstuff in aquaculture, for non-production animals (EU No 2017/893) and for poultry and porcine animals (EU 2021/1372) . Biomass conversion efficiency (BCE) in BSFL composting is lower for plant-based biodegradable waste than for food waste (Lalander et al., 2015; Meneguz et al., 2018). However, Gold et al. (2020) achieved BCE of 22.1% with vegetable canteen waste, possibly because vegetable canteen waste is more varied, containing various types of vegetables and legumes, compared with a single source of vegetable. The lower BCE when using single-source plant-based biodegradable waste can be a response to high carbon content, low availability to the larvae of lignin- and hemicellulose-rich materials, and lower protein content (Gold et al., 2018; Kumar et al., 2018; Meneguz et al., 2018; Nguyen et al., 2015; Nyakeri et al., 2017). Lalander et al. (2019) obtained considerably higher protein conversion efficiency for a mix of abattoir waste and fruit and vegetable

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waste than for each of these wastes separately. They attributed this to a more balanced composition in terms of available protein to carbohydrate ratio, enabling the larvae to utilize available protein in a more efficient way. Similarly, Gold et al. (2020) found that protein conversion efficiency was higher when using canteen waste than vegetable canteen waste and suggested that the larvae incorporated more lipids when fed vegetable canteen waste, as a response to the higher proportion of carbon per unit available protein. Nutritional parameters of the substrate considered to be important for high substrate-to-larval BCE are high protein content (Beniers and Graham, 2019; Lalander et al., 2019), similar non-fibre carbohydrate content to protein content (1:1 ratio of protein to carbohydrates) and low lipid and fibre content (Gold et al., 2020). Fruit and vegetable waste is typically low in protein and lipid and high in fibre, i.e. it does not meet the nutrient requirements of BSFL.

One way of dealing with the lower BCE of single-source plant-based biodegradable waste in BSFL composting is to apply a pre-treatment to improve the digestibility of substrates low in protein and high in fibre (Isibika et al., 2019). For example, on pre-treating banana peel with fungi combined with addition of ammonia solution for 7–14 days, Isibika et al. (2019) found that the BCE of the waste increased from 7 to 15% on a volatile solids (VS) basis. The ammonia solution likely promoted microbial breakdown of complex molecules (Tadele and Amha, 2015) and enzymes secreted by the fungi degraded the substrate into more easily available carbohydrates, rendering the substrate more available to the larvae (Godliving, 2009; Sánchez, 2009; Sigoillot et al., 2012).

Instead of relying on fungi to generate enzymes, pure enzymes could be added to biodegradable waste, which would provide more control over the process. Enzyme pre-treatment could also be less time-consuming, as the enzymes would be present immediately, rather than having to wait for enzyme production by the fungi (Hankin and Anagnostakis, 1975; Schumann et al., 2013). Using an enzyme cocktail and performing enzymatic hydrolysis could further shorten the pre-treatment time in comparison with fungi pre-treatment, the effect of which has been demonstrated to improve with time (Isibika et al., 2019). The hypothesis tested in the present study was that directly added enzymes degrade complex molecules into carbohydrates that can be easily digested by the larvae, in a shorter time than fungal pre-treatment and without consuming the substrate. To our knowledge, enzyme pre-treatment prior to BSFL composting has not been studied previously. However, previous studies have examined enzyme pre-treatment prior to other biological waste treatments, particularly anaerobic digestion. Total concentration of enzymes in the substrate, duration of enzyme hydrolysis and treatment temperature are key aspects of process efficiency evaluated in previous studies on enzymatic hydrolysis, e.g. of sawdust (Baksi et al., 2019) and municipal waste (Izaguirre et al., 2019). Baksi et al. (2019) investigated two different concentrations of enzymes added to sawdust and used the final concentration of glucose as an indication of how well the enzymes degraded the sawdust. They found that 2.56% and 26.5% enzymes in the substrate generated a solution with a glucose concentration of 15 g/L and 17–21 g/L, respectively, after 10–20 h. They also found that delignification was 25% at 50 °C and 29–33% at 100 °C (Baksi et al., 2019). Using 13.5% addition of enzymes to municipal waste for 48 h at 50 °C, Izaguirre et al. (2019) achieved a glucose concentration of 19 g/L. Moreno et al. (2021) added 0.08% enzyme blend (Cellic CTec2) to a solution with 5% dry weight of tomato plants at 50 °C and achieved a peak glucose concentration of 8 g/L at 24 h, after which the glucose concentration levelled off. However, all those studies were performed on a small scale and the samples were diluted with water to achieve a simple set-up for continuous mixing of the substrate. Plant-based biodegradable waste consists of >80% water, which is close to the critical water content if successful dry separation of larvae is wanted as a final step after BSFL composting (Cheng et al., 2017; Lalander et al., 2020). When using enzymatic hydrolysis as a pre-treatment in BSFL composting, diluting the substrate with water is therefore not advisable if the final products are to be dry separated. Fewer revolutions per minute due to lower substrate water content would also decrease the shearing force and spare the mixing tool. In compensation, longer duration could be applied in enzyme pre-treatment, to allow the enzymes to degrade complex molecules and make enough substrate easily available for the larvae.

In order to improve degradation of vegetable waste, often with a high content of cellulose, a mix of different enzymes can be expected to be more efficient than one specific enzyme, due to the complex and heterogeneous structure of cellulose (Teeri, 1997). Cellulolytic enzymes have endo- and exo- modes of action. Endoglucanase cleaves cellulosic bonds along the length of the cellulose chains, yielding progressively shorter chains that are as short as glucose monomers, and creating free chain ends on the cellulose surface that exoglucanases can use for further degradation (Teeri, 1997). Other enzymes involved in hydrolysis are glucanase, protease and lipase, which degrade complex sugars, proteins and lipids, respectively (Fernandes, 2010).

To our knowledge, there are no reported studies on the impact of enzyme pre-treatment duration on BSFL composting efficiency, but there have been studies on pre-treatment duration prior to anaerobic digestion (Arelli et al., 2020; Fisgativa et al., 2018). However, BSFL composting is rather dissimilar to anaerobic digestion and it cannot simply be assumed that efficiency in BSFL composting will improve after enzyme pre-treatment. The aim of this study was thus to assess the impact of enzyme pre-treatment duration on process efficiency in terms of BCE and material reduction in BSFL composting of lettuce and cabbage.

2. Materials and methods

2.1. Materials

Lettuces and cabbages used in the study were provided by the vegetable and fruit wholesaler Grönsakshallen Sorunda (Stockholm, Sweden). Upon arrival at the BSFL composting facility, the lettuces and cabbages were immediately minced (Universal Kross, model BG2, Austria), bagged separately and stored for up to 11 days at −18 °C before use.

The BSFL used in the study were taken from a BSF colony that has been run continuously by the Environmental Engineering group at the Department of Energy and Technology, SLU, since 2015 (Uppsala, Sweden). The BSFL used for treatment passed through a sieve with 1 mm mesh and had an average weight of 0.18 ± 0.09 g per 100 larvae.

2.2. Experimental set-up

The main aim of the study was to assess the impact of bio-chemical (enzyme) pre-treatment duration on process efficiency in BSFL composting of lettuce and cabbage, and it was expected that longer pre-treatment would generate more readily available carbohydrates for the larvae, and thus improve BCE (Baksi et al., 2019; Izaguirre et al., 2019). The substrate investigated was a 1:1 (wet weight) mixture of lettuce and cabbage, which was pre-treated with enzymes for 0, 2 and 4 days (treatments Enz-0d, Enz-2d and Enz4d, respectively). The BSFL VS load was similar in all treatments except Enz-2d, which had 9% higher BSFL VS load due to experimental inconsistencies, however still within the margin of error in empirical experiments (±10%) (Table 1).

2.2.1. Pre-treatments

An enzyme cocktail (SAE0020 Sigma-Aldrich), consisting of cellulases, ß-glucosidases and hemicellulases was added to the pre-treatment substrate to a concentration of 1% (w/w). During the pre-treatment, the substrate was placed in a bucket and a drill (SKIL 550 W 1020AA, United States) fixed on a stand and rotating on the lowest setting (100 revolutions per minute) was used to stir the material. The pre-treatment step was carried out in a tent at 28.8 ± 0.8 °C. There was a surplus of substrate in the pre-treatment, so the load of VS per larva was adjusted to

Table 1
List of pre-treatments used in each treatment, duration of pre-treatment and black soldier fly larvae (BSFL) composting, volatile solids (VS) load per larva and total amount of substrate on a wet weight (ww) basis added in each treatment. Values presented are mean ± SD (n = 3).

	Pre-treatment duration [days]	BSFL composting duration [days]	BSFL VS load [g VS/larva]	Total substrate [g ww/treatment]
Control	–	14	0.219 ± 0.0001	863 ± 0.4
Enz-0d	0	14	0.218 ± 0.0002	861 ± 0.7
Enz-2d	2	14	0.243 ± 0.00004	1225 ± 0.2
Enz-4d	4	14	0.220 ± 0.0002	1213 ± 1.0

0.2 g in the subsequent BSFL composting step.

2.2.2. BSFL composting

All treatments were performed in a growth tent with dimensions 120 cm × 120 cm × 200 cm (Secret Jardin, Hydro Shoot 120). A car heater connected to a temperature regulator (Trixie) and a fan for circulating the air were placed on the floor in the tent to keep an average temperature of 28.8 ± 0.8 °C over the course of the composting treatment. Each treatment was performed in triplicate. The treatment boxes (21 cm × 17 cm × 11 cm) were kept in a separate larger box placed in a rack, with 3 or 8 cm spacing between the larger boxes. Every second day, the boxes were rotated in the rack to erase effects of the temperature gradient (1 °C difference from bottom to top) and differences in air flow in the tent. The larval feeding load was set to 0.2 g VS/larva and the total amount of substrate allocated to each replicate was adjusted after completion of pre-treatment (Table 1). In each treatment, 300 larvae were added, giving a density of 1.5 larvae/cm^2 based on VS per larvae needed and on not exceeding 5 cm depth of substrate in the boxes. The larvae were fed on days 0, 3 and 6 of the treatment, by adding new substrate without stirring. BSFL composting was terminated 7 days after the last feeding (Table 1).

2.3. Sampling

The total amount of substrate was weighed before and after the pre-treatment step. After the BSFL composting treatment, larvae and residues were weighed together and then wet-separated by pouring into a sieve with 10 mm mesh and washing with water. The larvae were weighed and the total amount of residues was calculated by subtracting the larvae weight from the total weight before separation. The total number of larvae remaining at the end of the treatment was calculated by dividing the total weight of all larvae by the average larval weight. Material in each replicate was sampled in triplicate for analysis of total solids (TS) and VS.

2.3.1. Physical-chemical sampling

Samples for TS and VS analysis were taken from the substrate before the start of pre-treatment, before BSFL composting and after BSFL composting, at which point samples from both larvae and residues were collected. Three TS and VS samples were taken from each replicate and each sample contained substrate from five random areas in the treatment box (10–15 g per sample). The substrate removed for sampling was taken into account when calculating the feeding rate, BCE and material reduction.

2.4. Physical and chemical analysis

The TS content was determined by drying at 60 °C, to avoid evaporation of VS (Vahlberg et al., 2013), for a minimum of 48 h or until the weight of the substrate remained constant over two weighings. The VS content was determined by combusting at 250 °C for 2 h and at 550 °C for 4 h (modified ISO 18122:2015).

2.5. Calculations

The percentage waste-to-biomass conversion efficiency on a VS basis (BCE_{VS}) was calculated as:

$$BCE_{VS} = \left(\frac{mVS_{larvae}}{mVS_{initial}}\right)\cdot 100 \quad (1)$$

where, mVS_{larvae} and $mVS_{initial}$ is the total mass of VS of larvae and of initial substrate given to larvae, respectively. For BCE for the entire treatment, mVS_{larvae} and $mVS_{initial}$ is total mass of VS of larvae and of the initial material, respectively.

The percentage material reduction on a VS basis (Red_{VS}) for the entire treatment was calculated as:

$$Red_{VS} = \left(1 - \frac{mVS_{res}}{mVS_{initial}}\right)\cdot 100 \quad (2)$$

where, mVS_{res} and $mVS_{initial}$ is the total mass of VS of residues and of initial material, respectively. For material reduction after pre-treatment, mVS_{res} and $mVS_{initial}$ is the total mass of VS of pre-treatment residue and of the initial material, respectively. For material reduction after BSFL composting, mVS_{res} and $mVS_{initial}$ is the total mass of VS of residues and of the initial substrate given to larvae, respectively.

2.6. Statistical analysis

General linear regression with 95% confidence interval was used to assess correlations between response variables and substrate properties. To compare whether the different treatments gave statistically significant differences in BCE or material reduction, analysis of variance (ANOVA) with 95% confidence interval was used. Tukey's Honest Significant Difference (Tukey's HSD) was used when a significant difference was found. Normality was verified in the model residuals using the Shapiro-Wilk test. Paired *t*-test with 95% confidence interval was used for comparing TS and VS values in different stages in each treatment. All statistical tests and graphical representations were made in R (R Core Team, 2019).

3. Results

The initial substrate was similar in terms of TS and VS in all treatments (Table 2). However, the percentage TS and VS in the substrate changed significantly in Enz-2d, and the percentage TS in Enz-4d, after the enzyme pre-treatment.

The end-products from all BSFL treatments had similar TS and VS with the exception of Enz-0d, for which larval TS was somewhat higher than in the other treatments, while residue TS and VS were lower (Table 2). The residues were significantly different from the initial substrate and the substrate after pre-treatment in all cases, with lower amounts of TS and VS. The TS of larvae in the enzyme pre-treatment step decreased with pre-treatment duration, but only the difference between Enz-0d and Enz-4d was statistically significant.

Direct addition of enzymes (Enz-0d) resulted in 22% higher BCE_{VS} and 14% higher Red_{VS} on a VS basis compared with the control (Table 3, Fig. 1a-b). For BCE_{WW}, on the other hand, only Enz-2d differed significantly from the control, with 28% lower BCE_{WW}. The material reduction during the entire process (pre-treatment plus BSFL composting) was greater when the pre-treatment duration was longer, while the opposite was seen for the BSFL composting step, where a pre-treatment of 2 and 4 days gave a significantly lower material reduction compared with the control and direct enzyme addition. There was no significant difference in material reduction on a VS basis between the control and the pre-

Table 2
Total solids (TS) and total volatile solids (VS) content in initial substrate, substrate after pre-treatment, larvae and residues from black soldier fly larvae (BSFL) composting, and larval VS load during the treatment. Significant differences ($p < 0.05$) in TS and VS from initial substrate are denoted *. Different letters within columns indicate significant difference ($p < 0.05$). Values presented are mean ± SD (n = 9).

	Initial substrate		After pre-treatment		Larvae		Residues	
	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]
Control	8.6 ± 0.4^{a}	89.0 ± 0.5^{a}			$26.1 \pm 0.8^{*ab}$	$84.7 \pm 0.4^{*a}$	$3.9 \pm 0.8^{*a}$	$66.6 \pm 5.9^{*a}$
Enz-0d	8.6 ± 0.4^{a}	89.0 ± 0.5^{a}			$28.9 \pm 1.6^{*a}$	$86.8 \pm 0.3^{*b}$	$2.2 \pm 0.3^{*b}$	$54.0 \pm 1.0^{*b}$
Enz-2d	7.5 ± 0.2^{b}	88.7 ± 1.0^{a}	$8.0 \pm 0.1^{*a}$	88.7 ± 0.1^{a}	$27.1 \pm 0.7^{*ab}$	88.9 ± 0.2^{c}	$3.6 \pm 0.2^{*ab}$	$73.2 \pm 2.9^{*a}$
Enz-4d	7.5 ± 0.2^{b}	88.7 ± 1.0^{a}	7.4 ± 0.1^{b}	$85.9 \pm 0.1^{*b}$	$24.4 \pm 2.3^{*b}$	$88.5 \pm 0.5^{*c}$	$3.5 \pm 0.5^{*ab}$	$71.9 \pm 3.3^{*b}$

Table 3
Material reduction (Red) and biomass conversion efficiency (BCE) on a volatile solids (VS) and wet-weight (WW) basis during pre-treatment, black soldier fly larvae (BSFL) composting and the entire process. Values presented are mean ± SD (n = 3).

	Pre-treatment	BSFL composting		Entire process			
	Red_{VS} [%]	Red_{VS} [%]	BCE_{VS} [%]	Red_{VS} [%]	BCE_{VS} [%]	Red_{WW} [%]	BCE_{WW} [%]
Control		78.4 ± 0.3^{a}	19.7 ± 0.2^{a}	78.4 ± 0.3^{a}	19.7 ± 0.2^{a}	34.6 ± 11^{a}	6.8 ± 0.2^{a}
Enz-0d		89.4 ± 0.6^{b}	24.1 ± 0.2^{b}	89.4 ± 0.6^{b}	24.1 ± 0.2^{b}	33.1 ± 10^{a}	7.2 ± 0.5^{a}
Enz-2d	9.3^{a}	70.6 ± 4.6^{c}	20.2 ± 0.8^{a}	73.6 ± 4.1^{a}	18.2 ± 0.7^{a}	34.9 ± 3.4^{a}	4.9 ± 0.3^{b}
Enz-4d	14.8^{a}	69.0 ± 7.1^{c}	23.3 ± 1.9^{b}	74.6 ± 5.8^{a}	19.1 ± 1.5^{a}	33.8 ± 2.8^{a}	5.9 ± 1.0^{ab}

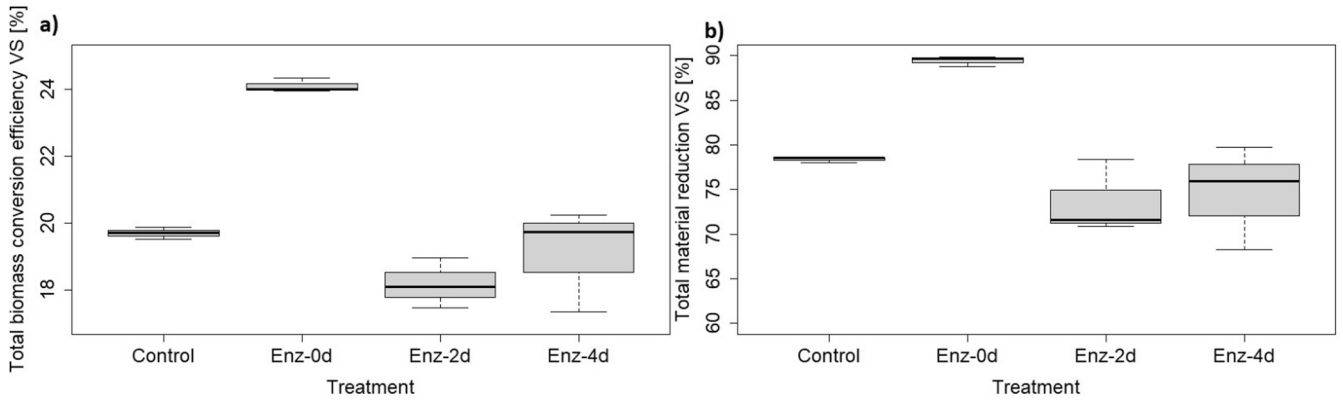

Fig. 1. Process efficiency parameters for the entire process on a total volatile solids (VS) basis in the control and after pre-treatment with directly added enzymes (Enz) for 0, 2 or 4 days: a) biomass conversion efficiency and b) material reduction.

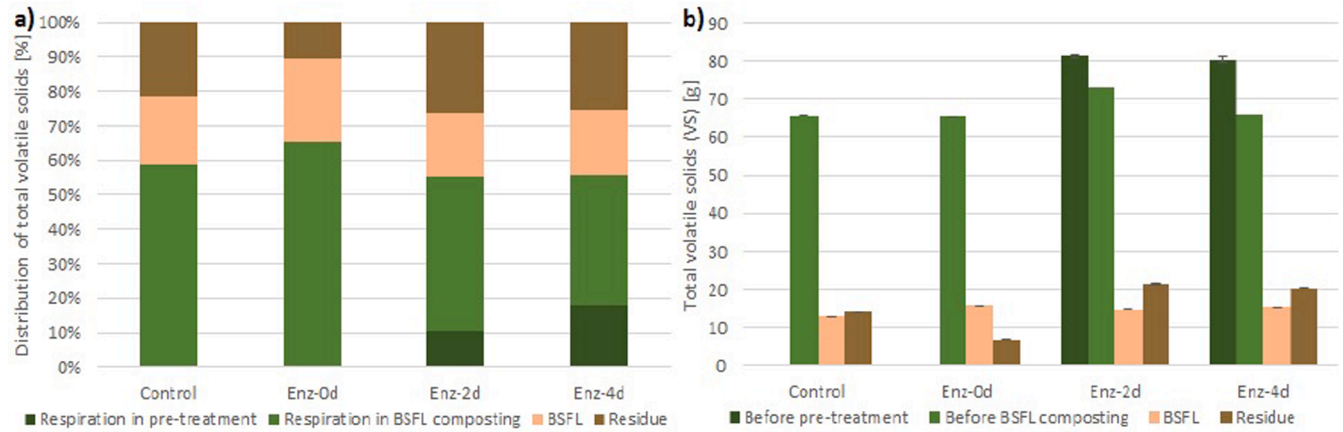

Fig. 2. Mass balance (volatile solids (VS) basis) during black soldier fly larvae (BSFL) composting of a mixture of cabbage and lettuce in the control and after pre-treatment with directly added enzymes (Enz) for 0, 2 or 4 days: a) distribution of initial VS during treatment divided into four fractions, respiration in pre-treatment (dark green), respiration in BSFL composting (light green), larvae (beige) and residues (brown); b) total VS content in substrate before pre-treatment (dark green), substrate before BSFL composting (light green) and in the end-products larvae (beige) and residues (brown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatments of 2 and 4 days.

The VS were distributed into four fractions: respiration during pre-treatment, respiration during BSFL composting, larvae and residues (Fig. 2a). Respiration of VS during pre-treatment and BSFL composting in all treatments ranged between 55 and 65 %, with Enz-0d having a larger proportion of VS as respiration than the other treatments. Enz-0d differed from the other treatments by yielding less residues and a larger mass of larvae from the initial VS. The amount of VS before BSFL composting was 66 g VS in all treatments except Enz-2d, which had 73 g VS (Fig. 2b). The pre-treatments with enzymes for 2 days and 4 days had similar mass of VS before pre-treatment, larvae and residues.

The mass of residues produced per unit mass of substrate was similar (±10%) in all treatments (Table 4). However, the mass of larvae produced was significantly lower in Enz-2d compared with the control and Enz-0d. Treatment Enz-0d produced the largest mass of larvae, but it was not significantly different on a wet-weight basis from neither the control nor Enz-4d.

4. Discussion

4.1. Impact on treatment efficiency of enzyme pre-treatment

Enzyme pre-treatment of different duration (0–4 days) resulted in different BCE and material reductions. The treatment with enzymes added with the larvae at the start of the BSFL composting (Enz-0d) resulted in 22% higher BCE and 14% higher material reduction compared with the control and the longer enzyme pre-treatments (Table 3). This contradicted the initial hypothesis that longer pre-treatment duration leads to more easily available substances for the larvae, resulting in increased BCE.

Previous studies with enzyme pre-treatment, although not before BSFL composting, have found that the efficiency of the subsequent treatment increases with longer pre-treatment duration (Baksi et al., 2019; Izaguirre et al., 2019; Moreno et al., 2021). Longer pre-treatment (48 h) of municipal waste in Izaguirre et al. (2019) achieved the highest glucose concentration (19 g/L) seen in all previous studies. Degree of hydrolysis reached 49% in that enzyme pre-treatment, compared to ~5% in a control with no added enzymes (Izaguirre et al., 2019). However, after a sugar concentration peak, longer pre-treatment did not give an additional increase in any of the studies (Baksi et al., 2019; Izaguirre et al., 2019; Moreno et al., 2021). In those studies, the structure of the substrate after enzymatic hydrolysis was not important and the residues were intended for use in extraction of bioplastics (Izaguirre et al., 2019) and biofuels (Baksi et al., 2019; Moreno et al., 2021). The structure of the substrate is important in BSFL composting, as it enables aeration so that the larvae can actively move through the substrate without respiration through their spiracles being hindered (Barros et al., 2019). Longer pre-treatment led to visibly reduced structure in the substrate in the present study, which could explain why BCE did not improve as hypothesised. However, it does not explain why BCE and material reduction were higher in the treatment with directly added enzymes (Enz-0d) compared with the control. Hou et al. (2021) found that the polysaccharide concentration only increases during the first 12 h of enzyme pre-treatment and since only protease is added, and not cellulose, the protease may have promoted microbial degradation of polysaccharides. An increase in polysaccharides during the first 12 h could be one reason why longer pre-treatment did not generate higher BCE or material reduction in the present study, while immediate enzyme addition (Enz-0d) increased BCE and material reduction in comparison with the control.

Another reason why longer pre-treatment did not increase BCE further could be that the total VS in the substrate was reduced during pre-treatment, suggesting that the microorganisms consumed monomers produced (Table 3). The control had lower BCE than treatment Enz-4d for the BSFL composting step, but not for the entire process, which was likely an effect of the material reduction in the pre-treatment step. Treatment Enz-4d had lower VS (86%) than Enz-0d (89%), so even if carbohydrates were used to the same degree in both treatments, there was less left for the larvae to assimilate into their biomass in Enz-4d.

The presumed higher activity of microorganisms during enzymatic pre-treatment compared with that found in previous studies (Baksi et al., 2019; Izaguirre et al., 2019; Moreno et al., 2021), could be a result of differences in temperature during the pre-treatment step. In this study, hydrolysis was performed at 28 °C, while it was performed at 50 °C or higher in the other studies. Microorganisms in food are usually mesophilic and their temperature range for growth is 20–45 °C (Keenleyside, 2019), while the activity of enzymes, as biological catalysts, increases with temperature up to 67 °C (Peterson et al., 2007). During BSFL composting, the temperature in the substrate increases (Johannesdottir, 2017; Parodi et al., 2020). Further, Paz et al. (2015) found that higher larval density results in a temperature increase of 1.5 °C in the substrate. This could be a reason why longer pre-treatment at 28 °C with mixing gave no advantage over direct use of enzymes before BSFL composting, as larval movements might be more efficient in mixing the substrate than the mixing tool used here (electric drill), thus increasing the enzyme activity. It would be interesting to test whether pre-treatment with enzymes at 50 °C can inhibit microbial degradation, increase enzyme activity and promote larval growth in the BSFL composting step.

4.1.1. Impact of larval VS load

The VS content after pre-treatment was significantly lower than in the initial substrate in Enz-4d, but not in Enz-2d (Table 2). This decrease in VS can be related to microbial respiration during the pre-treatment step. The VS composition was not evaluated in this study, but the results indicate that the microorganisms present in the substrate consumed easily available nutrients, leaving more complex molecules for the larvae, counteracting the purpose of the enzyme pre-treatment. Treatment Enz-2d had the lowest BCE in the study, possibly due to the slightly higher VS load (0.24 g VS/larva) (Table 2). Paz et al. (2015) found that a feeding rate of 200 mg/larva/day resulted in lower relative growth of the larvae than a lower feeding rate (60 mg/larva/day), but obtained the highest relative larval growth at 130 mg/larva/day. The impact of the feeding rate was especially pronounced at the highest larval density tested in that study (6 larvae/cm^2), while at the lowest larval density (2 larvae/cm^2), the reduction in relative larval growth on increasing the feeding rate from 130 to 200 mg/larva/day (54% increase) was very

Table 4
Estimated production of pre-treated substrate, larvae and residues from 1 ton wet weight (WW) initial substrate in black soldier fly larvae (BSFL) composting. Values presented are mean ± SD (n = 3). Different letters within columns indicate significant difference ($p < 0.05$) in substrate after pre-treatment, larvae and residues.

	1 ton substrate, wet weight basis						
	Substrate	After pre-treatment	BSFL		Residues		
	Protein [kg]	Mass [kg]	Mass [kg]	Change to control [%]	Mass [kg]	Change to control [%]	Water content [%]
Control	9.7[i]		67.8 ± 2.4[a]	0	654 ± 107[a]	0	96.1 ± 1.0
Enz-0d	9.7[i]		72.3 ± 4.6[a]	6.6	669 ± 103[a]	2.3	97.8 ± 0.3
Enz-2d	9.7[i]	822[a]	48.7 ± 2.6[b]	−28	651 ± 28[a]	−0.5	96.4 ± 0.2
Enz-4d	9.7[i]	856[b]	59.2 ± 8.2[ab]	−13	662 ± 23[a]	1.3	96.5 ± 0.4

[i]Theoretically calculated based on data from Livsmedelsverket (2021)

small (Paz et al., 2015). The larval density in the present study was 1.5 larvae/cm^2, so the 9% higher larval feeding rate in Enz-2d likely did not have a significant impact on the results.

4.1.2. Impact of nutritional composition of the substrate on BCE

Treatment Enz-0d yielded the highest BCE, followed by the control. The value obtained was similar to that reported for vegetable canteen waste and combined waste by Gold et al. (2020) (Table 5). The carbon/nitrogen (C/N) ratio is similar for mixed lettuce and cabbage, vegetable canteen waste and combined waste substrates (Table 5), however it is not known how the enzyme pre-treatment affected this ratio.

Isibika et al. (2019) compared two 14-day pre-treatments with fungi (*Rhizopus oligosporus* and *Trichoderma reesei*), both of which led to significant improvement (61–100% increase) in BCE compared with non-pre-treated banana peel (Table 5). The fibre content was reduced in both pre-treatments, but in particular in the *Trichoderma reesei* pre-treatment (42% reduction). In the present study, the BCE improvement was 22% on a VS basis (Table 5), but the fibre content of the substrate (lettuce-cabbage) was considerably lower than that of the banana peel used by Isibika et al. (2019) (20% compared with 70%). This suggests that enzyme pre-treatment could result in a larger difference in BCE compared with the control when using substrate with a higher fibre content. It also suggests that enzyme pre-treatment is preferable to fungi pre-treatment when using substrates with a low amount of fibre, since the enzymes do not consume what they degrade. However, fungi pre-treatment can add other benefits in terms of production of different enzymes and in distribution of the enzymes throughout the substrate. Isibika et al. (2019) found that the C/N ratio and protein to carbohydrate (Pt/CH) ratio changed in the fungi pre-treatments. It is known that substrate composition greatly affects BCE (Table 5). In a study by Gold et al. (2020), BSFL composting with vegetable canteen waste with similar C/N ratio, but lower Pt/CH ratio and fibre content than the substrate used in this study, gave BCE of 23%. Higher feeding rate in that study had no effect on BCE, indicating that Pt/CH ratio and fibre content are of higher importance. According to Gold et al. (2020), a Pt/CH ratio of 1:1 is optimal for achieving high BCE in BSFL composting. As concluded in other studies (Beniers and Graham, 2019; Gold et al., 2020; Lalander et al., 2019), high content of protein and carbohydrates, together with a low content of fibre, increases the process efficiency. The combined substrate in Gold et al. (2020), containing vegetable canteen waste, had a higher Pt/CH ratio and fibre content than the treatment with only vegetable canteen waste, and resulted in lower BCE. This suggests that Pt/CH ratio close to 1 is not enough to improve BCE if the fibre content is too high. As the fibre content was not analysed in the present study, but theoretically calculated, it is not known how much the enzymes reduced the fibre content. However, the results suggest that enzyme pre-treatment converts fibre into carbohydrates, which results in increased BCE.

5. Conclusions

The hypothesis that enzyme pre-treatment increases readily available carbohydrates was confirmed in this study, while the hypothesis that longer pre-treatment duration increases treatment efficiency was rejected. Mixed lettuce and cabbage waste pre-treated with enzymes added directly with the larvae improved BCE_{VS} by 22% compared with the control. On a wet-weight basis, BCE and material reduction were not significantly different between the control and Enz-0d. Longer pre-treatment with enzymes appeared to improve the environment for microorganisms, which likely consumed the most easily available carbohydrates. This counteracted the purpose of enzyme pre-treatment, which was to increase BCE in the BSFL composting step. Enzyme pre-treatment is likely to improve BCE in BSFL composting of fibre-rich materials. Further studies are needed to determine whether longer pre-treatment is needed for substrates higher in fibre and for substrates with high TS content.

Table 5
Substrate carbon to nitrogen ratio (C/N), protein to carbohydrate ratio (Pt/CH) and fibre content in relation to biomass conversion efficiency (BCE) in different studies. TS = total solids, DM = dry matter.

	C/N	Pt/CH	Fibre [% of TS]	Feed rate [mg DM/larva per day]	BCE_{TS}	Reference
Lettuce and cabbage, control	27.4[ii]	0.3[ii]	19.9 [ii]	18	20.7	This study
Lettuce and cabbage, Enz-0d				18	24.7	This study
Banana peel	53.8	0.4	68.6	40	7.2[i]	Isibika et al. (2019)
Banana peel $Rhiz_{14d}$	49.3	0.03	61.9	40	15[i]	Isibika et al. (2019)
Banana peel $Trich_{14d}$	83.2	0.2	40.8	40	11.6[i]	Isibika et al. (2019)
Mill by-products (1)	22.5	0.6	51.7	27	14.9	Gold et al. (2020)
Canteen waste (2)	10.0	4.3	36.2	27	15.3	Gold et al. (2020)
Cow manure (3)	25.2	6.2	58.4	27	3.8	Gold et al. (2020)
Vegetable canteen waste (4)	25.9	0.8	31.5	27	22.7	Gold et al. (2020)
Combined (1–4)	22.1	1.1	48.7	27	20.9	Gold et al. (2020)

[i]BCE on a VS basis.
[ii]Theoretically calculated based on data from (Livsmedelsverket, 2021).

Declaration of Competing Interest

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

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References

Arelli, V., Juntupally, S., Begum, S., Anupoju, G.R., 2020. Significance of pretreatment in enhancing the performance of dry anaerobic digestion of food waste: an insight on full scale implementation strategy with theoretical analogy. Processes 8 (9), 1018. https://doi.org/10.3390/pr8091018.

Baksi, S., Sarkar, U., Saha, S., Ball, A.K., Chandra Kuniyal, J., Wentzel, A., Birgen, C., Preisig, H.A., Wittgens, B., Markussen, S., 2019. Studies on delignification and inhibitory enzyme kinetics of alkaline peroxide pre-treated pine and deodar saw dust. Chem. Eng. Process. 143, 107607. https://doi.org/10.1016/j.cep.2019.107607.

Barros, L.M., Gutjahr, A.L.N., Ferreira- Keppler, R.L., Martins, R.T., 2019. Morphological description of the immature stages of *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). Microsc. Res. Tech. 82 (3), 178–189.

Beesigamukama, D., Mochoge, B., Korir, N., Musyoka, M.W., Fiaboe, K.K.M., Nakimbugwe, D., Khamis, F.M., Subramanian, S., Dubois, T., Ekesi, S., Tanga, C.M., 2020. Nitrogen fertilizer equivalence of black soldier fly frass fertilizer and synchrony of nitrogen mineralization for maize production. Agronomy-Basel 10.

Beniers, J.J.A., Graham, R.I., 2019. Effect of protein and carbohydrate feed concentrations on the growth and composition of black soldier fly (*Hermetia illucens*) larvae. J. Insects Food Feed 5 (3), 193–199.

Cai, M., Zhang, K.e., Zhong, W., Liu, N., Wu, X., Li, W.u., Zheng, L., Yu, Z., Zhang, J., 2019. Bioconversion-composting of golden needle mushroom (*Flammulina velutipes*)

root waste by black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) larvae, to obtain added-value biomass and fertilizer. Waste Biomass Valori 10 (2), 265–273.
Cheng, J.Y.K., Chiu, S.L.H., Lo, I.M.C., 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste Manage. 67, 315–323.
Chiam, Z., Lee, J.T.E., Tan, J.K.N., Song, S., Arora, S., Tong, Y.W., Tan, H.T.W., 2021. Evaluating the potential of okara-derived black soldier fly larval frass as a soil amendment. J. Environ. Manage. 286, 112163. https://doi.org/10.1016/j.jenvman.2021.112163.
Čičková, H., Newton, G.L., Lacy, R.C., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Manage. 35, 68–80.
European Union, 2016. Combating Food Waste: an opportunity for the EU to improve the resource-efficiency of the food supply chain, Luxembourg, p. 74.
Fernandes, P., 2010. Enzymes in food processing: a condensed overview on strategies for better biocatalysts. Enzyme Res 2010, 1–19.
Fisgativa, H., Tremier, A., Saoudi, M., Le Roux, S., Dabert, P., 2018. Biochemical and microbial changes reveal how aerobic pre-treatment impacts anaerobic biodegradability of food waste. Waste Manage. 80, 119–129.
Godliving, Y.S.M., 2009. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. Afr. J. Biotechnol. 8, 1398–1415.
Gold, M., Cassar, C.M., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S., Mathys, A., 2020. Biowaste treatment with black soldier fly larvae: increasing performance through the formulation of biowastes based on protein and carbohydrates. Waste Manage. 102, 319–329.
Gold, M., Tomberlin, J.K., Diener, S., Zurbrügg, C., Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. Waste Manage. 82, 302–318.
Hankin, L., Anagnostakis, S.L., 1975. The use of solid media for detection of enzyme production by fungi. Mycologia 67 (3), 597–607.
Hou, J., Liu, W., Hu, W., Chen, J., Wang, J., Li, P., Li, Y., 2021. Isolation, production and optimization of endogenous alkaline protease from in-situ sludge and its evaluation as sludge hydrolysis enhancer. Water Sci. Technol. 83, 2700–2713.
Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C., Lalander, C., 2019. Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. Waste Manage. 100, 151–160.
Izaguirre, J.K., da Fonseca, M.M.R., Fernandes, P., Villarán, M.C., Castañón, S., Cesário, M.T., 2019. Upgrading the organic fraction of municipal solid waste to poly (3-hydroxybutyrate). Bioresour. Technol. 290, 121785. https://doi.org/10.1016/j.biortech.2019.121785.
Johannesdottir, S., 2017. Scaling-up of fly larvae composting treatment: Aeration need and ventilation, Disciplinary Domain of Science and Technology, Earth Sciences, Department of Earth Sciences, Department of Energy and Technology. Uppsala University and Swedish University of Agricultural Sciences, Uppsala.
Keenleyside, W., 2019. 9.6 Temperature and microbial growth. In: Keenleyside, W. (Ed.), Microbiology: Canadian Edition. Pressbooks.
Kumar, S., Negi, S., Mandpe, A., Singh, R.V., Hussain, A., 2018. Rapid composting techniques in Indian context and utilization of black soldier fly for enhanced decomposition of biodegradable wastes - A comprehensive review. J. Environ. Manage. 227, 189–199.
Lalander, C., Diener, S., Zurbrügg, C., Vinnerås, B., 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). J. Clean. Prod. 208, 211–219.
Lalander, C., Ermolaev, E., Wiklicky, V., Vinnerås, B., 2020. Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. Sci. Total Environ. 729.
Lalander, C.H., Fidjeland, J., Diener, S., Eriksson, S., Vinnerås, B., 2015. High waste-to-biomass conversion and efficient *Salmonella spp.* reduction using black soldier fly for waste recycling. Agron. Sustain. Dev. 35 (1), 261–271.
Livsmedelsverket, 2021. Livsmedelsverkets livsmedelsdatabas, Livsmedelsverket.se.
Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agr. 98 (15), 5776–5784.
Moreno, A.D., Duque, A., González, A., Ballesteros, I., Negro, M.J., 2021. Valorization of greenhouse horticulture waste from a biorefinery perspective. Foods 10 (4), 814. https://doi.org/10.3390/foods10040814.
Nguyen, T.T.X., Tomberlin, J.K., Vanlaerhoven, S., 2015. Ability of black soldier fly (diptera: stratiomyidae) larvae to recycle food waste. Environ. Entomol. 44 (2), 406–410.
Nyakeri, E.M., Ogola, H.J.O., Ayieko, M.A., Amimo, F.A., 2017. Valorisation of organic waste material: growth performance of wild black soldier fly larvae (*Hermetia illucens*) reared on different organic wastes. J. Insects Food Feed 3 (3), 193–202.
Parodi, A., De Boer, I.J.M., Gerrits, W.J.J., Van Loon, J.J.A., Heetkamp, M.J.W., Van Schelt, J., Bolhuis, J.E., Van Zanten, H.H.E., 2020. Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing - A mass balance approach. J. Clean. Prod. 271, 122488. https://doi.org/10.1016/j.jclepro.2020.122488.
Parra Paz, A.S., Carrejo, N.S., Gómez Rodríguez, C.H., 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.), (Diptera: Stratiomyidae). Waste Biomass Valori. 6 (6), 1059–1065.
Peterson, M.E., Daniel, R.M., Danson, M.J., Eisenthal, R., 2007. The dependence of enzyme activity on temperature: determination and validation of parameters. Biochem. J. 402, 331–337.
R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Sánchez, C., 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol. Adv. 27 (2), 185–194.
Schumann, U., Smith, N.A., Wang, M.-B., 2013. A fast and efficient method for preparation of high-quality RNA from fungal mycelia. BMC Res. Notes 6, 71.
Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M.A., Ross, C., St-Hilaire, S., 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquacult. Soc. 42, 34–45.
Sigoillot, J.C., Berrin, J.G., Bey, M., Lesage-Meessen, L., Levasseur, A., Lomascolo, A., Record, E., Uzan-Boukhris, E., 2012. Fungal strategies for lignin degradation. Adv. Bot. Res. 61, 263–308.
Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R., Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renewable Energy 98, 197–202.
Tadele, Y., Amha, N., 2015. Use of different non protein nitrogen sources in ruminant nutrition: a review. International knowledge sharing plattform IISTE. Adv. Life Sci. Technol. 26.
Tan, J.K.N., Lee, J.T.E., Chiam, Z., Song, S., Arora, S., Tong, Y.W., Tan, H.T.W., 2021. Applications of food waste-derived black soldier fly larval frass as incorporated compost, side-dress fertilizer and frass-tea drench for soilless cultivation of leafy vegetables in biochar-based growing media. Waste Manage. 130, 155–166.
Teeri, T.T., 1997. Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. Trends Biotechnol. 15 (5), 160–167.
Tomberlin, J.K., van Huis, A., Benbow, M.E., Jordan, H., Astuti, D.A., Azzollini, D., Banks, I., Bava, V., Borgemeister, C., Cammack, J.A., Chapkin, R.S., Čičková, H., Crippen, T.L., Day, A., Dicke, M., Drew, D.J.W., Emhart, C., Epstein, M., Finke, M., Fischer, C.H., Gatlin, D., Grabowski, N.T., He, C., Heckman, L., Hubert, A., Jacobs, J., Josephs, J., Khanal, S.K., Kleinfinger, J.-F., Klein, G., Leach, C., Liu, Y., Newton, G.L., Olivier, R., Pechal, J.L., Picard, C.J., Rojo, S., Roncarati, A., Sheppard, C., Tarone, A.M., Verstappen, B., Vickerson, A., Yang, H., Yen, A.L., Yu, Z., Zhang, J., Zheng, L., 2015. Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. Insect. Food. Feed 1 (4), 307–309.
Vahlberg, C., Nordell, E., Wiberg, L., Schnürer, A., 2013. Method for correction of VFA loss in determination of dry matter in biomass. Svenskt Gastekniskt Center AB, Malmö, Sweden, p. 53.