Life-history traits of black soldier fly reared on agro-industrial by-products subjected to three pre-treatments: a pilot-scale study

C.D. Heussler[1,2*], H. Insam[2], A. Walter[3], B.C. Schlick Steiner[1#], F.M. Steiner[1#] and T. Klammsteiner[1,2#]

[1]Department of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria; [2]Department of Microbiology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria; [3]Department of Biotechnology & Food Engineering, MCI – The Entrepreneurial School, Maximilianstraße 2, 6020 Innsbruck, Austria; carina.heussler@uibk.ac.at; # these authors contributed equally to this paper as senior authors

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Abstract

Agrarian production generates approximately 190 million tons of agro-industrial by-products (AIBP) per year that are often disposed of without proper treatment, causing health hazards and environmental pollution. The black soldier fly (BSF; *Hermetia illucens*) has gained popularity as an organic waste recycler owing to its suitability for large-scale insect farming. Despite their valuable components, AIBPs are rich in fibres with low digestibility and provide a breeding ground for potentially pathogenic microbes, which necessitates proper caretaking. This study focuses on a pilot-scale life-cycle analysis of BSF, comparing three different pre-treatments for agro-industrial wastes. We assessed the effect of the pre-treatments on larval and pupal biomass yield and development time of life stages, pupal and adult body properties, adult emergence and sex ratio, and oviposition and fertilization rates. A mix of residues from pasta production and wheat bran (basis substrate, BS; control treatment) was pre-treated using a chemical (with 0.15% potassium sorbate, BSSORB), an organic (with 10% biochar, BSCHAR), and a microbiological (with a 10-day lactic acid fermentation, BSFERM) approach. We report that the BSSORB treatment had the significantly highest larval and pupal biomass yield as well the significantly largest pupae and adults, and the most successful adult emergence but had low oviposition success. BS and BSFERM were similar in terms of larval and pupal biomass yield, BSF development time, pupal and adult body properties, adult emergence, and oviposition. Nonetheless, BSFERM had the highest share of unfertilized eggs. With BSCHAR, BSF developed faster. This study indicates that all treatments have advantages and disadvantages and that thus their application should be selected based on breeding strategy. By analysing and adapting methods that facilitate the conversion of AIBPs to BSF biomass, the treatment of agricultural wastes and by-products can be made more efficient and sustainable.

Keywords: *Hermetia illucens*, waste valorisation, insect farming, sustainable agriculture, circular economy, fertilizer

1. Introduction

Crop residues and agro-industrial by-products (AIBP), like damaged/spoilt foods, seeds, husks, brans, and grains, are a growing environmental problem (Barcelos *et al.*, 2020; Kumari *et al.*, 2018). According to the FAO (2013), agricultural production generates approximately 190 million tons of by-products per year (Barcelos *et al.*, 2020; FAO, 2013). Often, these are not taken care of in an adequate way and consequently may harm human, animal, and plant health and contribute to environmental pollution. Furthermore, AIBPs are often disposed of by incineration or landfilling, causing an increase in greenhouse-gas emissions (Barcelos *et al.*, 2020; Sadh *et al.*, 2018). Notably, these wastes are rich in bioactive compounds such as vitamins, carotenoids, and minerals (Barcelos *et al.*, 2020). Therefore, developing sustainable solutions to reuse and recycle AIBPs will be essential for economic and environmental prospects (Boukid *et al.*, 2021; Sadh *et al.*, 2018).

Insects like the black soldier fly (BSF; *Hermetia illucens* L.) have shown great potential as agents for biological waste

management (Liu *et al.*, 2022). The BSF's larvae (BSFL) have the ability to digest a wide range of organic residues (Nguyen *et al.*, 2015). Due to their high resilience and nutritional value, these larvae are suitable for large-scale insect farming, yielding biomass rich in fat and protein as well as insect frass suited as organic fertilizer (Barragan-Fonseca *et al.*, 2017; Klammsteiner *et al.*, 2020). However, large-scale BSF farming still faces obstacles, including the selection and preconditioning of a suitable, safe, and readily available rearing substrate. Within the current European legislative framework, AIBPs represent a highly suitable substrate for BSF rearing (IPIFF, 2021). Large-scale operations rely on a continuous and guaranteed supply of feeding substrate, ideally showing a consistent nutritional composition (Chia *et al.*, 2020). AIBPs are often rich in microbes, among which pathogens potentially harmful to humans could contaminate the substrate (Barcelos *et al.*, 2020; Villas-Bôas *et al.*, 2002). Besides the hygienisation potential of AIBP treatment by BSF, pathogens affecting the BSF larvae themselves are an important issue for industrial-scale BSF farming (Awasthi *et al.*, 2020). Pre-treatment methods of the rearing substrate have been shown to improve the BSFL conversion and palatability of substrate and even reduce emissions of CH_4 and N_2O (Isibika *et al.*, 2019; Liew *et al.*, 2022; Lindberg *et al.*, 2022). Pre-treating AIBPs may both reduce the bioburden and increase digestibility of lignocellulosic fibres and thereby result in higher BSF yield and product safety.

Here, we assessed the effects of substrate pre-treatments on larval biomass generation and the entire life cycle of the BSF on pilot-scale (that is, beyond lab scale and tested in an industrially relevant environment in accordance with a commercial breeder). We conducted selected life cycle trials, using approximately 75,000 larvae obtained from a commercial breeder. Among the numerous AIBPs available, we chose wheat bran and waste from pasta production. Wheat bran is a by-product of milling wheat grain where the bran fraction constitutes about 11-15% of the total milling product and is often used for livestock feed (Balandrán-Quintana *et al.*, 2015; Hossain *et al.*, 2013). Approximately, 10% of pasta is lost due to cracking and breaking during production (Tagliasco *et al.*, 2021). We hypothesized that pre-treatment of these by-products would result in: (1) better digestibility and nutrient recovery measurable in increased larval biomass gain; (2) larger pupae and female adults; and (3) an improved egg biomass yield. To test these hypotheses, we applied three pre-treatments, representing a chemical, an organic, and a microbiological approach. For the chemical approach, we used potassium sorbate, a commercially widespread preserving agent (Woolford, 1975). For the organic approach, we used biochar, as it has been shown to improve nutrient availability to BSF larvae (Tan *et al.*, 2021). For the microbiological approach, we subjected the fresh substrate to lactic acid fermentation, a method frequently used to increase the shelf-life of feed and food (Van Campenhout, 2021; Waqas *et al.*, 2019; Yang *et al.*, 2006).

2. Material and methods

Study location

The study was carried out in a 12-m^3 climate chamber at 27±2 °C and 60% relative humidity at the Department of Ecology, University of Innsbruck, Austria (N 47° 15' 52.7, E 11° 20' 34.3). Relative humidity was measured and maintained using a hygrometer (HY/WE, RO/SE, Bad Birnbach, Germany) connected to a mobile humidifier (Fog Box IX 8.7L, Taifun, MiHa, Laatzen, Germany).

Black soldier fly origin, breeding of larvae, and pre-treatments

Approximately 9-day-old larvae were obtained from a commercial producer. During a preliminary trial, we streamlined the rearing system and protocols to achieve rearing conditions comparable with those of the commercial producer. The BSF colony was maintained at 27-30 °C and grown on a 1:2 mixture (w/w) of residues from pasta production (ground pasta with particles <2 mm) and wheat bran. This dry blend was mixed with tap water to obtain a substrate with 31.5% dry matter content and was used as the control treatment (BS), according to the protocol used by the commercial producer. It was subjected to three separate substrate pre-treatments; that is, firstly, a chemical, by adding 0.15% (w/w) potassium sorbate (Gustav Ehlert GMBH & CO KG, Verl, Germany), the maximum effective level to prevent mould according to Ray and Bullerman (1982) (BSSORB); secondly, an organic, by adding 10% biochar (w/w) (made from untreated wood chips, Innsbrucker Kommunalbetriebe, Innsbruck, Austria) according to Beesigamukama *et al.* (2020) (BSCHAR); and thirdly, a microbiological pre-treatment was performed by fermenting the BS prior to the start of the experiment (BSFERM) by adding 0.5% NaCl and 1.4% natural yoghurt (3.6% fat, Berglandmilch, Wels, Austria) as starter and incubating it at room temperature (25±2 °C) for 10 days. In the latter, two 12-l plastic bags filled with water were used to seal the surface of the substrate during the fermentation process while still allowing gases to escape. A study performed by (Cho *et al.*, 2020) showed that a NaCl concentration of 1% can affect larval growth performance; therefore, we aimed for a concentration below 1%. The fermentation process was performed according to Katz (2012). After pre-treatment, the water content was determined gravimetrically based on loss of mass after drying the substrates at 105 °C for 24 h, resulting in a similar dry matter content of 28.5±0.9% and water content of 71.5±0.9% for all pre-treated substrates and the control.

Each treatment was replicated three times using a total of twelve standardized plastic boxes (400×300×120 mm). These boxes were randomly arranged on three shelves within the climate chamber. To account for unknown spatial influences, the boxes were randomly rearranged every other day. In total, 6,250 larvae (100.64 g 9-day-old larvae were added to each of the 12 boxes (75,000 larvae in total), equalling a larval density of 5.2 larvae/cm^2. The larvae were batch-fed once at the beginning of the experiment with 3,125 g substrate per box. The average biomass of the larvae was determined by weighing 50 randomly selected larvae on an analytical balance (Mettler Analytical Balance AE 166 Delta Range, Mettler-Toledo Ltd., Columbus OH, USA; accuracy of scale display 0.001 g). This sampling procedure was repeated with larvae from five locations within each of the twelve boxes following a Z-scheme (with sampling points at the upper left, upper right, centre, bottom right, and bottom left of each box; Figure 1) to ensure standardized sampling throughout the experiment (Figure 1). After the emergence of the first pupae on day 8 of the experiment, pupae were collected, weighed, and measured every other day (Figure 1). The larval breeding was terminated after 12 days when samples contained circa 50% pupae as determined by visual inspection (Figure 1). At the end of the experiment, overall larval and pupal biomass production was determined using a digital scale (DZD DJKF6000A, G&G, Kaarst, Germany; accuracy 0.1 g).

Table 1. Elemental and nutrient composition of the dry basic substrate (BS) subsequently subjected to three pre-treatments.

	Basic substrate component	Content
Nutritional content	Water (%)	10.8
	Crude protein (%)	40.9
	Crude fat (%)	3.8
	Crude ash (%)	3.8
	Sucrose (%)	4.4
	Starch (%)	33.9
	Calcium (%)	0.085
	Metabolizable energy (MJ/kg)	13.9
Elemental composition	C (%)	45.66±0.28
	H (%)	7.25±0.08
	N (%)	2.95±0.02
	S (%)	0.64±0.12
	O (%)	43.49±0.61

Elemental, nutrient, and texture analysis of substrates

The elemental composition of BS was determined at the Technical University Vienna (Vienna, Austria) using a Vario MACRO elemental analyser (Elementar, Langenselbold, Germany) for CHNS and an OXY Cube (Elementar) for

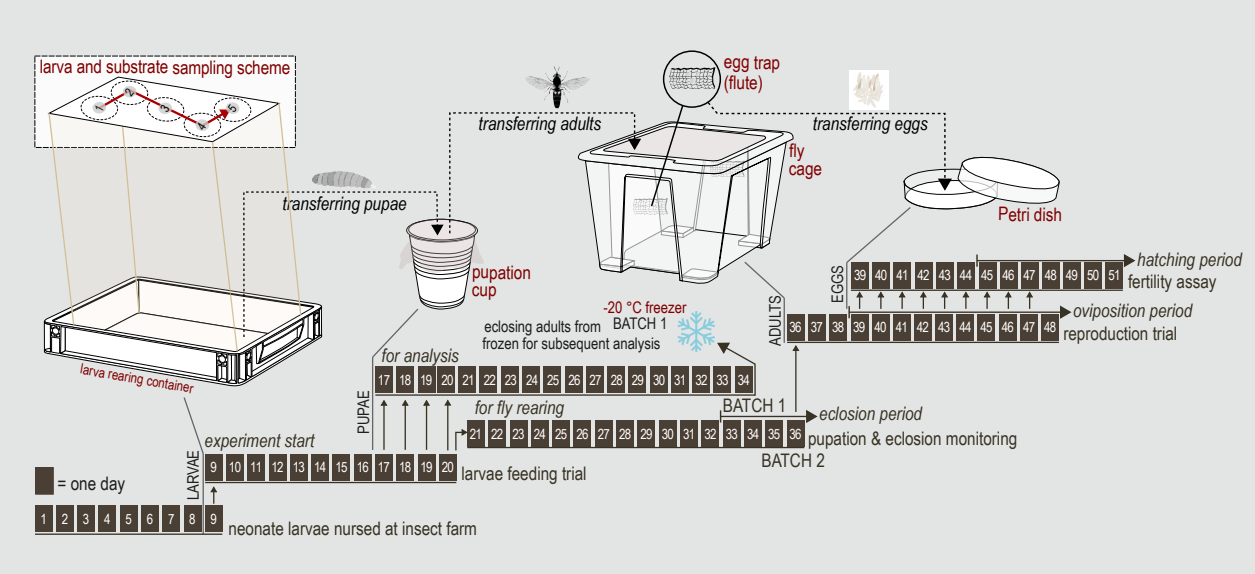

Figure 1. Illustration of the sampling scheme. Nine days after hatching, larvae were transferred to the basic substrate (BS) as a control and the three pre-treatment variants of the BS were supplemented with potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a 10-day fermentation process (BSFERM) (n=3 replicate boxes per treatment). Larvae were batch-fed once at the start and incubated until day 20. A Z-scheme was used to take samples of larvae and substrates in a standardized and reproducible way (with sampling points at the upper left, upper right, centre, bottom right, and bottom left of each box). Two batches of pupae were collected. Pupae Batch 1 was collected during larvae feeding trial. Pupae Batch 2 was collected at the end of the feeding trial. The emerging black soldier flies (BSF) of Batch 1 were frozen for further analysis. The emerging BSF of Pupae Batch 2 were transferred into fly cages for oviposition. Oviposited egg clutches were transferred to Petri-dishes to monitor fertilization success and hatching time.

O contents. The contents of water, crude protein, crude fat, crude ash, sucrose, starch, calcium, and metabolizable energy in BS were determined by the Austrian Agency for Health and Food Safety (Vienna, Austria) following nationally standardized protocols for food analytics (Table 1). Texture properties were analysed using a TA.XTplus texture analyser (Stable Micro Systems, Godalming, UK) as described in Shim and Lim (2013) with some modifications: Substrate samples were maintained over night at 27 °C to mimic experimental conditions (Supplementary Table S2). A flat cylindrical probe (Ø=25 mm) was used as adaptor. Aliquots of each sample were carefully poured into sample beakers and then firmly secured centrally on the texture analyser stage. The substrate's hardness and adhesiveness were measured in replicates (n=5) by using the following instrumental test parameters: the mode was set to compression; pre- and post-test speeds were set to 50 mm/min; the entry depth was set to 10 mm; the trigger type was set to auto 0.1 N.

Experimental set-up for adult life-history traits

Two batches of pupae were collected for each triplicate of the treatments. The first batch (Pupae Batch 1) contained the pupae that emerged during the larval rearing stage up until the termination of the feeding experiment. This batch was used to determine the number, sex, biomass, and length of eclosing flies. After eclosion, adult BSFs were devitalized by transferring them to -20 °C for 60 min to facilitate subsequent analysis (Figure 1).

The second batch (Pupae Batch 2) was collected at the end of the larval rearing stage, that is, at the end of the feeding experiment. This batch was used for further fly rearing and to determine the oviposition success of the treatments. All pupae were collected into white plastic cups (0.5 l) containing approximately 600 pupae each and wood litter for rodents (Dehner Terra, Rain, Germany). Cups were incubated under the same environmental conditions as the larvae (Figure 1). The cups were covered with fibreglass net (150×150 mm, mesh size 2×2 mm) that was kept in place with rubber bands (Heussler *et al.*, 2018). Four days after the first flies of Pupae Batch 2 had emerged, 50 male and 50 female flies from each replicate box were transferred into transparent polypropylene cages (390×280×280 mm; 0.031 m^3) with a fibreglass net (200×300 mm, mesh size 2×2 mm) integrated into the lid of each cage (Figure 1). The cages were randomly placed in triplicates under a separate LED panel as described by Heussler *et al.* (2018) in the above-mentioned climate chamber. The light sources were set to a light:dark photoperiod of 16:8. On two opposed walls of each cage, a piece of corrugated plastic (50×30×3 mm, henceforth termed flutes, Figure 1) held by a magnet was placed for oviposition. A glass test tube containing tap water sealed with a cellulose paper plug was provided as a water supply. The flutes were checked every day for fresh egg clutches. If oviposition had occurred, the flutes were collected into Petri dishes to avoid desiccation. All eggs of each cage were collected using a plastic spatula and placed on a small piece of aluminium foil and weighed (Mettler Analytical Balance AE 166 Delta Range). To determine the fertilization rate of the eggs, the aluminium foil was placed in the middle of a Petri dish filled with water (Figure 1). Thereby, hatching BSFL would accumulate in the water as a sign of successful fertilization of the respective egg clutches. Visual control for hatching larvae was performed daily. Additionally, the biomass of single eggs was determined on the first day of oviposition. After weighing the collected egg clutches of each cage, a small portion of the egg clutch was removed as a subsample and transferred onto a black plastic piece (PET; 50×30 mm) marked with a grid. This subsample was reweighed and dispersed on the grid for counting:

$$\text{Single egg biomass} = \frac{\text{egg clutch subsample biomass}}{\text{number of eggs within the subsample}}$$

This process was repeated five times for each cage.

Statistical analysis and data visualisation

Analysis of variance (one-way ANOVA) and a Bonferroni correction (α=0.05) using SPSS v26 (IBM, Armonk, NY, USA) were conducted for each treatment. Substrate (for analysis of adults, also sex) was used as the main effect and the data for the four developmental stages, namely larvae, pupae, adults, and eggs, as the response variables. For the analysis of larvae, development time and biomass production were used; for that of pupae, development time, biomass production, individual biomass, and length; for that of adults, percentage of adult emergence, individual biomass, length, and longevity; and for that of eggs, egg biomass, time until hatching, and percentage of unfertilized eggs. Data were visualized using Microsoft Excel 365 Office 2021 (Microsoft Corporation, Redmond, WA, USA) and Sigmaplot v.14.5 (Inpixon, Düsseldorf, Germany).

3. Results

The total larval and cumulative biomass (larval + pupal biomass) yields were each significantly different for BSSORB (234 g and 576 g, respectively), and BSCHAR (122 g and 495 g, respectively). No significant difference was observed in the total pupal biomass over all pre-treatments and cumulative biomass yield for BS (522 g) and BSFERM 527 g). By the end of the feeding trial, BSSORB yielded the highest larval biomass at 234 g and the lowest pupal biomass at 341 g (Figure 2, Supplementary Table S1). BSCHAR had the lowest larval biomass at 122 g whilst having the highest pupal biomass yield at 373 g. The pupae collected from Pupae Batch 2 at the termination of the feeding trial (as opposed to the Pupae Batch 1 collected during the feeding trial) were used for further fly rearing and oviposition analysis.

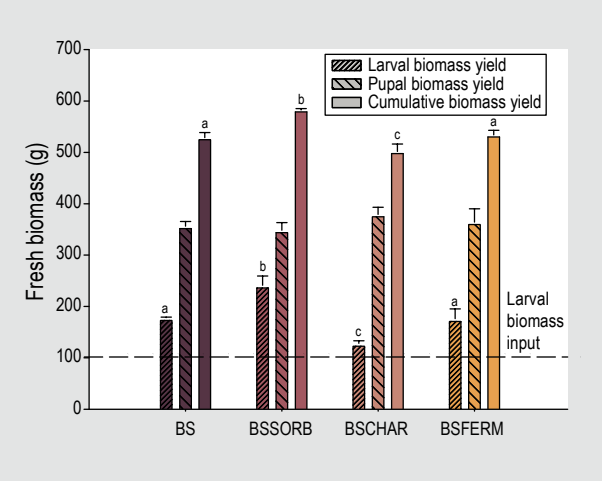

Figure 2. Average total larval, pupal, and cumulative biomass yield (g) with standard deviation for larvae raised on the basic substrate (BS) as a control and the three pre-treatment variants of the BS supplemented with potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a 10-day fermentation process (BSFERM) (n larvae=48; n pupae=12). Significant differences are shown as lowercase letters and were calculated via ANOVA (α=0.05) followed by a Bonferroni correction. The horizontal dashed line denotes the initial larval biomass input per box.

The BSCHAR pre-treatment significantly increased the hardness of the substrate as determined by the texture analysis (Supplementary Table S2) and resulted in the shortest period until the first pupation (17.0±0.0 days) (Figure 3, Supplementary Table S1). The remaining periods until the first pupation ranged from 18.0 to 19.7 days, with insignificant differences. Pupal development time did not differ significantly across the pre-treatments and the control (BS), ranging from 18.7±1.2 days in BSSORB to 19.3±1.5 days in BSFERM. Likewise, neither adult longevity nor time until eggs hatched were significantly different. The shortest adult life span was observed for BSSORB at an average of 14.7±1.2 days, the longest was observed for BS and BSCHAR at an average of 16.0±0.0 days. The shortest and longest hatching periods at an average of 1.0±0.0 days and 4.5±2.1 days were observed for eggs from BSCHAR and BS, respectively. Two small clutches of fertilised eggs with a total biomass of 0.0225 g oviposited on the floor of the cage were excluded from calculating the hatching time in BS, as their exact date of oviposition could not be determined. The BSCHAR pre-treatment resulted in the fastest life cycle at approximately 46 days from neonate to neonate.

The pupae collected during the feeding trial showed that larvae on BSSORB transformed into significantly heavier and longer pupae, at an average biomass of 0.125±0.015 g and an average length of 18.2±1.7 mm (Table 2). In comparison, the significantly lighter and shorter pupae from

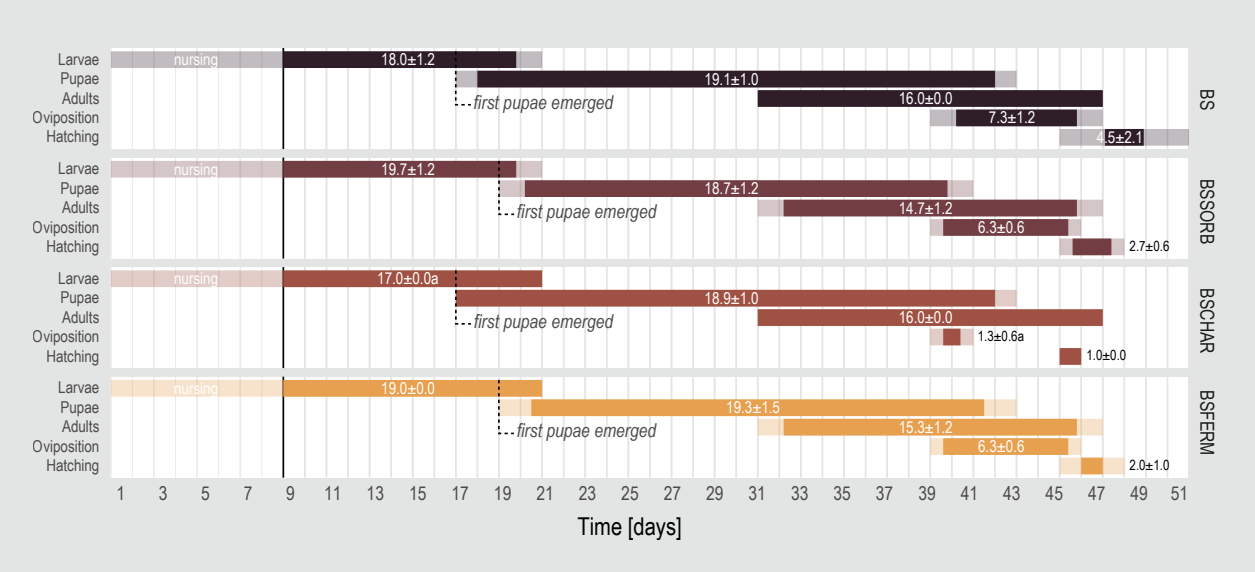

Figure 3. The average larval and pupal development time, adult life span, oviposition period, and hatching time frame of eggs in days ± standard deviation for black soldier fly larvae raised on the basic substrate (BS) as a control and the three pre-treatments of the BS with supplemented potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a 10-day fermentation process (BSFERM). The left and right tails of bars denote the minimum and maximum of each range, respectively, with the clear areas illustrating standard deviations (n larvae=36; n pupae=36; n flies=36; n eggs=57). The values accompanying each bar describe the average time in days ± standard deviation. Significant differences are shown as lowercase letters and were calculated via ANOVA (α=0.05) followed by a Bonferroni correction.

BSCHAR measured at 0.103±0.014 g and 16.4±2.1 mm, respectively. The female and male adults emerging from the BSCHAR pupae were consequently also significantly lighter and shorter, at an average biomass of 0.048±0.008 g and a length of 12.8±1.1 mm for females and an average biomass of 0.043±0.013 g and a length of 13.2±1.0 mm for males. Otherwise, no significant difference in biomass was observed among the emerging adults. While male flies differed significantly in length across all treatments ranging from an average length of 13.2±1.1 mm in BSCHAR to 14.3±0.5 mm in BSSORB, female lengths differed significantly only between BSCHAR and BSSORB, at an average length of 12.8±1.1 and 14.7±0.2, respectively. Pupae from BSCHAR also showed the lowest adult emergence (87.3±3.4%), while pupae from BS showed the highest adult emergence (94.8±2.8%).

During the period of adult emergence, the sex ratio (males:females; Table 2, Figure 4A and B, Supplementary Table S1) was male-dominated across the four treatments, resulting in a higher percentage of total male emergence. Significant differences were observed for the percentage of emerging females and males on different days but not for the total number of adults emerged.

The BS treatment had the highest and fastest oviposition rate at 0.165 g of egg biomass on the first oviposition day (Figure 5A, Supplementary Table S1). Generally, BS generated the highest total egg yield (0.936 g; approx. 37,440 eggs) and an average total egg yield of 0.0312±0.113 g per replicate (Figure 5B, Supplementary Table S1); however, none of the total egg biomasses differed significantly at the end of the oviposition period. BS also showed the longest oviposition period at 7.3±1.2 days. Alike BS, BSFERM generated similar total egg biomass of 0.874 g (approx. 32,370 eggs) with an average of 0.291±0.123 g per replicate The females of BSCHAR showed a high oviposition rate at the beginning of the oviposition period, though the oviposited egg biomass declined gradually and ended on day 41, resulting in the significantly shortest oviposition period of 1.3±0.6 days. As a result, BSCHAR generated the lowest total egg biomass at only 0.410 g (approx. 17,083 eggs) with an average of 0.137±0.051 g per replicate, over the entire oviposition period. Similar to those of BSCHAR, females of the BSSORB treatment oviposited the most on the first day of the oviposition period, gradually decreasing over time. BSSORB yielded total egg biomass of 0.510 g (approx. 18,888 eggs) with an average of 0.169±0.029 g per replicate.

The biomass of egg clutches and single eggs did not differ significantly among the treatments, ranging from 0.008 g (BSFERM) to 0.011 g (BSCHAR) for egg clutches and 0.024 mg (BSCHAR) to 0.027 mg (BSSORB; BSFERM) for single eggs (Table 2). The average percentage of unfertilised eggs was highest for BSSORB 15.9%, followed by BSFERM 6.4% and BS at only 1.6%. From the BSCHAR treatment, all egg clutches were fertilised (Table 2).

4. Discussion

In this study, selected life-history traits of BSF were analysed using three pre-treatment methods – BSSORB, BSCHAR, and BSFERM – and a control treatment, BS. The BSF successfully developed on all substrates. Larval and cumulative biomass yield, as well as adult male length differed significantly among treatments.

Table 2. Life history data for black soldier flies reared in a pilot-scale trial on the basic substrate (BS) as a control and the three pre-treatments of the BS with supplemented potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a 10-day fermentation process (BSFERM). Significant differences are shown as superscript letters and were calculated via ANOVA (α=0.05) followed by a Bonferroni correction.

		BS	BSSORB	BSCHAR	BSFERM
Pupae	Average biomass (g)	0.112±0.011^{ab}	0.125±0.015^{a}	0.103±0.014^{b}	0.115±0.016^{ab}
	Average length (mm)	17.5±1.9^{ab}	18.2±1.7^{a}	16.4±2.1^{b}	17.4±1.9^{ab}
	Adult emergence (%)	94.76±2.77^{a}	90.89±4.82^{a}	87.25±3.38^{a}	88.40±2.82^{a}
Adults	Female average biomass (g)	0.060±0.005^{a}	0.065±0.008^{ab}	0.048±0.008^{b}	0.060±0.003^{a}
	Male average biomass (g)	0.050±0.005^{a}	0.054±0.005^{a}	0.043±0.013^{b}	0.051±0.007^{ab}
	Female average length (mm)	14.14±0.44^{a}	14.69±0.16^{b}	12.80±1.12^{c}	13.75±0.58^{a}
	Male average Length (mm)	13.75±0.53^{a}	14.27±0.46^{b}	13.16±1.07^{c}	13.75±0.69^{d}
	Male:female ratio	1.44	2.14	1.23	1.54
Eggs	Egg clutch biomass (g)	0.010±0.003^{a}	0.010±0.004^{a}	0.011±0.003^{a}	0.008±0.003^{a}
	Single egg biomass (mg)	0.025±0.004^{a}	0.027±0.003^{a}	0.024±0.003^{a}	0.027±0.004^{a}
	Unfertilised eggs (%)	1.57±0.00^{a}	15.88±0.02^{a}	0.00±0.00^{a}	6.44±0.01^{a}

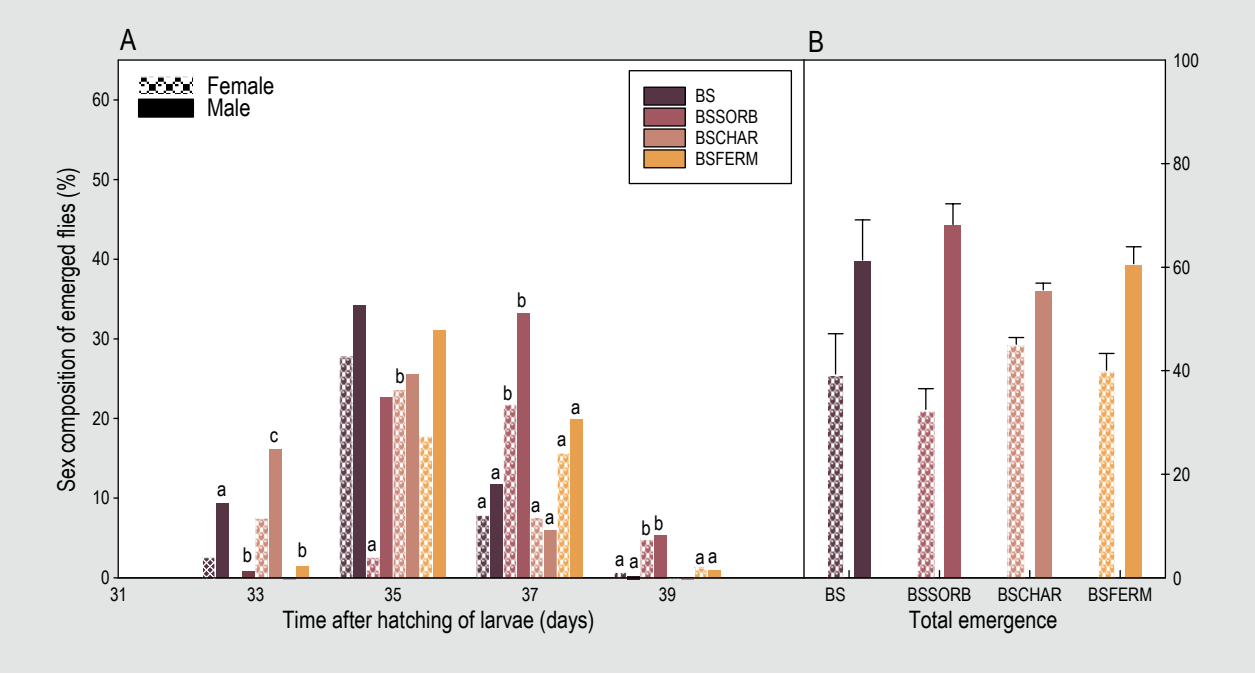

Figure 4. (A) Percentage of black soldier fly females and males over time (days) emerged from Pupae Batch 1 and (B) average percentage of black soldier fly female and male of all adults emerged from Pupae Batch 1 for larvae raised on the basic substrate (BS) as a control and the three pre-treatment variants of the BS with potassium sorbate (BSSORB); biochar (BSCHAR) and after exposure to a 10-day fermentation process (BSFERM) (n=5,590). Significant differences are shown as lowercase letters and were calculated via ANOVA (α=0.05) followed by a Bonferroni correction.

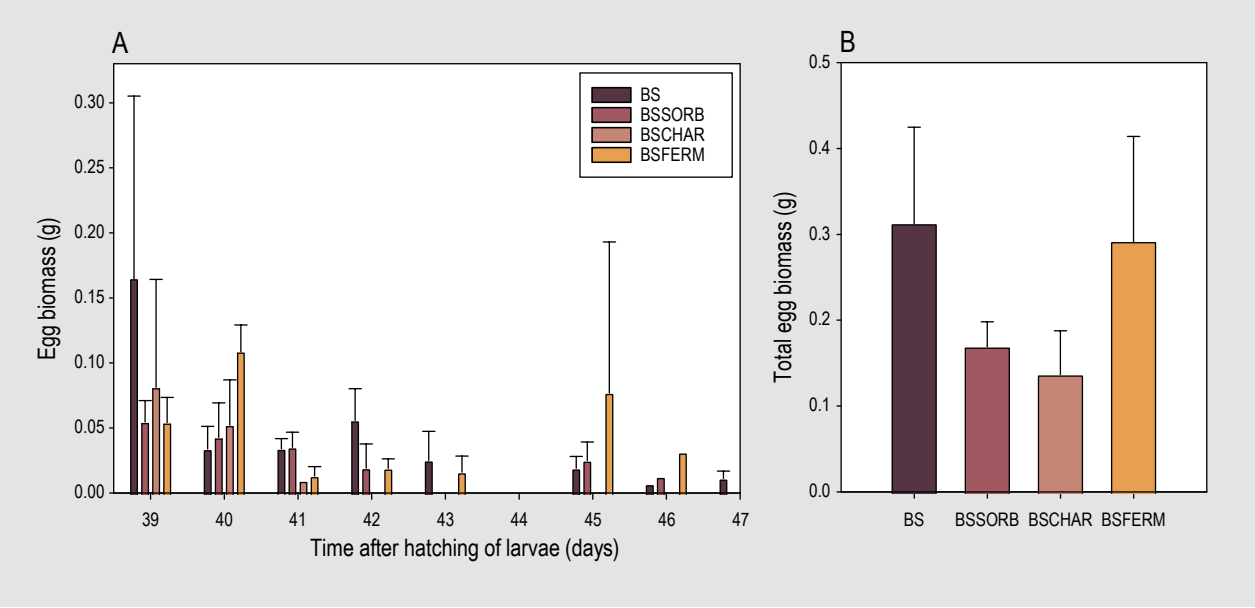

Figure 5. (A) Average oviposited black soldier fly egg biomass (g) per day and (B) the average total egg biomass (g) with standard deviation for the basic substrate (BS) as a control and the three pre-treatment variants of the BS with potassium sorbate (BSSORB); biochar (BSCHAR) and after exposure to a 10-day fermentation process (BSFERM) (n=56). No significant differences were observed.

Feeding on BSCHAR yielded a significantly lower total larval biomass, defined by the lowest cumulative total biomass made up by the significantly lightest and smallest pupae. This indicated insufficient nutrient availability for larval growth and pupal development. Beniers and Graham (2019) found that dietary nutrients, like protein and carbohydrates, have a significant influence on larval and pupal fresh weight. Biochar is a carbon-based material

known to promote organic matter degradation and thus represents a way to improve the physicochemical and microbiological dynamics involved in the reduction of organic wastes (Roberts *et al.*, 2010; Sánchez-García *et al.*, 2015; Sanchez-Monedero *et al.*, 2018). However, few studies have analysed the impact of biochar on BSFL and, to the best of our knowledge, no study has analysed its effect on the entire BSF life cycle. Qin *et al.* (2022) reported that biochar accelerates the development of BSFL, which is consistent with our data, as the fastest development was seen for BSCHAR. The latter might also indicate a stress reaction, considering the results of the other life-history traits. Those authors also reported no significant difference in larval growth between the control and treatments with 2, 5 and 8% of biochar added to the feed. According to Lalander *et al.* (2019), time until the first prepupation can vary between 12-42 days, depending on the substrate. This alone is not an indication of stress, but fast development in combination with considerably smaller larvae might indicate insufficient nutrient availability. Beesigamukama *et al.* (2020) conducted a feeding experiment in which spent grain from the brewing process was amended with different concentrations of biochar (5, 10, 15, 20%). In contrast to our results, they showed that larval biomass yield was significantly higher with higher concentrations of biochar than the control without biochar. An analysis

Figure 6. Replicates of black soldier fly larvae fed with the basic substrate (BS) as a control and the three pre-treatment variants of the BS with potassium sorbate (BSSORB); biochar (BSCHAR) and after exposure to a 10-day fermentation process (BSFERM).

of larval development reared at different concentrations of biochar mixed with BS would need to be conducted to assess the precise effects of biochar concentration on larval development. Nonetheless, our study indicated that apart from a faster larval development, all other results indicate a poorer performance of BSCHAR on selected life-history traits compared with the other pre-treatments and the control: smallest and lightest adults, with the lowest adult emergence, and the lowest total egg biomass. During the larval stage, sufficiently large amounts of proteins and fat have to be accumulated to avoid a need for the adult to feed. For this reason, the quality of food available plays a key role in the subsequent development (Gobbi *et al.*, 2013). In terms of textural properties, applying the biochar pre-treatment significantly increased the hardness of the substrate while it did not affect its adhesiveness. Biochar is difficult to be decomposed by BSFL, and therefore, its contribution to larval growth is limited. However, it can improve the growth environment of BSFL by enhancing substrate ventilation and bulkiness due to its large surface area and porosity (Qin *et al.*, 2022). Generally, biochar offers exchange sites for ions of various elements and also for potentially toxic chemicals. Thus, biochar might, in the case of contaminated substrates, beneficially act as a trap for undesired heavy metals, xenobiotics, pharmaceuticals, and so on (Gałwa-Widera, 2021). Furthermore, considering that BSF frass and biochar are growing in popularity as sustainable fertilisers (Beesigamukama *et al.*, 2020, 2021; Klammsteiner *et al.*, 2020), a combination of both should be further investigated. The effects of BSFL-based animal feed produced from larvae that have been reared on biochar-supplemented substrates have not been investigated yet. BSFL of the BSCHAR treatment had a darker colour, which could affect the protein and fat composition of the larvae (usually white, yellow colour) and the quality of products derived therefrom (Figure 6).

The BSORB treatment generated the significantly highest total larval biomass and highest cumulative total biomass, the heaviest and longest pupae, leading to the longest and heaviest adults with the highest adult emergence. The male:female ratio was the highest for BSSORB but not significantly different among treatments. Tomberlin *et al.* (2009) have shown that the development time of the larvae is sex-specific, meaning longer larval development leads to a higher occurrence of females. This can be explained by females being heavier and longer than males, and also needing more nutrients for egg production; hence, they need more time to develop (Hoc *et al.*, 2019). The feed was provided only for 12 days, and it would be interesting to compare the sex ratio for longer feeding periods using all pre-treatments. The total egg biomass was second lowest with the highest share of unfertilised eggs, though this was not statistically significant. Notably, most unfertilised eggs were oviposited at a late stage when no males were alive anymore, suggesting that females oviposit unfertilised eggs shortly before death. Moreover, studies have shown that food quality has a direct effect on fertility (Kim *et al.*, 2021). Further studies on the effects of potassium sorbate and the fertility of BSF eggs are needed. As far as we know, this is the first study analysing the effect of potassium sorbate on BSF. Potassium sorbate has various positive effects on biomass, larval development, and adult emergence, and may be useful to avoid competition stress exerted by the growth of yeasts and moulds. Further studies assessing the latter are needed. A study performed by Singh and House (1970) used potassium sorbate to improve the artificial diets of the flesh fly *Agria affinis*. They showed that high concentrations of potassium sorbate led to less effective larval growth as well as lower pupation and adult emergence, though they used much higher concentrations (the lowest was 100 mg/100 ml diet) than we did in this study.

A more natural, cheap, and sustainable way to prolong the shelf-life of organic substrates is lactic acid fermentation (Waqas *et al.*, 2019). The BSFERM treatment showed similar results to the control (BS) treatment in larval development time, total larval, and pupal total biomass, as well as pupal average biomass and length. The BSFERM treatment only differed significantly in terms of adult male length. Likewise, the results for the total egg biomass were similar to those of BS. BSFERM oviposited comparably large biomass of eggs on day 45. However, the period for oviposition was slightly but insignificantly longer for BS, where females were still able to oviposit on day 47, with a higher percentage of fertilised eggs than for the BSFERM treatment. According to Van Huis *et al.* (2020), BSF can oviposit from 3-5 to 10-17 days after eclosion, which is comparable with the oviposition periods measured for BS and BSFERM (Figure 3). Further studies should be performed to compare the effect on larval growth of different fermentation methods and fermentations performed at different temperatures. Substrate fermentation may enhance the digestibility of AIBPs used for insect farming: several studies conducted with different substrates (e.g. corncob, coconut endosperm) and fermentation treatments indicated that fermentation prior to feeding the BSFL can increase larval protein and lipid content (Li *et al.*, 2015; Van Campenhout, 2021; Wong *et al.*, 2019). Another study performed by Kuttiyatveetil *et al.* (2019) showed that using *Lactobacillus plantarum* (NRRL B4496) for fermentation of starflower (*Borago officinalis*) improved not only larval growth but also the lipid and protein amount in the larval body. However, it is important to mention that these studies were performed to analyse the subsequent production of insect protein and/or biodiesel from insect biomass. Therefore, the authors did not focus on the entire life cycle of the BSF.

All pre-treatments have their advantages and disadvantages. The choice of pre-treatment will depend on the targeted final product. BSSORB offers a promising method to prolong the shelf-life of the substrate (Aminzare *et al.*,

2014), but it is not suitable to continuously maintain BSF colony, owed to the lower oviposition success. BSCHAR might be a good option for the production of a frass-based fertiliser but might be problematic for the production of a BSFL-based feed. Based on Kuttiyatveetil *et al.* (2019), BSFERM may be beneficial for the larvae's lipid and protein content and when animal feed is the end product (Barragan-Fonseca *et al.*, 2017); however, the preparation of substrate might be challenging and involves more work (Waqas *et al.*, 2019). The proposed pre-treatments can be extended to other AIBPs, but further investigations should be performed. Also, the optimum time and temperature of the fermentation would have to be determined. By comparing ways how to improve AIBPs as BSF rearing substrate, this study will hopefully help to reuse and recycle more AIBPs more often and on larger scales.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2022.0044

Table S1. Summary of all life history data for black soldier flies reared in a large-scale trial on the basic substrate (BS) as a control and the three pretreatments of the BS with supplemented potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a ten-day fermentation process (BSFERM).

Table S2. Texture analysis results of the basic substrate (BS) as a control and the three pretreatments of the BS with supplemented potassium sorbate (BSSORB), biochar (BSCHAR), or subjected to a ten-day fermentation process (BSFERM).

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Conflict of interest

The authors declare no conflict of interest.

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