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Research Paper

Dynamics of black soldier fly larvae composting – Impact of substrate properties and rearing conditions on process efficiency

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ABSTRACT

Inadequate organic waste management have detrimental impact on the environment and on public health. Black soldier fly (BSF) larvae composting is a biological treatment for biodegradable waste that align with circular economy principles. The bioconversion efficiency of bio-waste into larval biomass is influenced by various factors, such as substrate type and the process parameters employed in the larval rearing process. In this study, the influence of these parameters on survival, material reduction (Mat.Red), waste-to-biomass conversion efficiency (BCE) and larval yield per rearing unit was investigated through two sets of experiments. In Experiment 1, the impact of larval density in five distinct rearing substrates was evaluated, while the effect of larval feed dose and substrate depth was assessed in Experiment 2, using a model substrate (dog food). In Experiment 1 it was found that higher larval density lead to an increase in BCE and larval yield, up to a threshold (around 6.25 larvae cm^{-2}). Surpassing this threshold led to the production of smaller larvae, while the yield remained relatively consistent. In Experiment 2 it was found that supplying the substrate in a shallow layer (1–1.5 cm depth) and providing a low feed dose (0.1 g volatile solids (VS) $larva^{-1}$) led to higher BCE and Mat.Red, albeit with a reduced overall yield per unit. Increasing feed load and substrate depth reduced the conversion efficiency, Mat.Red and larval survival. This study enhances the understanding of the effect of various process parameters used in the BSF larvae treatment, and how they interrelate.

1. Introduction

There is a growing interest worldwide in bioconversion of biodegradable waste using black soldier fly (*Hermetia illucens*, BSF) larvae (van Huis, 2020). BSF can play a key role in helping societies efficiently handle bio-waste through enabling an effective and low-impact transformation of it into useful products, namely animal feed (fly larvae) (Lu et al., 2022) and organic fertiliser (treatment residue called frass) (Lopes et al., 2022).

Regulations governing insect rearing have been developed in different countries, but these vary widely in terms of the type of substrate that can be fed to the insects and how production and processing of insects can be done (Lähteenmäki-Uutela et al., 2021). In order to achieve high waste-to-biomass conversion efficiency (BCE), multiple variables known to have significant impact on the larval conversion process and process outcomes must be taken into account. For instance, temperature is a critical factor for the process, with higher temperature (up to around 30–37 °C depending on the substrate) increasing BCE (Chia et al., 2018). The nutritional composition of the substrate is another critical factor, with a major impact on larval development time, process efficiency and larval composition (Hopkins et al., 2021; Lalander et al., 2019; Seyedalmoosavi et al., 2022). The moisture content of the substrate fed to the larvae also has a strong impact, with an increase in moisture content leading to reduced BCE (Lalander et al., 2020) and more complicated separation of larvae from frass (Cheng et al., 2017). In addition, the scale at which larval production is performed brings multiple challenges to the process, meaning that the results from laboratory-scale experiments are not easily transferable to realistic set-ups or industrial-scale production (Yang and Tomberlin, 2020). In all cases, a more controlled process will lead to fewer challenges (Ribeiro et al., 2022). This means that the effects of process parameters, either alone or in combination, must be determined in order to enable better control of the bioconversion process as a whole and identify areas for further improvements.

One of the parameters affecting bioconversion is larval density (defined as larvae cm^{-2}), which generates a response similar to that seen

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for other livestock, *i.e.* the higher the density, the lower the individual growth (Opare et al., 2022). Larval feed dose (amount of feed substrate provided per larva) also affects bioconversion to a large extent, however different feed substrates provided at similar doses may not result in similar BCE and larval growth (Lalander et al., 2019). A parameter that has been less well studied is the depth of substrate provided for the larvae. Many studies report the feed dose used (often given as feed rate in mg larva^{-1} day^{-1}), larval density and surface area (cm^2) on which BSF larvae (BSFL) were reared, but few mention how deep the substrate in the rearing units were. However, there is evidence that substrate depth plays a significant role for how larvae interact with the substrate and utilise it. For instance, Dortmans et al. (2017), suggested that depths greater than 5 cm may lead to substrate at the bottom layer not being processed. Another parameter that can influence the efficiency of BSF treatment as a whole is larval survival. Efficient utilisation of added larvae is economically desirable, as overproduction of these larvae due to low survival involves extra cost, so an efficient process should aim to use the minimum number of larvae for the highest yield.

Parameters known to affect BCE are investigated separately in most previous studies, even though it is clear that the impact of larval density, larval feed dose and substrate depth are closely interconnected and that interactive effects should be taken into account. In this study, the impact of larval feed dose, substrate depth and larval density, alone and in combination, on BCE, material reduction (Mat.Red), larval survival and total yield per rearing unit was investigated. The study's hypothesis was that the substrate provided to BSFL influences larval growth and process efficiency through factors other than solely its nutritional quality.

2. Materials and methods

2.1. Larvae and feed substrates

The BSFL used in the study were obtained from a BSF colony that has been running continuously at the Swedish University of Agricultural Sciences (SLU Uppsala, Sweden) since 2015. Newly-hatched larvae were kept on substrate comprising chicken feed and water (30% dry matter, DM) for 5–7 days and then passively separated by sieving (1 mm mesh) and five batches of 100 larvae were counted and weighed for estimation of average larval weight. The average larval weights of larvae added to the treatments were 1.8 mg larva^{-1} in Experiment 1 and 2.5 mg larva^{-1} in Experiment 2.

Six distinct bio-waste substrates were used in this study (food waste, chicken feed, cow feed, bread, bread with vegetables and dog food). Minced source-segregated household food waste was obtained from a local municipal waste treatment plant (Eskilstuna och Strängnäs Energi och Miljö AB). Dry broiler feed (Granngården Hönsfoder Bas with metabolisable energy content of 10.9 MJ kg^{-1}) was dissolved in water to a predetermined moisture content. Dry cow feed (calf feed obtained from SLU's research station Lövsta, Sweden) was dissolved in water to a predetermined moisture content. A mixture of reclaimed bread from local grocery stores was obtained from Fazer (Uppsala, Sweden). The vegetables used as substrate were lettuce (50%) and cabbage (50%) obtained from the vegetable and fruit wholesaler Grönsakshallen Sorunda (Stockholm, Sweden). The vegetables were minced upon arrival (Univeral Kross, model BG2, Austria) and frozen at −18 °C until use. Dry dog food (Purina Pro Plan puppy, metabolisable energy content of 16.0 MJ kg^{-1}) was dissolved in water to a predetermined moisture content.

2.2. Experimental design

Two separate experiments were performed. Experiment 1 evaluated the impact of larval density on process efficiency and larval survival on five different feed substrates: bread (Br), 1:1 bread:vegetables (BrV), chicken feed (CF), cow feed (CoF) and food waste (FW). A factorial scheme was designed with three larval densities (4.16, 6.25 and 8.3 larvae cm^{-2}, equal to 10,000, 15,000 and 20,000 larvae per experimental unit), five feed substrates (described above) and three replicates, in 45 rearing units (Table 1). Substrate was provided on three occasions, on rearing day 1, 4 and 7. The experiment continued until the substrate was sufficiently dry to be sieved or until the first prepupae started to appear in the rearing units. The rearing units used were plastic crates with dimensions 40 cm × 60 cm × 12 cm (total surface area 2400 cm^2). The feed dose (wet weight basis) provided in each unit was the same for all feed substrates at each distinct larval density, and as a consequence the larval feed dose varied between the treatments of different densities and feed substrate. A second experiment (Experiment 2), was conducted to evaluate the effects of feed dose and substrate depth on process parameters, using a model substrate with constant nutritional value (dog food), in order to eliminate possible differences in the substrate that could affect the conversion performance (Table 1). Four treatments were designed for investigating the impact of substrate depth and TS: low depth/high TS (1 cm deep; TS 44%); low depth (1.5 cm deep; TS 30%); medium depth (3 cm deep; TS 27%) and deep (6.5 cm deep; TS 20%); in all these, the feed dose was kept at 0.2 g volatile solids (VS) per larva. In order to maintain this feed dose, different number of larvae had to be added in the different depth treatments. Four treatments were run to investigate the impact of feed dose: low (0.1 g VS per larva; depth 1.1 cm); medium (0.2 g VS per larva; depth 2.3 cm), high (0.4 g VS per larva; depth 4.5 cm) and very high (0.5 g VS per larva; depth 5.7 cm); in all these, the TS of the substrate was kept at 20% and 700 larvae were added to each rearing unit (Table 1).

Smaller plastic containers were used in Experiment 2 (21 cm × 11 cm × 17 cm, surface area 231 cm^{-2}). Each treatment was performed in triplicate rearing units with two exceptions (high feed dose, very high feed dose) (Table 1), due to high mortality in one replicate of each of these treatments.

2.3. Sample collection and calculations

At the end of the treatment, larvae were separated from the treatment residues (frass) by sieving. In Experiment 1, the frass was sufficiently dry for sieving using a vibration table fitted with a grid with 4 mm mesh size. In Experiment 2, the frass was in some cases quite wet and the larvae were separated out manually. Samples (10–30 g) of inflow substrate, larvae and frass were collected from each rearing unit for determination of TS content, by drying the samples at 70 °C to constant weight, and VS content, by combusting the dried samples at 550 °C for 4 h.

Larval survival was calculated by dividing the number of larvae at the end of the experiments by the initial number of larvae (Gold et al., 2020). The Mat.Red was calculated as:

$$\text{Mat.Red} = \left(1 - \frac{m_{\text{frass}}}{m_{\text{initial}}}\right) \times 100 \quad (1)$$

where m_{frass} and m_{initial} is total mass of frass and initial substrate, respectively.

Waste-to-biomass conversion efficiency (BCE) of substrate in both experiments was calculated as:

$$\text{BCE} = \left(\frac{m_{\text{lv}}}{m_{\text{initial}}}\right) \times 100 \quad (2)$$

where m_{lv} and m_{initial} is total mass of larvae and initial substrate, respectively.

2.4. Statistical analysis

All data were analysed for normality of distribution (Shapiro-Wilks's test) and homoscedasticity of variance (Levene's test). In Experiment 1, which involved a 5 × 3 × 3 factorial design, two-way analysis of variance (ANOVA) was performed, with feed substrate and larval density as factors, to evaluate the effects of each factor and their interaction on

Table 1
Descriptionof treatments in Experiment 1 and Experiment 2: substrate tested, its total solids (TS) content, feed dose, substrate depth inside the rearing unit, larval density and total of number of larvae added per treatment unit.

	Treatment name[a]	TS (%)	Feed dose (g VS lv^{-1})	Depth (cm)	Larval density[b] (lv cm^{-2})	Tot # larvae[c] (lv $unit^{-1}$)
Experiment 1						
Chicken feed (n = 3)	CF4.16–8.33	33	0.22–0.44	5.62	4.16–8.33	10,000–20,000
Food waste (n = 3)	FW4.16–8.33	20	0.12–0.24	5.62	4.16–8.33	10,000–20,000
Cow feed (n = 3)	CoF4.16–8.33	33	0.22–0.44	5.62	4.16–8.33	10,000–20,000
Bread (n = 3)	Br4.16–8.33	30	0.15–0.31	4.10	4.16–8.33	10,000–20,000
Bread & vegetables (n = 3)	BrV4.16–8.33	28	0.16–0.33	5.30	4.16–8.33	10,000–20,000
Experiment 2						
Low depth/high TS (n = 3)	TS44%,FD0.2,D1.0	44	0.2	1.0	3.03	700
Low depth (n = 3)	TS30%,FD0.2,D1.5	30	0.2	1.5	3.03	700
Medium depth (n = 3)	TS27%,FD0.2,D3	27	0.2	3.0	4.32	1000
Deep (n = 3)	TS20%,FD0.2,D6.5	20	0.2	6.5	8.65	2000
Low feed dose (n = 3)	TS20%,FD0.1,D1.1	20	0.1	1.1	3.03	700
Medium feed dose (n = 3)	TS20%,FD0.2,D2.3	20	0.2	2.3	3.03	700
High feed dose (n = 2)	TS20%,FD0.4,D4.5	20	0.4	4.5	3.03	700
Very high feed dose (n = 2)	TS20%,FD0.5,D5.7	20	0.5	5.7	3.03	700

[a] *TS*: total solids; *lv*: larvae; *FD*: feeding dose; *D*: depth.
[b] Treatments in Experiment 1 had larval density 4.16, 6.25 or 8.33 larvae cm^{-2}.
[c] The total number of larvae added per unit in Experiment 1 was 10,000, 15,000 and 20,000.

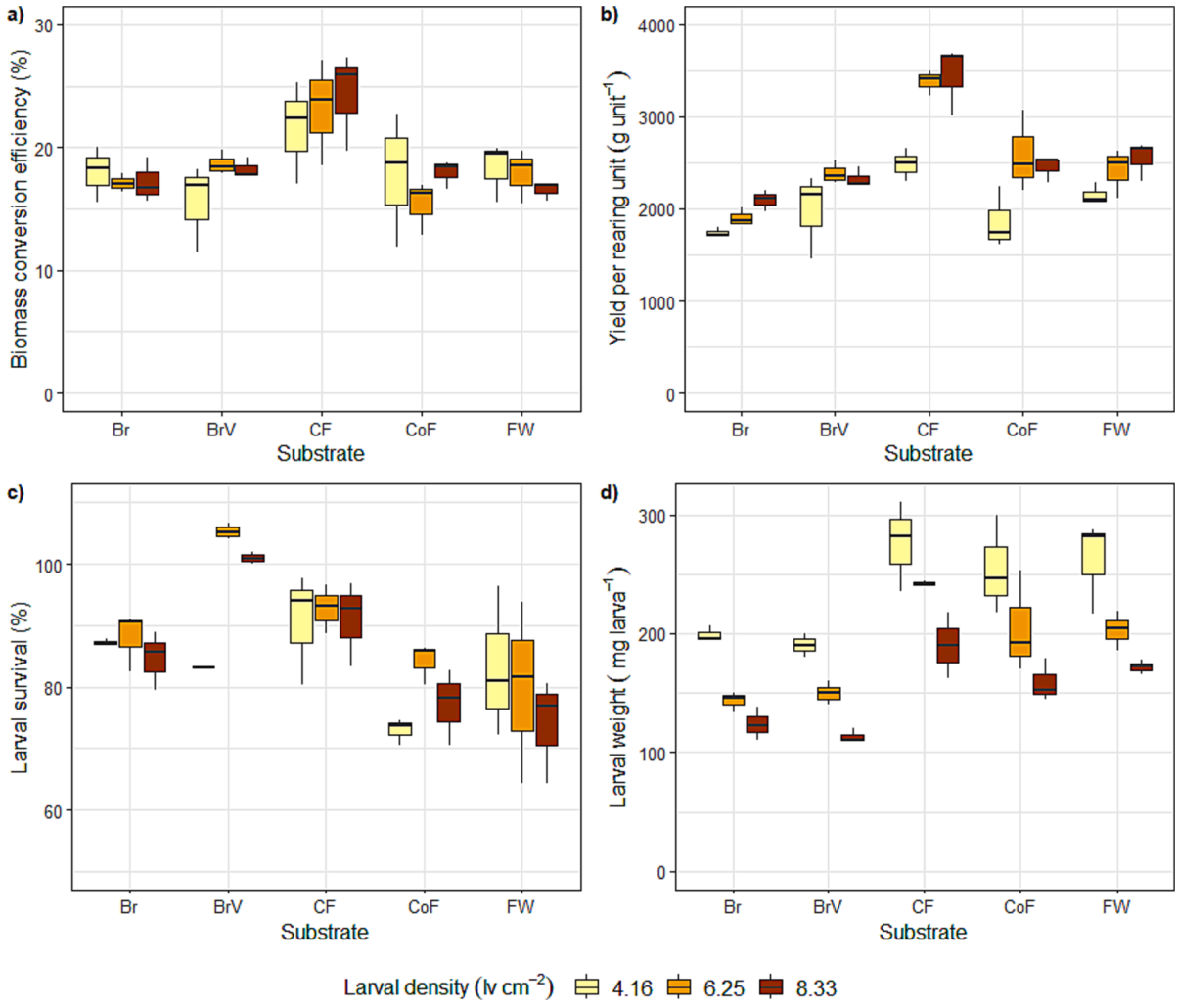

Fig. 1. Boxplots of the a) biomass conversion efficiency on a wet weight basis (%): b) yield per rearing unit (g $unit^{-1}$); c) larval survival (%) and d) larval weight (mg $larva^{-1}$), in the substrates provided to black soldier fly larvae stocked at different densities (4.16, 6.25 and 8.33 larvae cm^{-2}). Horizontal lines in boxplots indicates median. Br: bread; BrV; bread and vegetables, CF: chicken feed; CoF: cow feed; FW: food waste.

process parameters. In Experiment 2, the data obtained were first subjected to factor analysis with an extraction method using principal components, in order to identify the most influential variables for observed variance. Generalised linear regression (5% significance level) was then used to assess correlations between response variables, larval density and feed rate. Normality of modal residuals was verified with Shapiro-Wilks's test. Factor analysis was performed in the software STATISTICA 7.0, while the other statistical evaluations were made in R (R Core Team, 2019).

3. Results

3.1. Experiment 1

The biomass conversion efficiency was not greatly impacted by larval density or feed substrate except in the case of chicken feed (CF), which gave the highest BCE (>20%) (Fig. 1a). Higher larval densities (6.25 and 8.33 larvae cm^{-2}) resulted in significantly increased yield of larval biomass per rearing unit compared with the treatments with 4.16 larvae cm^{-2}, in particular in chicken feed (Fig. 1b). Larval survival was highest in the bread and vegetables (BrV) and lowest in cow feed (CoF) and food waste (FW) (Fig. 1c). Larval weight was greatly impacted by density and was considerably higher at the lowest density (4.16 larvae cm^{-2}) in all substrates (Fig. 1d).

Material reduction (WW basis) exceeded 80% amongst treatments and individual larval weight range was 190–276 mg at a density of 4.16 larvae cm^{-2}, 105–242 mg at 6.25 larvae cm^{-2} and 113–190 mg at 8.33 larvae cm^{-2} (Table 2). Substrate type and larval density both had significant effects on final larval weight ($F_{substrate} = 22.07$, $p < 0.001$; $F_{density} = 47.64$, $p < 0.001$, respectively) and yield of larvae per experimental unit ($F_{substrate} = 28.56$, $p < 0.001$; $F_{density} = 21.47$, $p < 0.001$, respectively), but there was no significant interaction between these factors on any of the variables analysed.

Larval survival differed significantly ($F = 14.05$, $p < 0.001$) between the feed substrates, with the lowest survival (78.1–79.0%) on food waste (FW) and cow feed (CoF), and the highest (100%) on mixed bread and vegetables (BrV). Larval density did not affect survival, but it did affect individual larval weight, with a significant reduction in weight with increasing larval density (from 236 to 189 and 152 mg $larva^{-1}$ at density 4.16, 6.25 and 8.33 larvae cm^{-2}, respectively).

Table 2
The average larval survival, larval weight, bioconversion ratio (BCE) and material reduction (Mat.Red) in the distinct feed substrates and stocking densities (4.16, 6.25 and 8.33 larvae cm^{-2}). BCE: bioconversion ratio, Mat.Red: material reduction.

	Survival (%)	Larval weight (mg $larva^{-1}$)	Larval yield (g $unit^{-1}$)	BCE ($\%_{WW}$)	Mat. Red ($\%_{WW}$)
Substrate[a]					
CF	91.5^{b}	236^{a}	3104^{a}	22.99^{a}	–
FW	79.0^{c}	212^{ab}	2372^{b}	17.57^{b}	82.4
CoF	78.1^{c}	206^{ab}	2301^{b}	17.04^{b}	83.0
Br	86.7^{bc}	155^{bc}	1911^{b}	17.41^{b}	82.6
BrV	103.8^{a}	151^{c}	2234^{b}	17.48^{b}	82.5
Densities					
4.16	87.9	236^{a}	2044^{b}	18.22	82.6
6.25	90.1	189^{b}	2534^{a}	18.43	82.7
8.33	85.6	152^{c}	2574^{a}	18.83	82.5
Statistics					
Substrate (SB)	14.051***	22.070***	28.556***	6.959***	0.088 ^{ns}
Density (DS)	1.068 ^{ns}	47.636***	21.472***	0.178 ^{ns}	0.008 ^{ns}
Interaction (SBxDS)	0.413 ^{ns}	0.311 ^{ns}	1.507 ^{ns}	0.694 ^{ns}	0.979 ^{ns}

[a] CF: chicken feed; FW: food waste; CoF: cow feed; Br: bread; BrV: bread and vegetables. Different superscript letters within columns indicate significant differences between mean values of the test factors (substrate, larval density) according to Tukey's test ($p < 0.05$). ns: not significant *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3.2. Experiment 2

Bioconversion of the dog food substrate differed between the treatments of different feed doses and substrate depths treatments in Experiment 2 and ranged from 31.9 ± 4.7%*vs* in the low substrate depth treatment to 12.5 ± 2.0%*vs* in the deep substrate treatment (Fig. 2a). Mat.Red was lower with increasing substrate depth, both on a WW and VS basis (Fig. 2b). Increasing the feed dose from 0.1 to 0.5 g VS lv^{-1} resulted in a lowering in BCE and Mat.Red.

Survival ranged from 76 to 99% in all treatments except that with a high feed dose (0.4 g VS lv^{-1}), in which survival was only 36.5 ± 7.5% (Fig. 3a). When substrate depth was increased from 1.5 to 6.5 cm, survival declined by 20% and individual larval weight decreased from 234 ± 13 mg to 132 ± 11 mg. When feed dose was increased from low (0.1 g VS lv^{-1}) to very high (0.5 g VS lv^{-1}), while larval density was maintained at 3 larvae cm^{-2}, individual larval weight was reduced by 36% (Fig. 3b). Greater substrate depths (3.0 and 6.5 cm) resulted in higher yield (>200 g larvae per rearing unit), while changing feed dose did not give a clear trend for this parameter (Fig. 3c).

The proportion of initial VS provided in each treatment in Experiment 2 that was transformed into larval biomass and frass or lost during the bioconversion process differed strongly between the treatments (Fig. 4). Increasing the feed dose resulted a higher proportion of the initial VS being either lost (treatment with high TS/low depth; lost VS 44.1 ± 2.3%) or not fully processed and recorded as frass (treatment with very high feed dose; 93.8 ± 4.6% as frass, 4.5 ± 1.9% as larvae and 1.7 ± 2.7% lost VS). Higher substrate depths (3.0–6.5 cm) also resulted in a larger proportion of frass and lower proportion of larval biomass (although at medium depth (3.0 cm) production of larval biomass was similar) in comparison with the low feed dose and low depth treatments.

Exploratory factor analysis was carried out by extracting the two principal components (PC) in the original dataset, which explained 82.5% of the total variance (PC1 = 66.7%, PC2 = 15.8%). The analysis distinguished the most important parameters affecting BSFL bioconversion of the feed substrate as being substrate TS content, substrate depth and larval feed dose. The most impactful variables related to process efficiency were found to be BCE and Mat.Red. Correlation analyses were carried out to assess how these variables were affected by different bioconversion process parameters. BCE was found to be correlated with four individual parameters: substrate VS content, substrate depth, larval feed dose and larval density. Linear models were then used to check whether the variance in the data could be better explained by a combination of these factors. BCE values modelled using substrate TS and depth as predictors resulted in higher adjusted R^2 ($R^2_{adj} = 0.82$ ($p < 0.0001$) than when these variables were analysed alone ($R^2_{adj} = 0.48$ for TS content, 0.74 for substrate depth). BCE on a VS basis modelled using larval feed dose and density as predictors resulted in a significant correlation ($p < 0.0001$) and higher R^2_{adj} (=0.73) than when these parameters were evaluated alone ($R^2_{adj} = 0.53$ for feed dose, 0.09 for larval density) (Fig. 5). This means that a combination of substrate TS and depth, and of feed dose and larval density, significantly impacted BCE, with a higher impact than when evaluated alone.

4. Discussion

4.1. Impact of larval densities

Black soldier fly larvae reared on chicken feed, a nutritionally well-balanced feed for the larvae, were heaviest (Fig. 1d) and had the greatest yield per rearing unit (Fig. 1b) and highest BCE of all treatments in Experiment 1 (Fig. 1a), which is in agreement with previous findings for chicken feed substrate (Spranghers et al., 2017). The main goal of

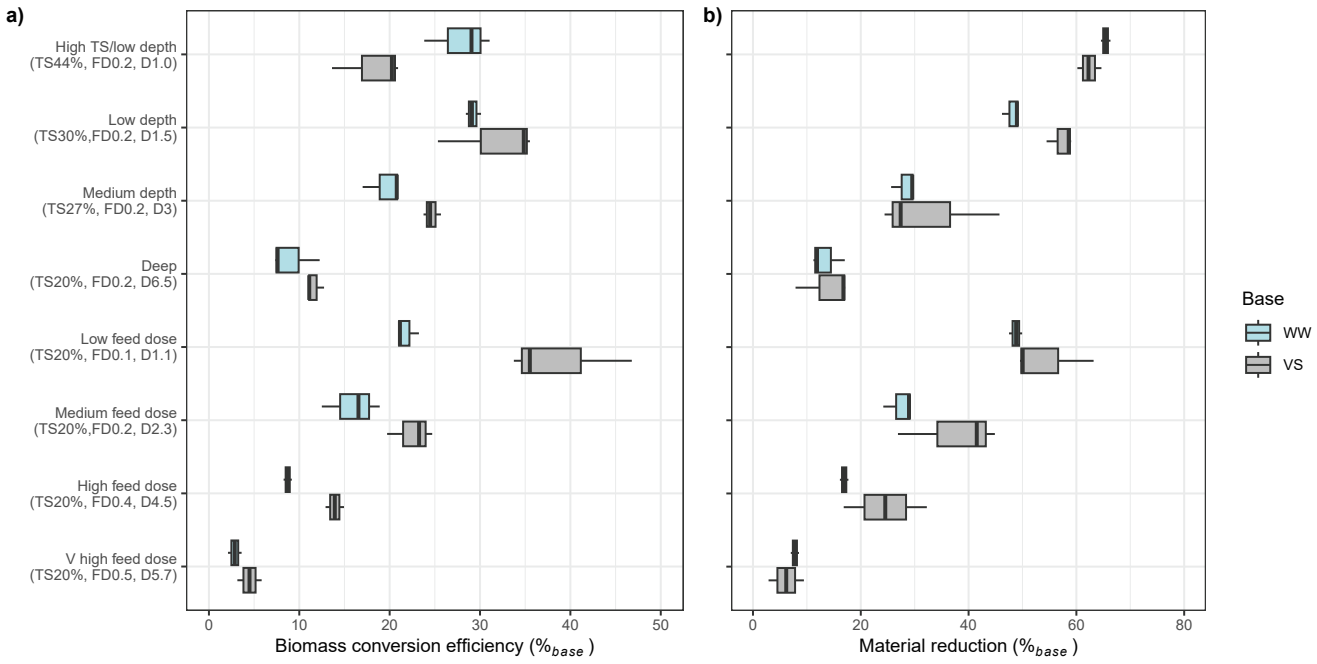

Fig. 2. Boxplots of a) biomass conversion efficiency (%) and b) material reduction (%), on a wet weight (WW) and volatile solids (VS) basis achieved in bioconversion of dog food with black soldier fly larvae at varying substrate depth (1.0–6.5 cm) and larval feed dose (0.1–0.5 g VS larva^{-1}). Vertical lines in boxplots indicates median.

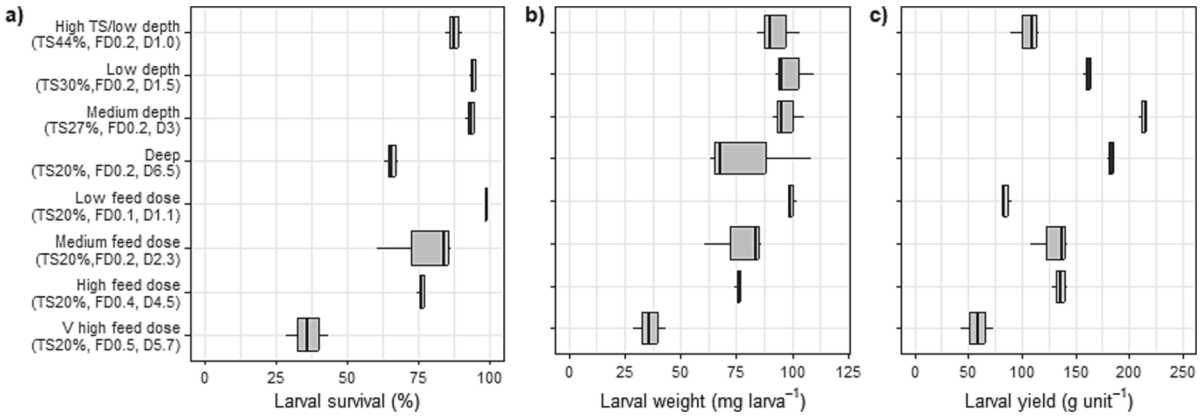

Fig. 3. Boxplots of a) larval survival (%), b) larval weight (mg larva^{-1}) and c) larval yield per rearing unit (g unit^{-1}) achieved in bioconversion of dog food with black soldier fly larvae at varying substrate depth (1.0–6.5 cm) and larval feed dose (0.1–0.5 g VS larva^{-1}). Vertical lines in boxplots indicates median.

Experiment 1 was to assess the impact of larval density on process performance. Interestingly, there was no statistically significant impact of density on Mat.Red (82.5–82.7%), BCE (18.2–18.8%) or survival (85.6–90.1%), while larval weight ranged between 152 mg (at density 8.33 larvae cm^{-2}) and 236 mg (at 4.16 larvae cm^{-2}). A similar trend was observed by Parra Paz et al. (2015), who reared BSFL at densities of 2, 4 and 6 larvae cm^{-2} in small reactors (49 cm^2 surface area) and found that density was the most influential parameter for larval growth performance.

In a study where BSFL were reared on a mixture of abattoir waste and vegetables at very low density (<1 larvae cm^{-2}), individual larval weight increased to >300 mg per larva (Lalander et al., 2019). However, such a low density is not commonly applied in industrial settings. In another study where BSFL were reared in a 2289 cm^2 container on manure (swine, dairy and poultry) at a density of 4.37 larvae cm^{-2}, which is similar to the 4.16 larvae cm^{-2} density tested in this study, Miranda et al. (2020) observed individual larva weighing more than 150 mg. That was comparable to the effect of the control diet used in that study (Gainesville diet, which is nutritionally a more balanced diet for BSFL than animal manure). However, as observed by Barragan-Fonseca et al. (2021), BSFL have high nutritional plasticity, with the larvae displaying similar growth when fed substrates with a wide range of nutritional quality. This observation was corroborated in Experiment 1, where similar BCE_{ww} was observed for all treatments except chicken feed, regardless of the larval density used (Fig. 1a).

Interestingly, larval yield per experimental rearing unit in Experiment 1 (2400 cm^{-2} plastic boxes) followed the same trend as BCE for the feed substrates, but was significantly lower at the lowest density (4.16 larvae cm^{-2}). Similar findings were made by Barragan-Fonseca et al. (2018), who evaluated densities of 0.31–2.47 larvae cm^{-2} for diets with different nutrient concentrations, and found lower individual weight gain and higher yield per treatment unit with increasing larval density. Jiang et al. (2022), evaluated the performance of BSFL on swine manure at weight-based densities (0.08, 0.24 and 0.40 % of substrate WW provided) and found that higher densities (in that case initial larval biomass of 0.40% of substrate weight) resulted in higher yield of prepupae. When

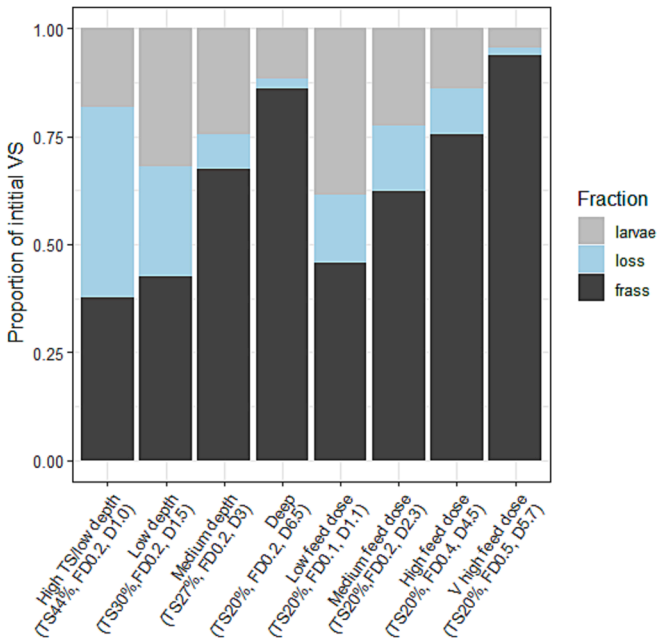

Fig. 4. Mean proportion of initial volatile solids (VS) in frass and larvae and lost through respiration during bioconversion by black soldier fly larvae of dog food at different substrate depths and feed rates.

recalculating the larval density used in this study into weight-based densities, we find that larval density 4.16 is equal to a weight-based density of 0.14%, larval density 6.25 is equal to 0.21%, while larval density 8.33 is equal to 0.28%. The findings in Experiment 1 is thus not in accordance with the ones found by Jiang et al. (2022). In both studies mentioned, there was a linear increase in larval yield with increasing larval density. In contrast, densities 6.25 and 8.33 larvae cm^{-2} in this study resulted in comparable yield, even though both surpassed the yield of the treatment with 4.16 larvae cm^{-2}. In Experiment 2 in this study, variations in weight-based densities were achieved through adjustments in the feed dose provided to the larvae: *e.g.* the low feed dose treatment (0.1 g VS $larva^{-1}$) had a weight-based density of 0.44%, while the very high feed dose treatment (0.5 g VS $larva^{-1}$) had a weight-based density of 0.09%. The 0.44% density indeed resulted in higher yield (~80 g $unit^{-1}$) than the 0.09% density (~60 g $unit^{-1}$), despite both treatments having the same initial number of larvae. Comparing to the results of Barragan-Fonseca et al. (2018), the assessed larval densities used in their study were lower than those assessed in this study. In fact their highest assessed density (2.47 larvae cm^{-2}) was lower than the lowest density investigated in Experiment 1 (4.16 larvae cm^{-2}). This suggests that there is a density threshold close to 6.25 larvae cm^{-2}, beyond which further increments in larval density does not lead to higher yields. As we lack the knowledge of the precise number of larvae per cm utilised by Jiang et al. (2022), it is likely that they had not yet reached the threshold of excessive larval density (lv cm^{-1}).

4.2. Impact of substrate TS and depth and larvae feed dose

Using dog food as model substrate in Experiment 2, it was found that substrate TS content, substrate depth and larval feed dose were the most influential variables affecting BCE, and that individually they explained less of the variance in the data than in combination. Thus the hypothesis that these parameters affect the bioconversion process in an interconnected way was confirmed. This was also demonstrated by Lalander et al. (2019), who found significant correlations for the interaction between larval feed dose and two other variables (feed substrate protein, VS feed rate) on BCE, protein conversion ratio, prepupal weight and prepupal emergence. Those findings and the results in present study highlight the need for considering multiple variables when converting bio-waste with BSFL.

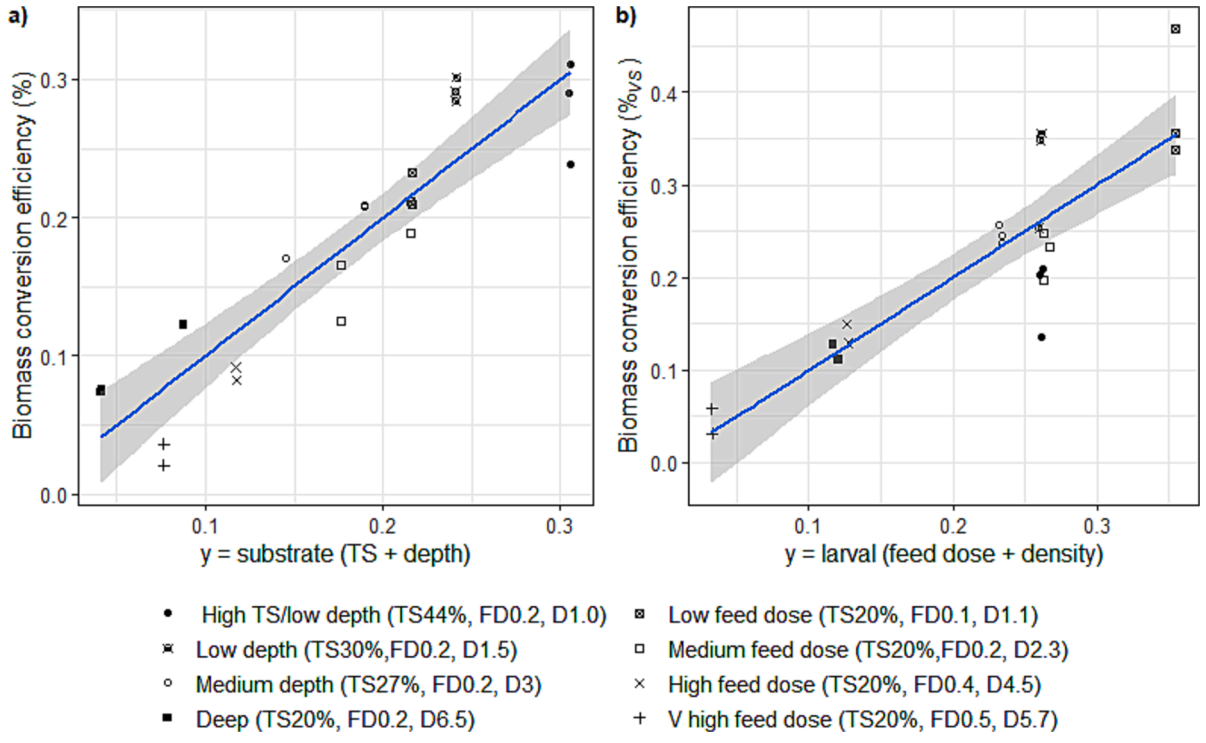

Fig. 5. Correlations between a) biomass conversion efficiency (BCE) on a wet-weight basis and BCE values modelled using substrate total solids (TS) content and substrate depth as predictors ($BCE_{WW} = 0.183 + 0.35 \times sub.TS + (-0.03)$ sub.depth; adjusted $R^2 = 0.82$, $p = 4 \times 10^{-8}$); and b) BCE on a volatile solids (VS) basis and BCE values modelled using larval feeding dose and larval density as predictors ($BCE_{VS} = 0.51 + (-0.78)$ feed dose $+ (-0.04) \times$ lv.density; adjusted $R^2 = 0.73$, $p = 1 \times 10^{-6}$).

Increasing substrate depth (from 1 to 5.7 cm) resulted in decreasing BCE and Mat.Red, even when larval density was increased with substrate depth (Fig. 2a-b). This indicates that there are limits on how much waste can be treated in a given time period in a rearing unit, regardless of the amount of larvae added, because at a certain depth the larvae will not be able to access and convert the material properly, as discussed by Dortmans et al. (2017). At the same feed dose, larvae subjected to the highest substrate depth in our study showed 21.5% lower survival (Fig. 3a) than larvae subjected to low or medium substrate depth, as well as lower individual weight (Fig. 3b). Opare et al. (2022), observed linear reductions in larval survival and pupal mass on increasing larva density from 1 to 5 and 10 larvae cm^{-2}. Interestingly, those authors also saw an interactive effect of variables affecting the process as a whole. They concluded that a combination of temperature and larval density gives a stronger response in terms of larval growth and development, and that these and other process parameters should be taken into account together in order to achieve good bioconversion. A noteworthy finding in our study was that the negative impact of a non-ideal parameter, such as greater substrate depth, can be reduced by adjusting another parameter, such as feed dose. In the treatment with high feed dose (0.5 g VS lv^{-1}) and substrate depth 5.7 cm, BCE_{ww} was > 5%, whereas it in the treatment with higher depth (6.5 cm) and lower feed dose (0.2 g VS lv^{-1}) BCE_{ww} was around 9% (Fig. 2a).

At higher substrate depths (4.5–6.5 cm), a smaller proportion of initial VS in the substrate was converted into larval biomass than at lower substrate depth, while a higher proportion of frass was produced (Fig. 4). However, a higher proportion of VS in frass does not necessarily make a good fertiliser and possibly indicates that the initial material was not well-processed by the larvae (Dortmans et al., 2017). It is important to assess the process efficiency both in terms of bioconversion efficiency (how efficient the substrate is being utilised by the larvae) as well as the total yield in the rearing unit, *i.e.*amount of larvae produced cm^{-2}. For example, the low depth treatments (1–1.5 cm deep) had the highest BCE, but considerably lower yield per rearing unit than the medium depth treatment (3 cm depth), likely because more larvae were added (1000 larvae compared to 700 larvae), while the larvae were provided with the same feed dose. In the treatment with highest substrate depth (6.5 cm), both the BCE and the yield were reduced, despite having the highest number of larvae in the rearing unit (2000 larvae) initially. To illustrate the inherent trade-offs that have to be made when assessing the over-all efficiency of a treatment, it could be argued that the medium depth treatment yielded the most optimal combination of BCE (15–20%$_{WW,}$ 20–25%$_{VS}$) and yield (200 g $unit^{-1}$) of assessed treatments. Despite not having the highest BCE or Mat.Red, it resulted in the highest yield per rearing unit, along with sufficiently high BCE. However, Mat.Red was relatively modest (around 30% on both WW and VS basis), indicating that the frass would comprise a relatively high proportion of unprocessed substrate. Lopes et al. (2022), suggested that there is a strong need to evaluate frass readiness level (maturity/stability) as a fertiliser, and in general frass should be submitted to post-processing steps (*e.g.* composting or digestion) in order to be considered a suitable fertiliser product.

Issues arising with increasing substrate depth in rearing boxes (such as lower survival and BCE in this study) could be tackled by the simple approach of providing substrate periodically, instead of as a thick layer at the start of the rearing process. In a study by Lopes et al. (2020), a mix of aquaculture waste and bread was provided either in bulk or in three feeding events to BSFL in 231 cm^2 rearing boxes, with a larval density of 4 larvae cm^{-2} and a feed rate of 0.25 g VS $larva^{-1}$. Those authors did not record substrate depths, but when the substrate was provided in split doses the depth was visibly smaller (personal obervation) and this resulted in larger larvae (165–170 mg) than when all substrate was provided at the start (109 mg), and also higher BCE on a dry matter basis (24% and 16.7%, respectively). In contrast, Banks et al. (2014) observed higher bioconversion and higher weight gain in BSFL fed a bulk amount of human faeces, which they attributed to lower nutritional quality of ageing faeces over time. However, a very small number of larvae per rearing unit were used in that study (1, 10 or 100 larvae per 50–324 mL container). At present, the insect industry generally provides substrate on one, two or three occasions, with each approach differing in terms of mechanization, equipment, labour requirement, *etcetera*.

When process parameters (*e.g.* depth, temperature, moisture or density) are combined within specific ranges, BSFL perform better and thus bioconversion as a whole is improved. This was shown by Opare et al. (2023), who subjected BSFL to temperatures of 23, 27 and 30 °C at varying densities (1–10 larvae cm^{-2}). Those authors observed higher immune response of the larvae reared at higher densities, measured as activity of phenoloxidase, an enzyme that plays a role in melanin production in invertebrates on exposure to pathogens, thus protecting the individual. As highlighted by Barrett et al. (2023), it is of paramount importance to determine how each parameter affects the bioconversion process in order to maintain an adequate level of insect welfare, as reflected in proper growth and development, and ensure good performance so that the insect industry can expand and make a real impact in handling waste from the feed and food sectors.

BSFL are usually fed based on predetermined parameters that will render expected results (Meneguz et al., 2018). However, substrates with differing composition, physical structure, water-holding capacity and other traits may end up generating varying depth, feed availability and other metrics when placed in rearing units, even if larval density, larval feed dose and other factors are kept constant. The two experiments in the present study showed that even when controlling one or two variables within the same design (*e.g.* larval density and feed load), other variables (*e.g.* depth) can end up being affected (Table 1).

5. Conclusions

In accordance with our hypothesis, it was demonstrated that factors beyond solely the nutritional quality of the substrate influenced the overall process efficiency in BSFL composting. In Experiment 1, it was observed that increasing larval density (lv cm^{-2}) led to an increase in yield per rearing unit, until reaching a density threshold (around 6.25 lv cm^{-2}), beyond which no further increase in the total yield was observed, but only a reduction in average larval weight. In Experiment 2, it was found that substrate TS and depths together significantly influenced BCE on a wet weight basis (which reflect the potential yield achievable per rearing unit within a given time period). Increasing substrate depth (>5 cm) was found to impair the process performance by lowering Mat.Red and BCE. However, supplying a dryer substrate (higher TS), allow for provision of higher larval feed dose without leading to increasing substrate depth. In terms of the BCE on a volatile solids basis (indicative of how effectively the organic fraction of the substrate is converted into larval biomass), larval feed dose (g VS lv^{-1}) and density were identified as the most influential parameters. As the feed dose increased, the BCE_{VS} decreased; a combination of high feed dose and large larval density led to a highly inefficient process. A trade-off balance between different efficiency variables has to be made when selecting treatment strategy; *e.g.* it was proposed that the treatment with medium depth (3 cm) and medium larval feed dose (0.2 g VS $larva^{-1}$) was the best balance between biomass conversion efficiency and larval yield of the assessed treatments, even though the demonstrated Mat.Red. was low. The findings of this study can provide guidance when devising laboratory-scale and industrial processes utilising novel bio-wastes as substrates for BSFL.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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