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Black soldier fly larvae composting of plant-based food and agro-industry waste

Process efficiency and impact of pre-treatment

Lovisa Lindberg

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Lovisa Lindberg
Faculty of Natural Resources and Agricultural Sciences
Department of Energy and Technology
Uppsala

SLU
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Cover: The black soldier fly's life cycle encircling plant-based waste evokes nature's quiet wisdom—where decay feeds renewal—and reflects a circular vision of waste management rooted in transformation rather than disposal. Created by me for this thesis.

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Abstract

Black soldier fly larvae (BSFL) composting is becoming increasingly recognised as a sustainable bioconversion technology aligned with circular economy principles. However, when applied to plant-based food and agro- industry waste—such as cucumber plant residuals, orange peels, and vegetable mixes—its biological efficiency and economic viability remains unclear. This study evaluated the effects of various pre-treatments (ammonia, fungal, enzymatic) and co-substrate additions (*e.g.*, frass) on BSFL process efficiency, greenhouse gas and ammonia emissions, and larval biomass yield across both small- and large-scale systems. Whilst fungal and enzymatic pre-treatments improved material reduction, their impact on biomass conversion efficiency (BCE) was substrate- and scale-dependent. The addition of both enzymes and frass significantly enhanced BCE and larval yield in small-scale systems but was less effective at larger scales. Direct emissions of CH_4 and N_2O were low relative to CO_2, however, ammonia pre-treatment increased NH_3 emissions. Despite reduced larval yields, BSFL composting achieved substantial waste volume reduction and partial degradation of pesticide residues. These environmental benefits suggest that the technology may still offer a viable treatment pathway for nutritionally imbalanced, fibrous waste streams, particularly where conventional composting or anaerobic digestion is unsuitable. For successful large-scale implementation, system modifications—such as improved environmental control, passive harvesting strategies, and decentralised processing models—will be essential. Overall, BSFL composting presents a promising waste management solution for plant-based residues, with potential value lying more in environmental services than in larval biomass production.

Keywords: Biological treatment; plant-based waste; *Hermetia illucens*; pre-treatment; co-treatment; ammonia; *Trichoderma reesei*; enzyme; frass

Fluglarvskompostering av växtbaserat livsmedels- och jordbruksavfall med den amerikanska vapenflugan. Processeffektivitet och påverkan av förbehandlingen.

Sammanfattning

Fluglarvskompostering med larver från den amerikanska vapenflugan uppmärksammas alltmer som en hållbar metod för biomassaomvandling i linje med principerna för en cirkulär ekonomi. När metoden tillämpas på växtbaserat avfall från livsmedels- och jordbruksindustrin – såsom gurkplantrester, apelsinskal och blandade grönsaksrester – är dess biologiska effektivitet och ekonomiska bärkraft dock fortfarande osäker. Denna studie utvärderade hur olika förbehandlingar (med ammoniak, svamp eller enzymer) samt tillsats av substrat (t.ex. frass) påverkar processeffektivitet, utsläpp av växthusgaser och ammoniak, samt larvbiomassans tillväxt i både småskaliga och storskaliga system. Svamp- och enzymbehandling förbättrade materialreduktionen, men effekten på biomassaomvandlings-effektiviteten (BOE) visade sig bero på både substrattyp och systemets skala. Tillsats av enzymer och frass ökade BOE och larvtillväxten i småskaliga försök, men gav begränsad effekt i större system. Direkta utsläpp av metan och lustgas var låga i förhållande till koldioxid, medan ammoniakförbehandling ökade ammoniak-utsläppen. Trots begränsad larvproduktion uppnåddes betydande reduktion av avfallsvolym och delvis nedbrytning av bekämpningsmedel. Dessa miljövinster tyder på att metoden kan utgöra ett relevant behandlingsalternativ för näringsmässigt obalanserade och fiberhaltiga avfallsströmmar, särskilt där konventionella metoder som kompostering eller rötning är mindre lämpliga. För att möjliggöra storskalig implementering krävs dock systemanpassningar – exempelvis förbättrad miljökontroll, passiva separeringsstrategier samt decentraliserade behandlings-modeller. Sammantaget erbjuder fluglarvskompostering en lovande lösning för hantering av växtbaserade restprodukter, där det huvudsakliga värdet snarare ligger i miljönytta än i larvbiomassa som produkt.

Nyckelord: Biologisk behandling; växtbaserat avfall; *Hermetia illucens*; förbehandling; sambehandling; ammoniak; *Trichoderma reesei*; enzym; frass

Preface

Becoming a doctor has been a dream of mine since I was sixteen. From an early age, I have been deeply fascinated by environmental issues and the urgent challenges facing our planet. Over time, this broad interest gradually took shape, into a focused passion for engineering solutions in resource management and recycling. It was through this journey that I found my true calling: to improve process efficiency in the recovery and reuse of valuable materials, contributing to a sustainable and circular economy.

This work reflects my commitment to applying engineering principles to real-world environmental problems, optimising processes to maximise resource utilisation and minimise waste. It is the result of years of learning, experimentation, and determination to make a meaningful difference in how we manage the Earth's finite resources.

I am grateful to everyone who has supported me along this path, helping me grow both personally and professionally, and enabling me to pursue this lifelong ambition.

Dedication

To my fiancé, William, whose unwavering love, patience, and encouragement have been my greatest strength. Your belief in me has given me the energy to keep going, especially knowing how hard you work to keep everything else running smoothly when I need to focus. Your quiet dedication and support have made every challenge feel possible, and it all means more than words can say.

To my wonderful children, who fill my life with joy, laughter, and purpose. You inspire me to be better and to strive for a future where you can thrive. This is for you — may you always chase your dreams with courage and kindness.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. L. Lindberg, E. Ermolaev, B. Vinnerås, C. Lalander (2022). Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment. Journal of Cleaner Production, 338

II. L. Lindberg, B. Vinnerås, C. Lalander (2022). Process efficiency in relation to enzyme pre-treatment duration in black soldier fly larvae composting. Waste Management, 137, pp. 121-127

III. L. Lindberg, B. Vinnerås, C. Lalander. Process efficiency and impact of enzyme and frass additions in black soldier fly larvae composting with fibrous plant-based waste. Journal of Environmental Chemical Engineering, 13(5)

IV. L. Lindberg, I. Lopes G., B. Vinnerås, C. Lalander. Pilot scale waste management using black soldier fly larvae composting with fibrous plant-based waste in Sweden (manuscript)

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Abbreviations

BCE	Biomass conversion efficiency
BSF	Black soldier fly
BSFL	Black soldier fly larvae
M.Red	Material reduction
WW	Wet weight
TS	Total solids
VS	Total volatile solids
GHG	Greenhouse gas
C/N	carbon-to-nitrogen ratio

1. Introduction

The rapid increase in global food production in recent decades has been crucial to meet the demands of a growing population (FAO, 2023). According to the Food and Agriculture Organization (FAO, 2023), global agricultural output has more than doubled since the 1960s, driven by advances in mechanisation, irrigation, synthetic fertilisers, and high yield crop varieties.

However, this intensification has also led to unprecedented levels of waste generation across the supply chain, from production and processing to retail and consumption. It is estimated that approximately one-third of all food produced globally is lost or wasted, amounting to around 1.3 billion tonnes per year (United Nations Environment Programme, 2024). This waste not only includes edible food but also large volumes of inedible plant material such as peels, stems, husks, and crop residues.

The inadequate handling of biodegradable waste (biowaste) presents serious environmental and logistical challenges (Ercoli et al., 2023). When biowaste is landfilled or dumped, it undergoes anaerobic degradation, releasing methane (CH_4), a greenhouse gas with a global warming potential more than 25 times greater than carbon dioxide (CO_2) over a 100-year period (Ercoli et al., 2023).

Additionally, the decomposition of nitrogen-rich biomass can result in emissions of reactive nitrogen, contributing to air pollution, eutrophication, acidification, and global warming (EEA, 2024). The main reactive nitrogen compound, ammonia (NH_3), can be emitted in particular high amounts from poorly managed compost heaps, especially when the carbon-to-nitrogen ratio is not balanced or not under aerobic conditions (Castro-Herrera et al., 2023). Methane, ammonia, and nitrous oxide (N_2O)—another potent greenhouse gas produced during incomplete nitrification/denitrification (IPCC, 2019)—are pollutants that exacerbate climate change either directly or indirectly.

Food loss and waste represent a significant inefficiency in the global food system because all resources used in food production—such as water, land, and energy—are wasted if the food is not consumed (Skawińska and Zalewski, 2022). Horticultural crops—including high-value fruits and vegetables such as tomatoes and cucumbers, as well as staple vegetables more broadly—experience some of the highest postharvest loss rates, often exceeding one-third of total production (Gage et al., 2025; Gage et al., 2024).

This is largely due to their perishable nature, stringent quality requirements, and susceptibility to diseases, pests, and climate variability, all of which exacerbate resource wastage and environmental impacts across the supply chain unless effectively managed. In horticulture, large and seasonal quantities of similar types of plant waste (*e.g.*, damaged fruits and vegetables, leaves and stems) are generated, creating challenges for conventional waste management methods, which often struggle to effectively process these high volumes of homogeneous, seasonal waste biomass (Mehta et al., 2024).

Although conventional composting methods, such as industrial-scale windrow systems, can be employed, the process typically requires several months (usually 3–4 months) to convert waste into stable compost. Substantial space is also necessary to accommodate the full processing of all biowaste (Andraskar et al., 2023).

If the material is too high in carbon, lignin and pectin (*e.g.*, dry leaves) and lacks nitrogen, microbial activity is reduced, leading to a significant increase in composting time (Eiland et al., 2001). As prolonged processing times may be associated with increased operational costs, there is an evident demand for more rapid treatment methods for such waste streams. This need is particularly pressing given that the resulting compost product generally holds low market value (approximately 300 SEK per tonne[1]), which is insufficient to offset treatment expenditures.

Another growing concern within agriculture and biodegradable waste management is the presence of pesticide residues in crop residuals. As intensive farming relies heavily on chemical pest control—targeting insects, weeds, fungi, and other harmful substances—and such residues are frequently found in crop residuals (Bajwa and Sandhu, 2014). These compounds can persist in the environment, leach into soil and water, accumulate in biomass and pose risks to non-target organisms as well as human health (Gunther et al., 2010; Löfkvist et al., 2009).

Circular waste management strategies that recover nutrients and reduce emissions are a potential pathway to mitigate nutrient losses and emitted emissions. One such approach is black soldier fly larvae (BSFL, *Hermetia illucens* L. (Diptera: Stratiomyidae)) composting, a biological process in

[1] https://www.sysav.se/privat/produkter-tjanster/jordprodukter/gronkompost

which insect larvae rapidly convert biodegradable waste into high-value products (Tomberlin et al., 2015). BSFL can consume a wide variety of biodegradable substrates, concentrating proteins and lipids into their biomass, allowing the larvae to be used as feedstock in biofuel production or as valuable feed ingredients (Suckling et al., 2021; Surendra et al., 2016).

The treatment residue, called frass, is a nutrient-rich material suitable for use as an organic fertiliser due to its high content of nitrogen (3%), phosphorus (1-5%), and potassium (0.5-4%) (Lopes et al., 2022), which is comparable to other organic fertilisers (Bhandari et al., 2019).

Compared to conventional composting or landfilling, BSFL composting has been shown to emit significantly less methane and ammonia, notably when operated under controlled conditions (Ermolaev et al., 2019; Mertenat et al., 2019). BSFL composting is comparatively simpler to operate than anaerobic digestion, requiring less stringent control of environmental and process parameters (Surendra et al., 2016).

Despite its environmental advantages, the efficiency of BSFL-based bioconversion—measured by biomass conversion efficiency (BCE) and material reduction—depends heavily on the type and quality of the organic input material (Gold et al., 2018). Single-stream fruit and vegetable waste sources, particularly those with low protein and high lignocellulosic content, tend to yield poor larval growth and result in incomplete degradation (Isibika et al., 2021; Lemme and Klüber, 2024). To overcome these limitations, studies investigating the impact of bio-chemical and biological pre-treatments of these biowaste streams on the bioavailability of nutrients and degradation have been conducted (Dzepe et al., 2025; Gold et al., 2022; Rehman et al., 2025).

Considering the growing need for circular waste management systems, there is a growing interest in innovative bioconversion approaches such as BSFL composting. However, further research is needed to assess the feasibility of such processes, with particular attention placed on the effects of pre-treatment methods, emission profiles, and whether the resulting products meet the relevant criteria in terms of nutrient content, pesticide residues, and volume reduction for their intended applications. Addressing these aspects is essential to determining whether BSFL composting is genuinely feasible, sustainable, and safe for large-scale implementation as a waste valorisation strategy for fibrous and homogeneous biowaste streams.

2. Aim and structure

2.1 Aim and objectives

The overall aim of this thesis was to investigate the feasibility of black soldier fly larvae (BSFL) composting as a treatment method for fibrous, homogeneous food and agro-industrial waste streams, and to evaluate the effects of biological and chemical pre-treatments on process efficiency—specifically in terms of biomass conversion efficiency and material reduction—using both laboratory- and pilot-scale setups. In addition, greenhouse gas emissions and pesticide degradation were examined in selected parts of the study.

Specific objectives were:

- To investigate the process efficiency in BSFL composting when pre-treating fruit and vegetable waste with fungi and ammonia, and to evaluate the associated direct emissions of greenhouse gases and ammonia during the two treatment steps of the process (Paper I).
- To investigate the impact of enzyme pre-treatment duration on process efficiency in BSFL composting of vegetable waste (Paper II).
- To identify an efficient treatment strategy for BSFL composting of fibrous cucumber plant residuals by applying additions of either enzymes, frass, or a combination of both, to increase process efficiency (Paper III).
- To assess the general feasibility of on-site BSFL composting of fibrous cucumber plant residuals using the treatment strategy identified in Paper III at pilot-scale, with a focus on process efficiency, impact on pesticide residues and system complexity (Paper IV).

2.2 Thesis structure

This thesis is based on four studies. Study I evaluated two different pre-treatments and their impact on both process efficiency in BSFL composting and direct greenhouse gas emissions (GHG) from the treatment. Study II investigated whether directly adding enzymes would be more effective than using fungi pre-treatment (evaluated in Study I) and determined the optimal pre-treatment duration needed to enhance process efficiency in BSFL composting. Study III assessed the impact of various additives, along with selected process parameters, on a fibrous substrate on process efficiency in BSFL composting. In parallel, a master's thesis project investigated the impact on pesticide residue in one studied set-up. The best performing treatment in terms of biomass conversion efficiency, material reduction, and harvestability in Study III was selected for further testing in Study IV. The selected treatment was evaluated in a pilot-scale experiment conducted on a cucumber production farm. In Study IV, the assessment focused on process efficiency, the impact on pesticide residues and the technical complexity of the process.

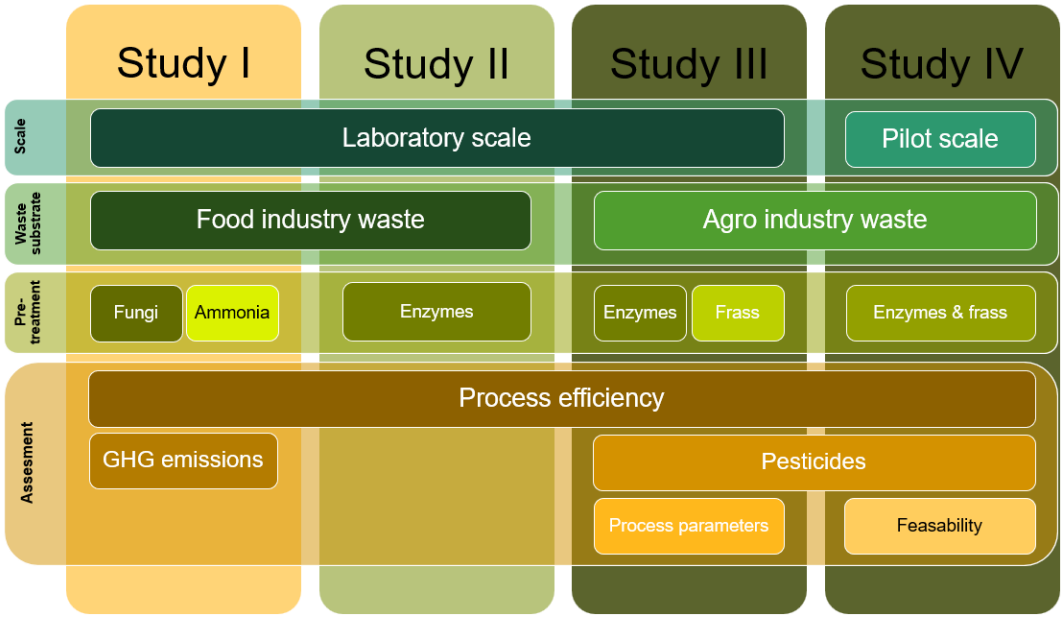

Fig 1. Schematic illustration of thesis structure.

3. Background

3.1 Life cycle of a black soldier fly

One insect species that can be efficiently applied to fly larvae composting is the black soldier fly (BSF), *Hermetia illucens,* which belongs to the order Diptera and the family Stratiomyidae. Optimal rearing conditions for this fly species vary depending on the developmental stage and involves the regulation and monitoring of temperature (~27°C), relative humidity (~75%), light exposure, and nutritional requirements (Nayak et al., 2024; Tomberlin and Sheppard, 2002; Tomberlin et al., 2009). The BSF is found worldwide, from 45°N to 40°S, and is thought to have originated from the American continent (Sheppard et al., 1994).

The BSF has four main life cycle stages, namely egg, larva, pupa, and fly (adult stage). In natural environments, female BSFs lay between 400 and 800 eggs in proximity to heaps of mixed decomposing organic matter, such as carcases, manure, and plant-based wastes (Laursen et al., 2024; Sheppard et al., 1994). At an ambient temperature, the eggs hatch after around four days and they begin to consume nearby food substrates. The larvae pass through six instars before metamorphosing into flies. In the final larval stage prior to pupation, called prepupal stage, the larvae stop eating (Myers et al., 2008; Sheppard et al., 1994) as their mouthparts transform into a hook-like structure, making it easier for them to exit the rearing substrate. Under optimal conditions, which include adequate nutrition and temperature, the larvae develop into the prepupal stage within approximately 16 d. During this stage, the larvae empty their digestive tract and actively migrate out of the substrate in search of drier soil for pupation (Sheppard et al., 1994).

Early BSF systems took advantage of the inherent migratory behaviour of the prepupae which facilitated the development and optimisation of autonomous self-harvesting systems (Diener et al., 2011a; Newton et al., 2005). In contrast to successful mating, the pupal stage necessitates a dry and dark environment to facilitate the eclosion of flies (Holmes et al., 2013). Adult BSFs measure 15–20 mm in length and have a lifespan of approximately one to two weeks, sustained by lipid reserves accumulated during the larval stage (Oliveira et al., 2015). As they do not feed during the adult stage, they are not vectors of disease transmission and, unlike houseflies, do not seek out waste or food sources (Nguyen et al., 2015).

3.2 BSFL treatment as a biowaste management strategy

In their natural habitat, BSFL can consume substantial quantities of organic matter, with reported intake rates reaching 507 mg/larva/day (Diener et al., 2011b). This capacity makes them particularly well suited for effective biological waste treatment, offering the potential to recover and recycle nutrients that would be otherwise lost, thereby helping to close the nutrient loop (Purkayastha and Sarkar, 2021). The treatment is applicable at both small and large scales and does not require advanced infrastructure, rendering it suitable for implementation in regions across the economic spectrum (Lalander et al., 2018; Salomone et al., 2017).

BSFL treatment is divided into two distinct components: a fly colony for oviposition and a composting facility for the bioconversion of organic waste. These two components require different environmental conditions regarding terms of temperature, humidity, and lighting (Nayak et al., 2024). The treatment approach may be implemented in centralised facilities for large-scale waste processing, decentralised systems suitable for community-level or on-site biowaste management, or a hybrid, which provides a flexible solution (Grau et al., 2022).

Using BSFL composting to recycle biowaste offers the opportunity to reduce the reliance on landfilling (Amrul et al., 2022). The landfilling of biowaste is commonly associated with a range of environmental issues, including GHG emissions, eutrophication, and public health hazards (Ferronato and Torretta, 2019).

Although larval composting can also be conducted using the common housefly (*Musca domestica*), this species poses additional sanitary risks due to its potential role as a disease vector (Kenis et al., 2018). Furthermore, its larvae are smaller and therefore more difficult to separate from the compost substrate (Kenis et al., 2018).

One advantage of the BSFL composting system is that it can facilitate the conversion of biowaste into valuable and marketable products (Lohri et al., 2017), potentially generating supplementary income that may offset the operational costs of the waste management system.

3.3 BSFL treatment products

3.3.1 Larval biomass

One of the two BSFL composting products is larval biomass, whose most studied components are proteins and lipids. The larval biomass typically contains a crude protein content of approximately 40% dry matter (DM), although this can vary by around ±5 percentage points depending on the substrate as well as the size and age of the larvae (Do et al., 2020; Ewald et al., 2020; Makkar et al., 2014). Variations in protein content related to larval age have been attributed to the presence of non-protein nitrogen compounds, such as chitin, which can result in an overestimation of protein content (Do et al., 2020; Lalander et al., 2019; Liland et al., 2017). Whilst no definitive conversion factor has been established for protein quantification in BSFL, it has been proposed that a factor of 4.76 is more appropriate than the conventional factor of 6.25 that is commonly used for generic protein determination (Janssen et al., 2017).

The variation in fat content of BSFL biomass is substantially influenced by the fatty acid composition of the substrate, and has been shown to vary by ±15 percentage points in larvae reared on different substrates (Ewald et al., 2020). The fatty acids in the BSFL biomass are primarily comprised of short-chain saturated fatty acids (Ewald et al., 2020; Meneguz et al., 2018b).

BSFL biomass has been shown to serve as a suitable alternative to conventional protein sources such as soybean meal and fishmeal in aquaculture and other animal feed formulations due to their high protein and lipid content (Fatima et al., 2023; Weththasinghe et al., 2022). Soybean-derived feeds frequently lack adequate quantities of certain essential amino acids, including methionine and lysine (Khodadoust et al., 2024). These amino acids are vital for optimal biomass growth in animals and thus typically require supplementation with synthetic amino acids or fishmeal.

Notably, these amino acids—lysine, methionine, tryptophan, threonine, cysteine, and valine—are present in the fifth and sixth larval instar stage at concentrations exceeding those found in soybean-based feeds, enabling the larvae to serve as a viable alternative protein source in animal nutrition (Alagappan et al., 2025; Khodadoust et al., 2024; Surendra et al., 2016). This substitution has the potential to reduce the demand for soybean cultivation which has been associated with significant biodiversity loss due to extensive

pesticide application and monocultural farming practices (Green et al., 2019).

Although an indirect relationship between soy cultivation and deforestation may exist, further research is needed to elucidate this linkage. Deforestation is primarily driven by the expansion of cattle farming, whilst soy cultivation often replaces former pasturelands. This displacement effect prompts the establishment of new pastures elsewhere, thereby indirectly contributing to deforestation (Kuschnig et al., 2021; Kuschnig and Giljum, 2019). Thus, limiting deforestation would reduce CO_2 emissions associated with the removal of forest biomass.

Regarding fishmeal utilisation in animal feeds, BSFL have been identified as a sustainable alternative. Although about 89% of total aquatic animal biomass is ultimately used for human consumption, a substantial portion of wild-caught fish (approximately 50%) and a significant share of aquaculture production (around 20-25%) are processed into fishmeal and fish oil used as animal feed, primarily sustaining the growing aquaculture sector itself, which highlights considerable indirect reliance on wild fish for farmed fish production (FAO, 2024; Roberts et al., 2024). Its use in pig and poultry feed involves notable inefficiencies, with approximately 100 kg of fishmeal-based feed producing only 20–25 kg of chicken meat or 13–17 kg of pork (University of Gothenburg, 2025). With tightening regulations aimed at sustainable fishing practices, expanding fishmeal production to meet the growing meat demand will become increasingly challenging (FAO, 2022).

Waithaka et al. (2022) found that incorporating BSFL meal into the diets of indigenous chickens in Kenya improved growth rates and provided a cost-effective protein source. According to the authors, this approach could support sustainable poultry farming practices in regions with limited access to conventional feed ingredients. Laying hens fed with diets that include BSFL biomass have been reported to have improved product quality such as increased eggshell thickness, contents of egg yolk, and albumin (Kawasaki et al., 2019). van Heugten et al. (2022) reported that supplementing nursery pig diets with BSFL oil, in replacement of corn oil, resulted in increased average daily gain, improved feed conversion ratios without affecting feed intake, and increased final pig body weight after a 40-d test period. This suggests that BSFL oil is a palatable and effective dietary fat source for pigs.

Emerging evidence suggests that BSFL and their derived products may exert probiotic-like effects in animals, particularly in poultry. These effects

are thought to be mediated through the larvae's natural microbiota as well as bioactive compounds such as antimicrobial peptides, chitin, and potential probiotic genera associated with nutrient absorption (Moll et al., 2022; Xu et al., 2025). When included in poultry feed, BSFL meal has been shown to modulate gut microbiota composition, often resulting in increased populations of beneficial bacteria such as *Lactobacillus* spp. and reduced levels of potentially pathogenic taxa such as Enterobacteriaceae (Saidani et al., 2025). Moreover, BSFL may enhance gut barrier function and stimulate immune responses, thereby contributing to improved gut health and disease resistance (Hender et al., 2021; Yu et al., 2019).

The provision of live BSFL as a feed supplement for laying hens has garnered attention due to its potential benefits for both animal welfare and nutritional performance (Dabbou et al., 2025; Machado Tahamtani et al., 2021). In addition to serving as a rich source of high-quality protein and lipids, live BSFL may stimulate natural foraging behaviours, which would improve behavioural enrichment among laying hens. Two studies have reported no negative effects on laying performance when live BSFL were incorporated into the diet (Dabbou et al., 2025; Machado Tahamtani et al., 2021). However, when given live BSFL *ad libitum*, the hens gained weight more rapidly, ultimately achieving a higher body weight and greater protein intake compared to those in the other treatment groups (Machado Tahamtani et al., 2021).

Although BSFL supplementation had a minimal impact on the overall microbial diversity in the excreta, it influenced specific microbial taxa and was linked to reduced stress levels among the hens (Dabbou et al., 2025). However, the nutritional variability of larvae, resulting from variability in their rearing substrate and developmental stage, necessitates careful formulation to ensure balanced dietary provision.

3.3.2 Treatment residue

The BSFL composting process also yields a treatment residue, known as frass and comprises of larval excreta, unprocessed waste fractions and larval exuviae (Amrul et al., 2022). With a functional resemblance to conventional organic fertilisers, BSFL residues possess the potential to serve as a nutrient-rich organic fertiliser (Lopes et al., 2022). However, recent findings indicate that frass is not a biologically stabilised and may continue to undergo active decomposition after collection (Lopes et al., 2024; Lopes et al., 2022). This

instability can lead to potential phytotoxic effects and ammonia volatilisation (Bohm et al., 2023).

Despite these limitations, frass has demonstrated several agronomic benefits. It has been shown to reduce nitrogen leaching by up to 87% compared to synthetic fertilisers applied at equivalent rates, without negatively impacting plant growth or leaf quality (Beasley et al., 2023). In addition to nutrient content, frass contributes to soil microbial enrichment, introducing viable microbial communities and enzymatic activity that can promote nutrient cycling and organic matter turnover in the substrate (Choi and Hassanzadeh, 2019; Lopes et al., 2024). It also contains biologically active compounds such as chitin and groups of microorganisms. Chitin contributes to enhanced plant health primarily by boosting defence and disease resistance mechanisms, whilst rhizobacteria (also present in frass) can colonize plant roots and promote growth by enhancing nutrient uptake, improving soil health, and possibly inducing systemic resistance in plants (Barragán-Fonseca et al., 2022).

Given frass' lack of biological stability, further processing steps such as composting, drying, or blending with stabilising agents may be necessary before field application to ensure agronomic safety and efficacy (Lopes et al., 2024). Alternatively, treatment residues may be directed into other valorisation pathways, such as anaerobic digestion for biogas production, although their heterogeneous composition and ongoing biological activity may require process optimisation (Lalander et al., 2018).

3.4 Risks with circular waste management

The integration of BSFL composting of biowaste within a circular economy framework—aimed at closing nutrient and energy loops in the food system—poses potential safety concerns due to the risk of toxic contaminants accumulating in the food chain. These include residues of pharmaceuticals and pesticides, mycotoxins, bacterial toxins, heavy metals, and pathogenic microorganisms (Mufungwe et al., 2025; Ogbon et al., 2025). Such contaminants are particularly concerning as they may form stable toxic compounds capable of disrupting biochemical processes and physiological functions in plants and animals upon ingestion (Alengebawy et al., 2021).

3.4.1 Pathogens

Lalander et al. (2015) and Erickson et al. (2004) observed that BSFL composting plays a role in reducing certain pathogenic microorganisms, including *Salmonella* spp. and *Escherichia coli.* Nevertheless, other studies have reported the presence of parasitic oocysts or eggs—such as *Eimeria tenella*, *E. nieschulzi*, and *Ascaris suum* (Müller et al., 2019)—as well as pathogenic bacteria including *Salmonella* spp., *Enterococcus* species (Lalander et al., 2013), and *Bacillus cereus* (van Kessel et al., 2024) within both the larvae and the resulting treatment residues. To minimise the risk of disease transmission to humans and animals, further processing of the larvae and residues has been suggested to eliminate any pathogenic microorganisms, with treatments such as ammonia or heat sanitation (Lalander et al., 2013; Müller et al., 2019), drying (Erickson et al., 2004; Lalander et al., 2013), and blanching (Bessa et al., 2020).

Drying both the larvae and the frass for 1 min at 100°C to mimic blanching demonstrated that *Bacillus cereus* endospores persisted and were transferred into both larvae and frass (van Kessel et al., 2024). It is recommended to test the substrate ingredients for *Bacillus cereus* endospores prior to BSFL rearing to prevent their introduction into the feed and food chain, therefore ensuring the safety of the final product for both animals and humans (van Kessel et al., 2024).

3.4.2 Pesticides

The presence of pesticides complicates the treatment of biodegradable waste, as certain compounds exhibit toxicity towards specific microbial communities and require specialised microorganisms that are less efficient at degrading waste (Fogg et al., 2003). In the context of BSFL composting, emerging evidence suggests that the larvae and their gut microbiota may help in degrading, or transforming, numerous pesticides, to varying degrees depending on the compound type and concentration (Alagappan et al., 2022; Lalander et al., 2016; Yang, C. et al., 2022). However, the efficiency of pesticide degradation may depend on the characteristics of the biodegradable waste used in BSFL composting. Substrates that are more readily digested by the BSFL could enhance the degradation of pesticides: *e.g.* nutrient-rich or well-balanced substrates could accelerate larval metabolism and associated microbial interactions, thereby facilitating more effective degradation of the pesticides (Meijer et al., 2021).

Simultaneously, the potential for bioaccumulation in larval biomass must be carefully monitored, particularly when the larval biomass is intended for use in animal feed. There is no evidence that shows the bioaccumulation of pesticides (*e.g.* λ-cyhalothrin, azoxystrobin, chlorpyrifos, chlorpyrifos-methyl, cypermethrin, imidacloprid, pirimiphos-methyl, propiconazole, propoxur, spinosad, and tebufenozide) in the larvae (Lalander et al., 2016; Mbokou Foukmeniok et al., 2024; Meijer et al., 2021). However, whilst some pesticides do not appear to interfere with the process efficiency, others do—especially at higher concentrations, where the survival and larval size have been shown to decrease (Mbokou Foukmeniok et al., 2024).

It remains unclear whether the pesticides are fully mineralised during BSFL composting or if they form intermediate compounds, where some potentially degrade into secondary products. These degradation products may themselves exhibit biological activity, thereby influencing the composting process. Further research is needed to understand these transformation pathways and their implications (Mbokou Foukmeniok et al., 2024).

3.5 Emissions

Climate dynamics are influenced, among other factors, by the concentration of greenhouse gases (GHGs) in the atmosphere (IPCC, 2023). Whilst studies on greenhouse gas emissions from fly larvae composting exist, there remains a need for more comprehensive data, particularly at larger scales and when using vegetable-based substrates. Most studies investigating GHG emissions from the BSFL treatment system have mainly focused on the BSFL composting phase and have been conducted at laboratory scale (Chen, Jiangshan et al., 2019; Ermolaev et al., 2019; Pang et al., 2020; Parodi et al., 2020; Wu et al., 2026), although some have been measured on somewhat larger scale (Parodi et al., 2021; Xiang et al., 2024a). These studies have examined larvae feed on various substrates, including food waste, agricultural residues, sludge and animal manure (Chen, Jiangshan et al., 2019; Ermolaev et al., 2019; Pang et al., 2020; Parodi et al., 2020; Parodi et al., 2021; Wu et al., 2026; Xiang et al., 2024a).

In contrast, other stages of the BSFL production cycle—such as the fly rearing unit—have received comparatively little attention in terms of their contribution to overall GHG emissions. Many of the earlier life cycle

assessment studies conducted on the BSFL treatment system used generic emission data for insects, rather than measurements derived from specific BSFL treatment systems (Halloran et al., 2016; Mondello et al., 2017; Smetana et al., 2016). Unlike many earlier studies that used generic emission factors, Pishgar-Komleh et al. (2022) measured both direct emissions from the nursery/rearing phase and indirect emissions such as electricity use, finding that energy consumption during the nursery phase is a significant contributor to the overall GHG emissions of BSFL production (Pishgar-Komleh et al., 2022). These findings are in line with the findings of Mertenat et al. (2019), who employed a life cycle assessment (LCA) approach to quantify the global warming potential (GWP) by measuring direct GHG emissions at a BSFL waste treatment facility during the BSFL composting phase, comparing the results with those from an open windrow composting system. Their findings indicated that the BSFL treatment system is associated with a lower GWP than conventional composting methods. This highlights that context-specific factors such as energy use for machinery operations and transport play a much larger role in the overall GHG emissions of BSFL production than direct biological emissions from larvae rearing alone.

The ammonia (NH_3) emissions have been shown to range between 0.05 and 0.5 g/kg initial wet weight (ww), whilst emissions of methane (CH_4) and nitrous oxide (N_2O) ranged between 0.03 and 8.6 g/kg initial ww from BSFL composting. These findings indicate that the emissions are largely dependent on the type of diet and rearing methods, among other factors (Chen, Jiangshan et al., 2019; Ermolaev et al., 2019; Pang et al., 2020; Parodi et al., 2020).

Compared to aerobic composting (Ermolaev et al., 2015; Ermolaev et al., 2012; Xiang et al., 2024a), emissions from BSFL composting were generally lower across all measured gases. However, this reduction of emissions may be attributed to the incomplete degradation of the substrate, suggesting that further emissions could arise during subsequent decomposition processes during post-treatment of the residue which can increase by 20-52% (Beesigamukama et al., 2023) and consumption of the larval biomass. In the study by Parodi et al. (2021), BSFL composting increased the emissions of NH_3 but is discussed by the authors that the emissions when applied on the field, might be lower than when fresh manure is directly applied on the field.

One approach to reducing GHG emissions from the BSFL treatment system involves the application of pre-treatment strategies. However, bacterial pre-treatment showed no observable effect on emissions during the BSFL treatment system of food waste, as emissions generated during the pre-treatment phase itself were not included in the assessment (Ermolaev et al., 2019).

3.6 BSFL bioconversion process

3.6.1 Substrate degradation

The degradation of biowastes in BSFL composting is a multifaceted process involving both larval enzymatic activity and the metabolic functions of their associated gut microbiota (Yu et al., 2023). Larvae primarily consume the more easily available and nutrient-rich components of the substrate, such as simple carbohydrates, proteins, and lipids, whilst the degradation of complex polymers such as cellulose, hemicellulose, and lignin is typically slower and possibly more heavily reliant on microbial enzymatic activity (Liu et al., 2020).

BSFL possess a well-developed digestive system capable of secreting a range of endogenous enzymes, including proteases, lipases, and amylases, which facilitate the breakdown of proteins, fats, and simple carbohydrates, respectively (Seyedalmoosavi et al., 2022). Upon ingestion, the substrate is sorted and small parts can break down into finer parts in the mouth, which is described as a “sort of tunnel boring machine” (Bruno et al., 2020), and is further processed in the gut, where enzymatic hydrolysis releases nutrients for absorption (Bonelli et al., 2019; Bruno et al., 2020).

In addition to larval digestion, the gut microbiome plays a crucial role in the decomposition of more recalcitrant compounds, such as cellulose, hemicellulose, and pectin, by producing specialised enzymes including cellulases and pectinases. This microbial contribution is particularly important for the degradation of fibrous plant materials, which are otherwise poorly digested by the larvae alone (Liu et al., 2020). Furthermore, BSFL movement within the substrate promotes mechanical disruption and aeration, altering physicochemical parameters—such as temperature, pH, and moisture—and creates a more favourable environment for microbial colonisation (Čičková et al., 2015).

The efficiency of substrate degradation is influenced by several operational and substrate factors, such as the carbon-to-nitrogen (C/N) ratio, particle size, and environmental conditions such as temperature and aeration, all of which affect microbial growth and larval feeding behaviour (Lu et al., 2021; Yakti et al., 2023). High-protein substrates generally result in higher larval biomass yields, whilst high-fibre or lignin-rich substrates may slow down the degradation process and reduce larval growth unless pre-treated or supplemented (Gold et al., 2020a; Liu et al., 2020).

3.6.2 Density, feeding rate, temperature, and moisture content

The efficiency of the treatment is affected by various factors such as larval density, feeding rate, and the temperature and moisture content of the substrate (Dzepe et al., 2020; Lopes et al., 2023). Higher larval densities typically enhance substrate degradation rates due to increased feeding activity and bioconversion, resulting in more rapid biomass accumulation and a greater reduction in organic matter (Dzepe et al., 2020).

However, excessively high densities can lead to overcrowding, which may reduce oxygen availability and increase competition for resources, ultimately impairing larval growth and survival (Jones and Tomberlin, 2019). Conversely, low larval densities may prolong processing times and reduce overall treatment throughput. Optimising larval density is therefore essential to balance rapid waste degradation with larval health and biomass yield, taking into account the nature of the substrate and environmental conditions (Dzepe et al., 2020).

Whilst the entire feed substrate can be supplied at the start of BSFL composting, it is generally recommended to divide the substrate into equal portions and administer these at 2 to 4 d intervals during the first week (Banks et al., 2014). This approach helps to reduce surface crust formation and minimises the risk of unprocessed substrate accumulating at the bottom of the treatment containers (Banks et al., 2014). High feeding rates, typically between 200 and 250 mg per larva per day, have been associated with shorter development times to the prepupal stage and increased final larval biomass when compared with lower feeding rates (Nyakeri et al., 2019).

Reduced feeding rates tend to limit larval weight gain, likely due to intensified competition for nutrients. However, when paired with a high larval density, low feeding rates have also been shown to enhance substrate degradation efficiency (Lopes et al., 2023; Paz et al., 2015). These findings

suggest that feeding regimes in BSFL composting systems can be optimised according to larval density and tailored to meet specific treatment objectives (Lopes et al., 2023; Paz et al., 2015).

Composting with a high BSFL density and elevated feeding rates should be avoided, as it may induce excessive temperature increases and a substrate pH drop below 6, as well as generate a large substrate depth, which can hinder larval development and minimise the total waste reduction (Lopes et al., 2023; Paz et al., 2015). Substrates with a high moisture content (>90%) tend to destabilise the treatment process by promoting anaerobic conditions, forcing the larvae to consume more substrate to obtain their nutritional requirements (Lalander et al., 2020). This may alter the microbial community, potentially shifting it towards a microflora less conducive to efficient decomposition, thereby reducing both BCE and overall waste reduction during BSFL composting (Lalander et al., 2020; Schreven et al., 2022). Throughout the BSFL composting process, the substrate's moisture content typically declines, whilst the temperature rises (even more so with higher larval densities) and pH shifts from acidic or neutral levels towards more alkaline conditions (Klammsteiner et al., 2025).

These parameters are interdependent, and optimal conditions for achieving higher BCE and material reduction seem to include a substrate moisture content below 90% (depending on the ventilation regime), a larval density between 2.5 and 6.5 larvae/cm^2 also to maintain moderate temperatures and substrate depth below 5 cm, and a feed dose of 0.1–0.2 g VS/larva to minimise unprocessed substrate (Klammsteiner et al., 2025; Lalander et al., 2020; Lopes et al., 2023; Paz et al., 2015).

3.7 Nutritional demand of BSFL

One of the key advantages of BSFL composting is its capacity to process a wide range of biowastes, including food waste, abattoir by-products, human waste, fruit and vegetable residues, and sewage sludge (Gold et al., 2020a; Lalander et al., 2019). Combining vegetables and legumes as feed substrates has been shown to enhance BCE to levels comparable with those achieved using mixed food waste (Gold et al., 2020a).

However, the protein content in the larvae were lower when they were fed with vegetable canteen waste compared to mixed food waste, which Gold

et al. (2020a) attributed to the higher C/N ratio of the former, leading to increased fat accumulation in the larvae.

Similarly, Lalander et al. (2019) observed that a mixture of abattoir waste and fruit and vegetable waste yielded higher protein conversion efficiency compared to the individual substrates. This improvement was attributed to a more balanced protein-to-carbohydrate ratio in the mixed substrate, facilitating more effective nutrient utilisation by the larvae.

3.7.1 Nutrients required

The most important nutritional parameters for the development of BSFL include protein, non-fibre carbohydrates (NFC), fibre, and lipids (Barragán-Fonseca et al., 2018a; Barragán-Fonseca et al., 2018b; Gold et al., 2018; Lalander et al., 2019), which are part of the total volatile solids and suggested to be 0.2 g VS per larva (Lopes et al., 2023). Imbalanced nutrient ratios within the substrate can prolong larval development, reduce biomass production, and waste reduction efficiency (Lu et al., 2021). Among the substrate's nutritional characteristics, protein content and the proportion of readily available carbohydrates have been identified as the most influential (Lu et al., 2021).

Depending on the intended application of the larvae, maintaining an approximate 1:1 ratio of protein to NFC has proven to be beneficial, as an excess of NFC increases larval biomass without enhancing the protein content (Gold et al., 2020a). A higher protein concentration in the substrate is desirable when the larvae are destined for use as a protein source in animal feed, as it elevates the larval protein content (Lopes et al., 2020).

Conversely, reducing lipid and fibre levels in the substrate is advantageous because a high fibre content—especially when combined with low protein and NFC concentrations—has been associated with reduced BCE and substrate reduction, and lower larval weight (Gold et al., 2020a). Notably, fruit and vegetable wastes are generally low in protein and lipids but high in fibre, thereby providing insufficient nutrition to support optimal BSFL growth.

3.7.2 Nutrients in fruit and vegetable waste

Fruit and vegetable wastes are widely generated biowaste streams that exhibit considerable variability in nutrient composition, depending on crop type, maturity, and post-harvest handling (Angulo et al., 2012). These wastes

are generally characterised by a high moisture content and are rich in easily degradable carbohydrates such as glucose, fructose, and sucrose, particularly in fruits. However, they typically contain low levels of protein and lipids, resulting in a relatively high C/N ratio (Song et al., 2020) that often exceeds the optimal range for efficient bioconversion by insects or microbes (Gold et al., 2020a).

Fibre content, including cellulose, hemicellulose, and lignin, is usually elevated, particularly in vegetable stems and peels, which can reduce digestibility and slow down decomposition (Gao et al., 2025). Despite these limitations, fruit and vegetable residues are valuable sources of micronutrients such as potassium, calcium, magnesium, and various vitamins and polyphenolic compounds, which may support microbial activity and influence larval development in bioconversion systems such as BSFL composting (Borel et al., 2021; Coman et al., 2020; FAO, 1998). Their compositional profile suggests that, although nutritionally imbalanced on their own, these residues could be effectively utilised when blended with protein- or nitrogen-rich substrates to enhance process efficiency.

3.7.3 Degradation of lignocellulose

Lignocellulose degradation is a complex biochemical process that involves the breakdown of plant biomass composed primarily of cellulose, hemicellulose, and lignin. Microorganisms, including fungi and bacteria, are key contributors to the process, secreting a range of enzymes that work together to break down the rigid lignocellulosic structure (Rehman et al., 2025). Lignin, a complex and irregular aromatic polymer, forms a protective barrier around cellulose and hemicellulose in plant cell walls, making it highly resistant to microbial and enzymatic attack, which limits degradation efficiency, necessitating pre-treatment strategies to disrupt the lignin matrix and improve microbial access to cellulose and hemicellulose (Rehman et al., 2025).

Whilst BSFL are not known to produce high levels of lignocellulolytic enzymes themselves, they rely heavily on their gut microbiota to assist in breaking down complex lignocellulosic materials such as agricultural and plant-based wastes (Kariuki et al., 2023; Xiang et al., 2024b). The symbiotic microorganisms within the larval gut secrete enzymes such as cellulases, hemicelluloses, and, to a lesser extent, ligninases, which facilitates the partial degradation of lignocellulose into simpler sugars and aromatic compounds

(Xiang et al., 2024b). This microbial-assisted process enhances nutrient availability, allowing the larvae to efficiently assimilate carbon and energy for growth (Kariuki et al., 2023). Although the degradation of lignin is limited, BSFL can still thrive on substrates with a moderate lignocellulosic content as long as lignocellulosic degrading microorganisms are present (Xiang et al., 2024b).

3.8 Pre-treatments

The main objective of pre-treating a substrate that is high in lignocellulose prior to BSFL composting is to facilitate lignin disruption, alter the crystalline structure of cellulose, increase the substrate's surface area, and ultimately improve the degradation efficiency (Rehman et al., 2025). There are a wide range of pre-treatment approaches divided into different categories such as physical, chemical, physicochemical, and biological techniques, as well as various combinations of these methods.

3.8.1 Physical

Physical pre-treatments predominantly aim to reduce particle size, increase surface area, and disrupt the rigid architecture of lignocellulose, thereby improving enzyme accessibility during subsequent enzymatic hydrolysis (Peguero et al., 2024). Ball or hammer milling are widely used techniques that breaks down biomass into finer particles, leading to a reduction in cellulose crystallinity and an increase in the surface-to-volume ratio (Rehman et al., 2025). However, milling can be energy-intensive, depending on the desired particle size (Amin et al., 2017).

Grinding and mixing of the feed substrate are generally recommended to produce a uniform mixture with optimal particle sizes, ensuring efficient consumption and digestion by BSFL (Paz et al., 2015; Peguero et al., 2024).

3.8.2 Chemical

Chemical pre-treatments prior to BSFL composting are employed to improve the digestibility of complex organic substrates (Isibika et al., 2019). These treatments involve the application of acids, alkalis, or oxidising agents to disrupt the structural integrity of lignin and hemicellulose, which increases the accessibility of cellulose and other nutrients for microbial and larval digestion (Rehman et al., 2025).

Alkaline pre-treatments, using substances such as sodium hydroxide or lime, are particularly effective in solubilising lignin and swelling biomass, which could make degradation easier for the larvae and their gut microbiota (Amin et al., 2017).

Acid pre-treatments, typically involving dilute sulphuric or hydrochloric acid, hydrolyse hemicellulose and partially open up the biomass structure, but they may generate inhibitory by-products such as furfurals if not properly controlled (Amin et al., 2017).

Integrating a chemical pre-treatment could potentially accelerate the bioconversion process, enhance larval growth, and increase biomass yield. However, careful optimisation is essential to avoid toxicity to the larvae and to ensure environmental safety in large-scale applications.

Ammonia pre-treatment

Ammonia pre-treatment is used to enhance the digestibility of lignocellulosic biomass by disrupting its complex structure (Peguero et al., 2023). This approach mainly targets lignin, since ammonia has a strong affinity for lignin and can partially solubilise or chemically modify it, particularly in non-condensed regions (Zhao et al., 2020). It breaks ether and ester linkages in lignin-carbohydrate complexes, which are responsible for cross-linking lignin to hemicellulose and cellulose (Zhao et al., 2020). This delignification process reduces the overall rigidity of the biomass and increases porosity, improving the accessibility of cellulose and hemicellulose for enzymatic or microbial degradation (Zhao et al., 2020).

Unlike acid treatments, ammonia does not extensively degrade sugars, which preserves the carbohydrate content of the substrate (Amin et al., 2017). Notably, cellulolytic bacteria (*e.g.*, species of *Bacillus* and *Streptomyces*) thrive in ammonia-pretreated environments, taking advantage of the increased substrate porosity and reduced lignin content to accelerate decomposition (Book et al., 2016; Sharma et al., 2016). The degradation process is typically synergistic: lignin-degrading microorganisms first modify or remove lignin barriers, allowing cellulolytic and hemicellulolytic organisms to access and deconstruct the polysaccharide components more efficiently (Su et al., 2018). In BSFL composting systems, these microbial processes occur both in the substrate and within the larval gut, where the microbiota further assists in digesting partially decomposed material (Xiang et al., 2024b; Yu et al., 2023).

In addition to its role as a delignifying agent, ammonia could also serve as a valuable supplemental nitrogen source in pre-treatment processes, particularly when applied to substrates with a low nitrogen content, such as lignocellulosic agricultural residues (Au - Chundawat et al., 2020). This dual function is especially beneficial in biological systems such as BSFL composting, where balanced C/N ratios are critical for efficient microbial activity and larval growth (Lu et al., 2021).

During ammonia pre-treatment, some of the applied ammonia becomes chemically bound to biomass components through ammonolysis, forming compounds such as ammonium salts or amides. However, a portion remains bioavailable in the substrate, thereby enriching its nitrogen profile (Balan et al., 2025). This additional nitrogen can stimulate the growth of microorganisms, which require nitrogen for protein synthesis and metabolic function (Balan et al., 2025).

In BSFL systems, enhanced microbial degradation can improve substrate digestibility, whilst the larvae themselves would directly benefit from increased nitrogen availability, which supports protein biosynthesis and biomass accumulation (Isibika et al., 2019). According to Pang et al. (2020), an improved C/N balance can benefit the process efficiency and reduce ammonia volatilisation during larval rearing.

Ammonia raises the pH of the treated substrate to alkaline levels, often exceeding pH 9. This shift can inhibit certain microbial contaminants and pathogens, potentially improving the hygienic quality of the substrate (Magri et al., 2015). This also requires careful pH adjustment prior to biological systems such as BSFL composting even though substrates with pH 9.5 have been shown to have no effect on BSFL development (Meneguz et al., 2018a).

However, some researchers argue that ammonia pre-treatments may be unsuitable for BSFL composting due to ammonia toxicity, as a dose-dependent response has been observed—wherein higher ammonia concentrations and/or prolonged exposure prior to larval feeding were associated with more pronounced negative effects (Peguero et al., 2023). Either way, careful control of ammonia concentration and treatment conditions is necessary to avoid ammonia toxicity and to ensure environmental and operational safety.

3.8.3 Biological

Biological pre-treatments involve the use of microorganisms—primarily fungi and bacteria—or their enzymes to break down the structural components of lignocellulosic biomass (Rehman et al., 2025). Biological pre-treatments are usually performed at near-neutral pH and at ambient or slightly raised temperatures. They produce fewer inhibitors compared to chemical methods, making them more compatible with downstream biological processes such as BSFL composting (Rehman et al., 2025). However, these methods are generally slower as they may require longer incubation times, which can limit their application at industrial scales unless integrated with other pre-treatment strategies (Amin et al., 2017).

Fungal pre-treatment

Fungal pre-treatments primarily employs ligninolytic fungi, particularly white-rot species such as *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus*, which can degrade lignin through the production of oxidative enzymes such as laccases, lignin peroxidases, and manganese peroxidases (Andlar et al., 2018). These enzymes selectively target and modify the lignin matrix and open the phenyl rings without significantly affecting the cellulose and hemicellulose fractions, thereby increasing the accessibility of polysaccharides for subsequent microbial or enzymatic hydrolysis (Amin et al., 2017; Andlar et al., 2018).

Fungal methods produce minimal toxic by-products but are frequently limited by their relatively slow processing time (often several weeks) to achieve significant delignification and biomass modification. They are also sensitive to environmental factors such as moisture, temperature, and oxygen availability, and thus require careful optimisation for practical applications (Amin et al., 2017; Suryadi et al., 2022).

It has been reported that carbohydrates are consumed during fungal pre-treatments, which is considered a disadvantage (Amin et al., 2017). However, some studies have demonstrated that the concentrations of nitrogen and crude protein per unit mass of pre-treated dry matter increase during fungal treatment (Jalc et al., 1997; Zeng et al., 2011). Despite a reduction in total dry matter, there does not appear to be a corresponding loss of nitrogen, and the incorporation of fungal biomass into the substrate may contribute to an overall increase of protein content (Jalc et al., 1997) which is particularly relevant when followed by BSFL composting.

Enzyme pre-treatment

Enzymatic pre-treatments offer a targeted approach for enhancing the degradability of lignocellulosic biomass by selectively hydrolysing structural polymers (Suryadi et al., 2022). This method employs purified or crude enzyme preparations to break down cellulose, hemicellulose, and, to a lesser extent, lignin into simpler, more bioavailable compounds (Suryadi et al., 2022). Enzymatic treatments provide distinct advantages due to their relatively rapid action compared to fungal colonisation, which enables shorter processing times.

By reducing polymer complexity and increasing soluble sugar content (Suryadi et al., 2022), enzymatic pre-treatment have been shown to enhance microbial activity and substrate digestibility for the larvae (Dzepe et al., 2025). The combination of heat and cellulase addition followed by a short fermentation improved the BCE compared to when using enzymes alone (Dzepe et al., 2025).

However, the effectiveness of the enzymatic pre-treatment approach is influenced by multiple factors, including enzyme specificity, loading rate, substrate composition, and reaction conditions such as pH and temperature (Suryadi et al., 2022). Furthermore, the relatively high cost of commercial enzymes can restrict their large-scale application (Dzepe et al., 2025; Suryadi et al., 2022). Thus, more research investigating enzyme recycling, synergistic enzyme cocktails, and microbial enzyme production aims is encouraged to address these economic constraints (Afedzi et al., 2025; Mohanram et al., 2013).

Frass as an amendment

Frass, the treatment residue from insect bioconversion, is defined by the European Union as "… a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects …" (European Commission, 2021). It is increasingly recognised as a multifunctional input in biological systems, with applications ranging from composting to crop production (Zunzunegui et al., 2025).

Rich in organic matter, nitrogen, phosphorus, and microbial communities, frass offers potential as a sustainable organic fertiliser capable of improving substrate quality, enhancing microbial activity, and increasing overall system efficiency (Choi and Hassanzadeh, 2019; Gold et al., 2020b; Lopes et al., 2024; Lopes et al., 2022). When incorporated into composting or BSFL

rearing systems, frass may contribute to optimising the C/N ratio—depending on feedstock origin—support microbial colonisation and effective organic matter decomposition (Lopes et al., 2024; Lopes et al., 2022).

Beyond its macronutrient profile, frass harbours an active microbial community that can facilitate nutrient mineralisation and biodegradation, whilst simultaneously contributing to plant health through bioactive compounds such as chitin (Barragán-Fonseca et al., 2022). Chitin residues, originating from insect exoskeletons, have been shown to stimulate beneficial microbial populations and suppress soilborne pathogens, thereby enhancing the biostimulant and protective qualities of frass as an organic fertiliser (Kisaakye et al., 2024). In integrated systems, frass may also function as a microbial inoculant or nutrient enhancer when reintroduced into fresh substrate batches, reducing the reliance on synthetic additives and promoting circularity of resources (Lopes et al., 2024)

However, the use of frass as a fertiliser is not without challenges. A major limitation is that it is not biologically stable. Fresh frass often exhibits elevated levels of reactive nitrogen (primarily ammonium), high electrical conductivity, and ongoing microbial respiration—conditions that can lead to uncontrolled nutrient mineralisation, ammonia volatilisation, and phytotoxicity if applied directly to soil or plants (Bohm et al., 2023; Lopes et al., 2024; Lopes et al., 2022). These characteristics are influenced by the type of feedstock the larvae process, as well as the conditions of frass collection and handling. Immature or unstable frass can impair seed germination, stunt early plant development, and alter soil microbial balance in undesirable ways (Bohm et al., 2023).

To mitigate these issues, post-processing strategies such as composting, or controlled recirculation have been proposed (Lopes et al., 2024; Lopes et al., 2022). In particular, recirculating frass within BSFL bioconversion systems has shown promise in enhancing microbial inoculation of fresh substrate whilst promoting gradual stabilisation of the frass itself (Lopes et al., 2024). This approach has the potential to support process efficiency by reducing the demand for external microbial starters or additives but could also improve the overall maturity of the frass before use for crop production.

Ultimately, the efficacy of frass as a safe and effective organic fertiliser depends on carefully managing its origin, composition, and degree of stabilisation (Lopes et al., 2022; Mertenat et al., 2019), which must be considered in any large-scale application strategy.

4. Methodology

4.1 Materials

4.1.1 BSFL

The BSFL used in the experiments were reared on chicken feed (Granngården Hönsfoder Start) prior to the experiments. To enumerate the larvae for the experiments, they were passed through a sieve with 1 mm mesh and had an average weight of 0.20±0.10 g per 100 larvae. The larvae were taken from a BSF colony that has been run by the Environmental Engineering group at the Department of Energy and Technology, SLU, since 2015 (Uppsala, Sweden).

4.1.2 Waste substrates

The orange peels, broccoli stems, cauliflower cuttings (Paper I), lettuce, and cabbage (Paper II) were provided by the fruit and vegetable wholesaler Sorunda Grönsakshall (Stockholm, Sweden). The source separated household food waste (Paper I) was collected from the municipalities of Eskilstuna, Strängnäs, and Örebro, and minced at the recycling plant Lilla Nyby in Eskilstuna (Eskilstuna & Strängnäs Energi och Miljö, Eskilstuna, Sweden) before being transported to the BSFL composting facility at SLU. The cucumber plant residuals were provided by Tinas Grönsaksodling AB (Helsingborg, Sweden) where they were coarsely grinded before being transported to the BSFL composting facility at SLU (Paper III) or provided directly to the mobile BSFL composting container on site (Paper IV).

Preparation of substrates

The food waste was already minced into a slurry before arriving (Paper I) and the plant residuals were also grinded before arriving, but into coarser pieces of 10 cm and smaller which were stored for up to three months at -18°C before use (Paper III) or stored at 4°C for one day (Paper IV). Orange peels, broccoli stems, cauliflower cuttings (Paper I), lettuce, and cabbage (Paper II) were minced separately on site using a blender (Robot Coupe Blixer 4 V, France or Universal Cross, model BG2, Austria). The substrates were either stored for up to 9 d at 15°C before being minced and then directly used in the experiments (Paper I) or minced directly and bagged separately

to be stored for up to 11 d at -18°C before use (Paper II). The frozen substrate was then thawed at room temperature (28°C) for 24 h and thoroughly mixed before being used in pre-treatments or treatments.

4.1.3 Microorganisms

Trichoderma reesei was pre-cultured on malt extract agar (MEA) at 28°C for 7 d and harvested using sterile 0.9% NaCl.

4.1.4 Enzymes

For Paper II, 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*). In Paper III, cellulase and pectinase were the selected enzymes added at a concentration of 1% (*w/w*), in accordance with Paper II.

4.2 Methodology

4.2.1 Experimental set-up

The primary aim of this study was to evaluate whether supplementing a biowaste stream consisting of orange peels, broccoli and cauliflower, lettuce and cabbage, or plant residuals with bio-chemical amendments could improve the process performance of fly larvae composting in terms of increasing BCE, material reduction, larval yield, and larval survival (Figure 1). A total of 24 treatments were evaluated (Table 1), with the food waste treatment serving as the reference for comparison across all other treatments. In Paper I, fungal and ammonia pre-treatments were applied to either orange peels or a mixture of broccoli and cauliflower. Paper II focused on enzyme pre-treatment and pre-treatment duration using a substrate composed of lettuce and cabbage. In Paper III, enzyme and/or frass pre-treatments were evaluated at varying concentrations on cucumber plant residuals. Lastly, Paper IV scaled up the most effective treatment from Paper III—based on BCE, material reduction, and larval yield—for validation at pilot scale (Table 1).

Pre-treatments

The evaluated pre-treatment methods included biological, chemical, bio-chemical, and microbial approaches, as well as combinations of some of these. In Paper I, a 14-16 d pre-treatment with either *Trichoderma reesei* or a 1% (*w/w*) addition of non-protein nitrogen in the form of ammonia was performed at 30°C. The hypotheses behind using these pre-treatments were that the fungi would degrade complex molecules into forms more available to the larvae. Ammonia was added as an additional nitrogen source, for the microorganisms in the substrate to convert non-protein nitrogen into protein through nitrogen assimilation and thereby generate amino acids to the larvae (Zhao et al., 2020), as described previously (see section 3.8.2).

In Paper II, enzyme treatment was investigated. The hypothesis behind using enzymes as a pre-treatment method was that a shorter pre-treatment duration would be possible compared with using fungi. Still, it was expected that a longer enzyme pre-treatment would generate more readily available carbohydrates for the larvae. Another advantage of using enzymes is that the substrate would not be consumed by the fungi. An addition of an enzymatic cocktail containing cellulases, ß-glucosidases, and hemicellulases at a 1% (*w/w*) concentration was added to the substrate to pre-treat for 0 d, 2 d, and 4 d. The substrate was stirred in a bucket that had been placed in a tent at a temperature of 28.8 ± 0.8°C.

Rather than using the concentration to predict how much enzyme to add, the addition was recalculated to reflect enzyme units per g VS in the plant residuals. One unit (1U) is the measurement of the enzyme's catalytic activity. The enzymatic activity is defined as the amount of enzyme that catalyses the conversion of 1 micromole of a chosen substrate per minute under defined conditions. The enzyme catalytic activity depends on conditions such as temperature, pH, and the concentration of the chosen substrate (Cornish-Bowden, 2014). Different amounts of enzymes were targeted: 300 units for cellulase per g PR and 150 units for pectinase per g plant residuals in the base treatment PR-EF (Table 1). These levels were selected to correspond to approximately 1% (*w/w*) enzyme addition and to fall within the activity range reported in the literature, between 60 U/g TS (Edwards et al., 2014) and 19,000 U/g VS (Izaguirre et al., 2019).

In Paper III, cellulose and pectinase were used in the enzyme pre-treatment (1% *w/w*) to degrade complex molecules in the fibrous cucumber plant residuals. The results from Paper II indicated that a 0 d pre-treatment

performed better than 2 and 4 d. To compensate for the high moisture content of vegetable and cucumber plant residuals (PR) – a non-optimal environment for the larvae – frass from BSFL composted pig feed was added. The frass addition was hypothesised to have multiple benefits: 1) make the nutrients in PR more available to the larvae in terms of modifying the protein to carbohydrates ratio; 2) provide more structure; 3) reduce the formation of large air pockets within the bulky plant residues by filling voids, thereby increasing the effective surface area available for microbial or enzymatic action; 4) add beneficial microorganisms from the beginning of the treatment; and 5) promote more efficient BSFL composting process, which could allow for efficient separation of larvae and treatment residue (*i.e.,* remove the need for manually picking the larvae out). Nine different pre-treatments were investigated and involved the use of enzymes, frass, or a combination of both. Variations included half and double enzyme concentrations, as well as larval densities at half, double, and four times the control level. One treatment also included a plastic foam cover placed on top of the substrate, based on observations that the plant residuals tended to dry out before the larvae could digest them. Due to the bulky nature of the plant residuals, varying larval densities were applied to reduce the substrate depth from 11 cm to 5 cm.

The treatment that achieved the highest BCE and material reduction—identified as the most effective in terms of process performance in the third study—was selected for validation at pilot scale in a mobile, temperature-regulated container located at the cucumber farm (Paper IV).

BSFL composting

All treatments were performed in triplicate. In the first and fourth studies, large boxes (60 cm × 40 cm × 11 cm) were used, whereas in the second and third studies, smaller boxes (21 cm × 17 cm × 11 cm) were placed inside larger boxes and arranged within a rack system. Every second to third day, the boxes were rotated in the rack to offset the effects of a temperature gradient (~1°C difference from bottom to top) and differences in air flow in the experimental setting. During the BSFL composting, the temperature ranged between 27 to 33°C in Papers I-III and averaged 25.4 ± 1.3°C in Paper IV.

In Paper I, approximately 15,000 larvae were introduced per large box, yielding a larval density of 6.25 larvae/cm² (Table 1). The larval feeding load

was not adjusted for VS content in this study and ranged from 0.03 to 0.22 g VS/larva.

In Papers II-IV, however, the substrate weight was recorded both before and after pre-treatment to allow for an adjustment to 0.2 g VS per larva.

For the smaller boxes used in Paper II, 300 larvae were added per treatment, resulting in a larval density of 1.5 larvae/cm² (Table 1). The larval number was determined based on a target of 0.2 g VS/larva, whilst ensuring the substrate depth did not exceed 5 cm. In both Papers I and II, larvae were fed three times by adding substrate on top without mixing.

In the third study, 0.2 g VS/larva was targeted for all treatments except two, where 0.1 g VS/larva was used (Table 1). The number of larvae ranged between 500 and 2000, resulting in a larval density of 2.0 – 8.2 larvae/cm^2. Feeding was carried out daily for seven days, except for treatments with half the substrate density, where feeding was distributed across three occasions during the same period.

In Paper IV, each treatment box received 3,500 larvae, corresponding to a larval density of 1.5 larvae/cm² (Table 1). All substrate was provided on the first day, as per the practical constraints of the commercial cucumber farm where the study was conducted. Due to this single feeding approach, the substrate depth was limited to approximately 5 cm, which resulted in a slightly lower larval density than the 2 larvae/cm² applied in Paper III.

Composting durations varied among the studies: in Paper I, the process was terminated upon the first observation of prepupae; in Papers II and III, composting continued for 7 and 9 d after the final feeding, respectively; and in Paper IV, treatment lasted for 19 d. However, all BSFL composting trials ran for a minimum of 14 d (Table 1).

Table 1. List of pre-treatments used in each treatment, duration of pre-treatment and black soldier fly larvae (BSFL) composting, and total amount of substrate on a wet weight (ww) basis added in each treatment. Substrates used in treatments: food waste (FW), orange peels (OP), broccoli and cauliflower trimmings (BC), lettuce and cabbage (LC), cucumber plant residuals (PR). Pre-treatments used: no pre-treatment (C), *Trichoderma reesei* (T), ammonia (A), enzymes for 0d, 2d, and 4d (E0d), added enzymes (E) of different concentrations with frass (E/2, E2), frass (F), different larval densities with enzymes and frass (L2, L4), half substrate density with enzymes and frass (H), and cover (B). Values presented are mean ± SD (n=3), where LD (low deviation) means SD < 0.0008.

Paper		Substrate	Pre-treatment/ addition	Pre-treatment time [d]	BSFL composting time [d]	Feeding occasions	Number of larvae	Larval density [larvae/cm^2]	BSFL total VS load[ii] [g VS/larva]	Total substrate [kg ww]
I	OP-C	Orange peels		-	27	3	15 000	6.25	0.20^{ab} ± 0.003	14.6 ± 0.1
	BC-C	Broccoli and cauliflower		-	28	3	15 000	6.25	0.08^{c} ± LD	15.7 ± 0.1
	FW-C	Food waste		-	17	3	15 000	6.25	0.22^{de} ± 0.004	15.4 ± 0.2
	OP-T	Orange peels	*Trichoderma reesei*	16	31	3	15 000	6.25	0.15^{f} ± 0.02	15.4 ± 0.1
	BC-T	Broccoli and cauliflower	*Trichoderma reesei*	14	23	3	15 000	6.25	0.03^{g} ± 0.004	15.8 ± 0.5
	OP-A	Orange peels	Ammonia	16	35	3	15 000	6.25	0.18^{h} ± 0.003	14.4 ± 0.1
	BC-A	Broccoli and cauliflower	Ammonia	16	30	3	15 000	6.25	0.07^{c} ± 0.004	15.9 ± 0.6
II	LC-C	Lettuce and cabbage		-	14	3	300	1.50	0.22^{de} ± LD	0.86 ± LD
	LC-E0d	Lettuce and cabbage	Enzymes	0	14	3	300	1.50	0.22^{de} ± LD	0.86 ± LD
	LC-E2d	Lettuce and cabbage	Enzymes	2	14	3	300	1.50	0.24^{i} ± LD	1.26 ± LD
	LC-E4d	Lettuce and cabbage	Enzymes	4	14	3	300	1.50	0.22^{de} ± LD	1.21 ± LD
III	PR-C	Plant residuals		-	16	7	680	2.79	0.20^{a} ± LD	1.38 ± LD
	PR-F	Plant residuals	Frass	-	16	7	1 000	4.10	0.21^{bd} ± LD	1.38 ± LD
	PR-E	Plant residuals	Enzymes	-	16	7	680	2.79	0.20^{a} ± LD	1.38 ± LD
	PR-EF	Plant residuals	Enzymes and frass	-	16	7	1 000	4.10	0.21^{bd} ± LD	1.38 ± LD
	PR-E/2	Plant residuals	Enzymes and frass	-	16	7	1 000	4.10	0.21^{bd} ± LD	1.38 ± LD
	PR-E2	Plant residuals	Enzymes and frass	-	16	7	1 000	4.10	0.20^{ab} ± LD	1.38 ± LD
	PR-L2	Plant residuals	Enzymes and frass	-	16	7	2 000	8.20	0.10^{j} ± LD	1.38 ± LD
	PR-L4	Plant residuals	Enzymes and frass	-	16	7	2 000	8.20	0.10^{j} ± LD	1.38 ± LD
	PR-H	Plant residuals	Enzymes and frass	-	16	3	500	2.05	0.18^{h} ± LD	0.69 ± LD
	PR-HB	Plant residuals	Enzymes and frass	-	16	3	500	2.05	0.18^{h} ± LD	0.69 ± LD
	F-C	Frass	[i]	-	11	1	1 000	4.10	0.23^{e} ± LD	0.32 ± LD
	F-E	Frass	Enzymes[i]	-	11	1	1 000	4.10	0.23^{e} ± LD	0.32 ± LD
IV	PR-HB	Plant residuals	Enzymes and frass	-	19	1	3 500	1.46	0.27^{k} ± LD	6.70 ± LD

[i] Water added to the treatment.
[ii] Normally distributed

4.2.2 Sampling

At the end of the BSFL composting process, the combined weight of the larvae and residue was recorded prior to dry separation (Papers I, III, and IV) or wet separation (Paper II). After the separation, the mass of the larvae was recorded, and the mass of the residue was calculated by subtracting the total larval mass from the total mass.

Physical and chemical

Samples for TS, VS, pH, and nitrogen analyses were collected from the substrate before the start of pre-treatment, before the start of BSFL composting and after BSFL composting, both from larvae and treatment residue. Three samples were collected from each replicate for TS and VS, whilst one sample per replicate was collected for pH and nitrogen. Nitrogen and pH were not sampled in Paper II.

Nutritional characterisation

Samples for crude lipids, crude protein, and fibre analyses were collected before and after BSFL composting from the initial substrate and from larvae and residue after the treatment (Papers III and IV). One composite sample was collected per replicate for all measured parameters. Subsamples from five areas of each replicate were merged, totalling to 100 g per sample. The samples were kept at -18°C and shipped chilled for analysis at Eurofins Food & Agro Testing Sweden AB (Swedac-accredited laboratory).

Gas emissions

The gases measured in Paper I were carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3). The gas sampling was performed at the start of the pre-treatment, after 1 w and 2 w. The occasion for initial gas sampling for the BSFL composting phase was the same as the gas sampling performed after 2 w of pre-treatment. During BSFL composting, gas sampling was performed four times in total, before each of the three feeding occasions and at the end of BSFL composting (Paper I).

The gas samples were collected using a chamber technique described in Ermolaev et al. (2019). Gas samples were extracted three times during one gas measurement: 1) directly upon sealing the box, 2) after 20 min, and 3) after 1 h.

4.2.3 Analysis

Physical and chemical

For TS determination, the samples were dried at 60°C for a minimum of 48 h. The lower drying temperature was used to avoid volatilisation of VS. After drying, the samples were combusted by heating at 250°C for 2 h, followed by heating at 550°C for 4 h (modified ISO 18122:2015).

The pH was determined by diluting 10 g of a sample into 50 ml deionised water in a centrifuge tube which was left to acclimatise for 1 h at room temperature prior to pH readings (Level One pH meter, Inolab, with a Sentix electrode, PHM210, MeterLab®, Radiometer, Copenhagen) in Paper I. In Papers III and IV, a solid-state probe (InLab Solids Pro ISM, Mettler Toledo) was inserted directly into the material in each replicate, without the probe touching the bottom of the treatment box.

Total nitrogen measurements were conducted as described in Lalander et al. (2015), using a Crack-test 10 (114544) and Spectroquant© kit number 114764 (Paper I) or samples were sent to Eurofins and determined by the Kjeldahl method (Papers III and IV). A protein conversion factor of 4.76 was employed to calculate the protein content (Janssen et al., 2017).

Nutritional analyses

All nutritional analyses were performed at an accredited Eurofins laboratory (EC reg 152/2009 mod. and NF V 18-122). The gravimetric lipids determination principal using Gerhardt instruments determined the crude lipid content. The Van Soest methodology was used to determine the content of neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL, Papers III and IV).

Gas emissions

Emissions from the gas chamber were extracted and flushed into a vial for later measurements of N_2O and CH_4 using a gas chromatograph (Perkin Elmer Clarus 500, USA) with a flame ionisation detector (FID) and a thermal conductivity detector (TCD). The concentrations of NH_3 and CO_2 were determined on site with a reagent tube connected to a pump (Gas Detector, Kitagawa, Japan, Paper I).

Pesticides

All pesticide analyses were conducted by an accredited Eurofins laboratory. The laboratory quantified the levels of active substances in the plant residuals, the treatment residues, and the larvae. Gas chromatography was employed to detect the active compounds in Floramite 240 SC, Flexity, and Switch 62.5 WG, whilst the remaining pesticides were analysed using liquid chromatography.

4.3 Calculations

The survival was calculated in two steps as:

$$n_{BSFL} = \frac{m_{BSFL}}{m_{larva}} \quad (1)$$

$$Survival_{BSFL} = \frac{n_{BSFL_{end}}}{n_{BSFL_{start}}} \quad (2)$$

where m_{BSFL} is the total mass of the larvae after BSFL composting and m_{larva} is the average weight of one larva, based on three samples totalling to approximately 100 larvae in each, and $n_{BSFL_{end}}$ and $n_{BSFL_{start}}$ being the number of larvae after and prior to BSFL composting, respectively.

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

$$\mathrm{BCE}_{VS} = \left(\frac{m\mathrm{VS}_{larvae}}{m\mathrm{VS}_{initial}}\right) \cdot 100\% \quad (3)$$

where $m\mathrm{VS}_{larvae}$ and $m\mathrm{VS}_{initial}$ is the total mass of VS in larvae and total mass of initial substrate, respectively.

The percentage of material reduction on a VS basis (Red_{VS}) was calculated as:

$$\mathrm{Red}_{VS} = \left(1 - \frac{m\mathrm{VS}_{residue}}{m\mathrm{VS}_{initial}}\right) \cdot 100\% \quad (4)$$

where $m\mathrm{VS}_{residue}$ and $m\mathrm{VS}_{initial}$ is the total mass of VS of residue and total mass of initial material, respectively.

Amount of produced larvae per area on a TS basis was calculated as:

$$\text{Larval yield}_{TS} = \frac{m\mathrm{TS}_{larvae}}{a_{box}} \quad (5)$$

where $m\mathrm{TS}_{larvae}$ is the total mass of TS of the larvae and a_{box} is the area of the treatment box.

The mass balance on a TS basis was calculated as:

$$m\mathrm{TS}_{initial} = m\mathrm{TS}_{larvae} + m\mathrm{TS}_{residue} + m\mathrm{TS}_{loss} \quad (6)$$

where mTS is the total mass of TS of protein, nitrogen, lipids, lignin, cellulose, or hemicellulose in either the initial material, larvae, residue, or lost.

Subtracting acid detergent lignin (ADL) from acid detergent fibre (ADF) gives the cellulose content, whereas the hemicellulose content is estimated as the difference between neutral detergent fibre (NDF) and ADF, whilst the lignin content corresponds to the ADL value.

4.4 Statistical analysis

The gas emission rates were calculated with the Linest function in Excel (Microsoft version 16, USA). Analysis of variance (ANOVA) with 5% significance level was used to evaluate whether the treatments differed significantly during pre-treatment and BSFL composting in terms of material reduction, BCE, gas emissions, larval yield, nutritional content, TS and VS, and degradation of lignin, cellulose, and hemicellulose. Normality was verified in the model residuals using Shapiro-Wilk test ($p>0.05$) and by inspecting the normal Q-Q plot. When normality was verified, a Tukey's Honest Significant Difference (Tukey's HSD) test was performed (5% significance level) to find significant differences between treatments. When normality was not verified, the non-parametric Kruskal-Wallis test was performed, followed by the Dunn test for multiple comparisons (5% significance level) using the Benjamini and Hochberg correction method (Benjamini and Hochberg, 1995) (Paper I). Box-Cox test was used to transformation data to achieve normality (Papers II-IV). General linear regression with 5% significance level was used to assess correlations between response variables and substrate properties. Paired t-test with 5% significance level was used for comparing TS and VS values in different stages in each treatment. ANOVA, regression analyses, non-parametric tests, and graphical representations were made in R (R Core Team, 2024).

5. Results

5.1 BSFL composting (Papers I-IV)

5.1.1 Total solids, total volatile solids, and pH

The total solids (TS) of food waste (FW-C) provided to the larvae were 25% and varied between 7 to 32% in all other treatments. The total volatile solids (VS) in the reference treatment (FW-C) were 89% and varied between 70 to 97% in all other treatments (Table 2). The TS in larvae from the reference treatment were 41% and varied between 19 to 39% in all other treatments. All pH values increased during the fly larvae composting, where the pH in treatments with food waste (FW-C) and broccoli and cauliflower (BC-C) increased by more than 4 pH units.

Table 2. Total solids (TS) and total volatile solids (VS) of all substrates measured before pre-treatment and before BSFL composting, larvae and residue with substrates of orange peels (OP), broccoli and cauliflower trimmings (BC), food waste (FW), lettuce and cabbage (LC), cucumber plant residuals (PR), and frass (F). Pre-treatments used: no pre-treatment (C), *Trichoderma reesei* (T), ammonia (A), enzymes for 0d, 2d, and 4d (E0d), added enzymes (E) of different concentrations with frass (E/2, E2), frass (F), different larval densities with enzymes and frass (L2, L4), half substrate density with enzymes and frass (H) and cover (B). Differences ($p<0.05$) in TS, VS, and pH of before BSFL composting compared with before pre-treatment, larvae, and residue are marked with *. Different superscript letters within columns indicate significant differences ($p<0.05$). Values are presented as mean ± SD (n=3), where LD (low deviation) means SD < 0.001.

Treatment	Before pre-treatment			Before BSFL composting			Larvae		Residues		
	TS [%]	VS [%]	pH	TS [%]	VS [%]	pH	TS [%]	VS [%]	TS [%]	VS [%]	pH
Paper I											
OP-C				21.5^{a} ±0.4	96.7^{a} ±0.4	3.37^{a}±0.04	25.4^{a*} ±1.2	90.0^{a*} ±0.2	31.8^{abc*} ± 1.4	95.0^{a*} ±0.1	3.70^{a*} ±0.04
BC-C				8.20^{b}±0.04	87.4^{bc}±1.1	5.71^{b}±0.17	19.5^{b*} ±1.4	83.0^{bc} ±1.6	29.7^{abc*} ± 5.6	70.5^{bcd*} ±0.6	9.66^{bc*} ±0.11
FW-C				24.7^{c} ±0.1	89.3^{b} ±0.3	4.13^{a}±0.22	40.6^{c*} ±0.9	83.6^{bcd*} ±0.1	69.2^{defg*} ± 3.0	82.4^{e*} ±0.2	8.30^{d*} ±0.11
OP-T	21.1^{a*} ±0.4	96.6^{a*} ±0.1	4.26^{ab*}±0.78	29.8^{d} ±2.9	94.0^{d} ±0.9	3.50^{a}±0.10	23.6^{ab} ±1.0	87.7^{ade*} ±1.7	37.5^{abch} ± 3.8	92.5^{a} ±0.2	3.89^{a} ±0.12
BC-T	8.24^{b} ±0.19	87.4^{b*} ±1.5	5.49^{ac*}±0.02	11.3^{e} ±1.5	70.8^{e} ±2.4	8.12^{c}±0.29	35.3^{cd*} ±2.6	77.3^{fg*} ±2.1	86.9^{d*} ± 2.0	57.5^{fghi*} ±1.2	10.1^{b*} ±0.3
OP-A	21.6^{a*} ±0.2	96.9^{a*} ±0.1	3.74^{b*} ±0.61	19.2^{fg} ±0.2	96.4^{a} ±0.1	6.39^{b}±2.29	24.3^{ab} ±2.0	88.0^{ac*} ±0.8	52.8^{aehi*} ± 4.2	94.2^{a*} ±0.2	7.55^{e} ±0.13
BC-A	8.60^{b*}±0.09	89.5^{c*} ±0.3	5.85^{c*} ±0.29	7.34^{b}±0.20	88.0^{b} ±1.3	7.95^{c}±0.60	23.9^{ab*} ±2.7	86.8^{abde} ±0.9	15.0^{bj} ± 5.1	76.9^{be*} ±1.4	8.19^{d} ±0.04
Paper II											
LC-C				8.55^{b}±0.41	89.0^{b} ±0.5		26.1^{a*} ±0.8	84.7^{bcde*}±0.4	3.87^{ij*} ± 0.82	66.6^{cdfh*} ±5.9	
LC-E0d				8.55^{b}±0.41	89.0^{b} ±0.5		28.9^{ac*} ±1.6	86.8^{abde*}±0.3	2.23^{ij*} ± 0.32	54.0^{g*} ±1.0	
LC-E2d	7.48^{c*}±0.22	88.7^{bc} ±1.0		7.96^{b}±0.01	88.7^{b} ±0.1		27.1^{a*} ±0.7	88.9^{a} ±0.2	3.55^{ij*} ± 0.25	73.2^{bce*} ±2.9	
LC-E4d	7.48^{c} ±0.22	88.7^{bc*}±1.0		7.37^{b}±0.02	85.9^{c} ±0.1		24.4^{ab*} ±2.3	88.5^{ac*} ±0.5	3.50^{ij*} ± 0.52	71.9^{bcd*} ±3.3	
Paper III											
PR-C				13.7^{h} ±LD	71.1^{e} ±LD	7.87^{c}±0.05	25.7^{a*} ±0.5	72.2^{hij} ±0.5	40.9^{achi*} ± 4.7	57.1^{fghi*} ±0.4	9.35^{cf*} ±0.16
PR-F				18.7^{fg} ±LD	75.7^{f} ±LD	7.87^{c}±0.05	24.0^{ab*} ±0.6	73.7^{fhij*} ±0.7	40.7^{achi*} ± 1.6	64.3^{cdfhi*}±0.8	8.96^{fg*} ±0.03
PR-E				13.7^{h} ±LD	71.1^{e} ±LD	7.87^{c}±0.05	25.3^{ab*} ±0.3	72.2^{hij} ±0.7	29.5^{bc*} ± 2.1	56.8^{ghi*} ±2.6	9.27^{cf*} ±0.06
PR-EF				18.7^{fg} ±LD	75.7^{f} ±LD	7.87^{c}±0.05	24.4^{ab*} ±1.1	73.4^{fhij} ±1.0	49.9^{acehi*}± 3.7	64.4^{cdfhi*}±2.4	8.92^{fg*} ±0.09
PR-E/2				18.7^{fg} ±LD	75.7^{f} ±LD	7.87^{c}±0.05	24.0^{ab*} ±1.0	70.7^{hi*} ±0.6	60.0^{efhi*} ± 3.8	64.4^{cdfhi*}±2.6	8.94^{fg*} ±0.24
PR-E2				17.8^{f} ±LD	75.8^{f} ±LD	7.69^{c}±0.11	23.7^{ab*} ±0.4	73.4^{fhij*} ±0.5	36.9^{abch*}± 1.6	64.4^{cdfhi*}±1.4	9.12^{cfg*}±0.04
PR-L2				17.8^{f} ±LD	75.8^{f} ±LD	7.69^{c}±0.11	19.4^{b*} ±0.2	70.0^{i*} ±1.3	47.9^{acehi*}± 2.5	62.7^{dfghi*}±3.5	9.12^{cfg*}±0.10
PR-L4				17.8^{f} ±LD	75.8^{f} ±LD	7.69^{c}±0.11	27.3^{a} ±5.3	74.5^{fhj} ±0.8	83.5^{dg*} ± 7.9	70.6^{bcd} ±2.9	8.66^{dg*} ±0.28
PR-H				17.4^{f} ±LD	70.5^{e} ±LD		33.3^{de*} ±1.0	72.1^{hij} ±0.7	91.1^{d*} ± 0.2	59.3^{fghi*} ±0.6	
PR-HB				17.4^{f} ±LD	70.5^{e} ±LD		26.6^{a*} ±0.4	72.1^{hij} ±1.3	61.2^{efgi*} ± 4.9	54.6^{gi*} ±1.8	
F-C[ii]				31.8^{d} ±0.1	78.6^{g} ±LD		35.0^{cd} ±3.3	75.7^{fj} ±1.6	84.6^{d*} ± 1.0	67.0^{bcdf*} ±0.4	
F-E[ii]				31.8^{d} ±LD	78.6^{g} ±LD		38.7^{cd} ±2.9	81.3^{cg*} ±0.6	80.1^{dfg*} ± 2.2	71.0^{bcd*} ±1.0	
Paper IV											
PR-HB				19.8$^{[i]ag}$	66.7^{ih}	7.70^{c}±0.33	27.0^{a*} ±2.4	74.2^{fj*} ±2.5	34.7^{ac*} ±16.4	58.3^{fghi*} ±7.0	9.17^{f*} ±0.28

[i] Calculated based on measured values from PR and frass separately.
[ii] Water added to the treatment.

5.1.2 Process efficiency

The reference treatment with food waste had 23% BCE on a VS basis for the entire process, which was the highest BCE together with the treatment of lettuce and cabbage (LC-E0d 24%, Table 3). For BSFL composting without pre-treatment, orange peels had the lowest BCE, 5.8% on a VS basis, whilst orange peels pre-treated with *Trichoderma reesei* had the lowest BCE across the entire process. Higher BCEs were reached when using lettuce and cabbage, ranging between 20 and 24% (Table 3).

The material reduction was 64% on a VS basis for the entire process for the reference treatment with food waste (FW-C). The lowest material reduction across the entire process was observed in the treatments with plant residuals and orange peels, more specifically, in the plant residual control at 34% (PR-C). The treatment with enzyme pre-treatment for 0 d with lettuce and cabbage (LC-E0d) had a material reduction of 89%, which was also the highest when compared across the entire process.

Larvae fed food waste reached a weight of 141 mg per larva (Table 4). Heavier larvae were produced in the treatments with lettuce and cabbage (LC, 213-273 mg/larva), whilst larvae from the treatments with orange peels (OP, 18-50 mg/larva) and treatments with plant residuals (PR, 44-48 mg/larva) were generally smaller.

The highest larval yield was obtained in the reference treatment with food waste (369 mg TS/cm²), followed by the treatments with frass, those pre-treated with enzymes (215 mg TS/cm²), and the frass control (104 mg TS/cm²). The treatments with lower larval yields ranged between 26-33 mg TS/cm^2 and were the ones pre-treated with *Trichoderma reesei*.

The survival in the reference treatment (FW-C) was 100% and for the other treatments, it ranged between 28 to 100%. The treatments with plant residuals generally had high survival (PR -EF, -E/2, -L2 among others), whereas the treatment with fungi pre-treated broccoli and cauliflower (BC-T) had the lowest survival at 28%.

Table 3. Material reduction (Red) after pre-treatment; Red and biomass conversion efficiency (BCE) after black soldier fly larvae (BSFL) composting on a volatile solids (VS) basis and for the entire process on a VS and wet weight (ww) basis for the different substrates. Substrates used in treatments: food waste (FW), orange peels (OP), broccoli and cauliflower trimmings (BC), lettuce and cabbage (LC), cucumber plant residuals (PR). Pre-treatments used: no pre-treatment (C), *Trichoderma reesei* (T), ammonia (A), enzymes for 0d, 2d, and 4d (E0d), added enzymes (E) of different concentrations with frass (E/2, E2), frass (F), different larval densities with enzymes and frass (L2, L4), half substrate density with enzymes and frass (H), and cover (B). Values presented are mean ± SD (n=3). Different superscript letters within columns indicate significant differences ($p<0.05$).

	Pre-treatment	BSFL composting		Entire process			
	Red_{VS} [%]	Red_{VS} [%]	BCE_{VS} [%]	Red_{WW} [%]	Red_{VS} [%]	BCE_{WW} [%]	BCE_{VS} [%]
Paper I							
OP-C		38.7^{abc} ±3.1	5.74^{abc} ±1.66	57.8^{ab} ± 1.1	38.7^{ab} ±3.1	5.18^{abcd} ±1.20	5.74^{abc} ±1.66
BC-C		81.2^{de} ±0.5	14.4^{d} ±1.5	93.4^{cd} ± 1.2	81.2^{cd} ±0.5	6.45^{abefg} ±0.06	14.4^{d} ±1.5
FW-C		63.9^{fg} ±0.9	22.7^{ef} ±0.9	86.6^{cde} ± 0.8	63.9^{efg} ±0.9	14.2^{h} ±0.8	22.7^{e} ±0.9
OP-T	24.8^{a} ±8.0	43.0^{abh} ±7.0	2.57^{g} ±0.71	82.1^{cde} ± 0.5	60.1^{ef} ±4.2	1.33^{i} ±0.27	1.78^{f} ±0.37
BC-T	65.4^{b} ±4.9	52.2^{afhi} ±8.9	16.3^{dh} ±1.0	97.8^{c} ± 0.1	84.4^{cd} ±1.3	1.43^{i} ±0.27	5.38^{ab} ±0.77
OP-A	10.9^{c} ±0.02	51.6^{afhi} ±3.1	4.95^{ag} ±0.65	83.7^{cde} ± 0.6	57.5^{efh} ±2.7	3.85^{cj} ±0.19	4.34^{af} ±0.57
BC-A	15.3^{ac}±0.18	58.8^{fgi} ±3.4	15.0^{d} ±0.54	76.4^{acde} ± 7.5	66.2^{egi} ±2.8	4.46^{acdj} ±0.55	12.3^{dg} ±0.45
Paper II							
LC-C		78.4^{de} ±0.3	19.7^{i} ±0.2	34.6^{f} ±11	78.4^{cdi} ±0.3	6.78^{befgk} ±0.24	19.7^{h} ±0.2
LC-E0d		89.4^{d} ±0.6	24.1^{e} ±0.2	33.1^{f} ±10	89.4^{c} ±0.6	7.31^{efk} ±0.47	24.1^{e} ±0.2
LC-E2d	9.37^{i}	70.6^{eg} ±4.6	20.2^{fi} ±0.8	34.9^{f} ± 3.4	73.6^{dgi} ±4.1	4.87^{abcdj} ±0.32	18.2^{h} ±0.7
LC-E4d	14.8^{i}	69.0^{eg} ±7.1	23.3^{e} ±1.9	33.8^{f} ± 2.8	74.6^{dgi} ±5.8	5.92^{abdfg} ±1.00	19.1^{h} ±1.5
Paper III							
PR-C		34.3^{j} ±4.7	5.90^{abc} ±0.30	72.6^{abde} ± 1.4	34.3^{ab} ±4.7	3.09^{ij} ±0.18	5.90^{abc} ±0.30
PR-F		44.7^{bc} ±1.5	7.85^{bcj} ±0.46	70.1^{abe} ± 0.8	44.7^{ajk} ±1.5	6.29^{abefg} ±0.28	7.85^{bci} ±0.46
PR-E		46.5^{bc} ±4.2	7.70^{abcj} ±0.71	68.9^{abe} ± 1.0	46.5^{ahjk} ±4.2	4.10^{cdj} ±0.37	7.70^{bci} ±0.71
PR-EF		45.6^{bc} ±1.0	8.11^{bj} ±0.34	76.0^{acde} ± 1.1	45.6^{ahjk} ±1.0	6.41^{abefg} ±0.05	8.11^{ci} ±0.34
PR-E/2		42.4^{bcj} ±4.4	7.17^{abc} ±0.80	78.8^{acde} ± 1.2	42.4^{ajk} ±4.4	5.98^{abdefg} ±0.48	7.17^{bc} ±0.80
PR-E2		52.4^{abh} ±1.8	7.66^{abcj} ±0.21	72.9^{ade} ± 0.3	52.4^{fhjk} ±1.8	5.94^{abdfg} ±0.14	7.66^{bci} ±0.21
PR-L2		54.0^{abhi} ±1.8	7.28^{abc} ±0.30	79.2^{acde} ± 0.9	54.0^{efhk} ±1.8	7.23^{efgk} ±0.26	7.28^{bc} ±0.30
PR-L4		29.6^{cj} ±9.4	6.84^{abc} ±1.30	83.9^{cde} ± 0.3	29.6^{b} ±9.4	4.75^{abcdj} ±1.67	6.84^{abc} ±1.30
PR-H		42.4^{bcj} ±1.5	10.3^{j} ±0.7	86.9^{cde} ± 0.3	42.4^{ajk} ±1.5	5.28^{abgij} ±0.46	10.3^{gi} ±0.7
PR-HB		54.5^{ab} ±1.8	13.6^{d} ±0.7	83.2^{cde} ± 1.6	54.5^{efhk} ±1.8	8.72^{k} ±0.48	13.6^{d} ±0.7
F-C		44.3^{bc} ±1.7	8.41^{bj} ±0.41	75.5^{ade} ± 0.7	44.3^{ajk} ±1.7	7.99^{ek} ±1.16	8.41^{ci} ±0.41
F-E		41.2^{bc} ±1.3	18.7^{hi} ±0.9	74.2^{ade} ± 0.7	41.2^{abj} ±1.3	14.9^{h} ±0.7	18.7^{h} ±0.9
Paper IV							
PR-HB		53.6^{fhi} ±6.7	5.75^{ac} ±1.10	54.9^{b} ±16.0	53.6^{fhk} ±6.7	3.89^{cj} ±0.70	5.75^{ab} ±1.10

[i] Performed in singlets.

Table 4. Larval weight, survival rate, and larval yield for all treatments. Substrates used in treatments: food waste (FW), orange peels (OP), broccoli and cauliflower trimmings (BC), lettuce and cabbage (LC), cucumber plant residuals (PR). Pre-treatments used: no pre-treatment (C), *Trichoderma reesei* (T), ammonia (A), enzymes for 0d, 2d, and 4d (E0d), added enzymes (E) of different concentrations with frass (E/2, E2), frass (F), different larval densities with enzymes and frass (L2, L4), half substrate density with enzymes and frass (H), and cover (B). Values presented are mean ± SD (n=3). Different superscript letters within columns indicate significant differences ($p<0.05$).

Paper		Larval weight [mg/larva]	Survival[i] [%]	Larval yield$_{TS}$ [mg TS/cm^2]
I	OP-C	50.5^{abcd} ±11.9	100[ii]	80.7^{abcde}±22.7
	BC-C	108^{efghi} ± 8	62.8^{ab}± 4.5	82.0^{abcde}± 6.7
	FW-C	141^{e} ± 5	100^{ab} ± 6.3	369^{f} ± 7
	OP-T	17.9^{a} ± 3.7	100[ii]	26.3^{g} ± 4.9
	BC-T	61.2^{bcdj} ±23.1	28.1^{a} ±14.6	33.2^{gh} ± 4.7
	OP-A	42.5^{ab} ± 0.2	96.0^{ab}± 4.5	62.1^{adij} ± 8.3
	BC-A	95.3^{fghijk}±25.5	55.7^{ab}±25.0	72.5^{abcdi} ± 6.4
II	LC-C	213^{l} ± 9	68.9^{ab}± 4.5	62.6^{adij} ± 0.8
	LC-E0d	234^{l} ±10	67.5^{ab}± 7.0	74.5^{abcdi} ± 0.9
	LC-E2d	273^{m} ± 4	56.2^{ab}± 4.5	68.1^{abdij} ± 2.7
	LC-E4d	239^{l} ±19	76.0^{ab}±17.4	71.2^{abcdi} ± 6.0
III	PR-C	81.7^{cfhjk} ± 6.3	76.7^{ab}± 3.5	44.8^{ghj} ± 2.1
	PR-F	96.5^{fghik} ± 5.6	96.5^{ab}± 3.8	91.4^{bce} ± 5.8
	PR-E	85.1^{fghjk} ± 7.1	97.5^{ab}± 0.7	58.6^{aij} ± 5.8
	PR-EF	90.3^{fghjk} ± 5.9	105^{ab} ± 8	94.9^{ce} ± 4.8
	PR-E/2	85.6^{fghjk} ± 4.8	103^{ab} ± 3	87.0^{bce} ± 9.8
	PR-E2	119^{egi} ± 7	74.0^{ab}± 5.2	85.5^{bcde} ± 2.6
	PR-L2	48.5^{abcd} ± 3.5	111^{ab} ± 6	85.2^{bcde} ± 2.4
	PR-L4	44.1^{abd} ± 7.5	78.0^{ab}±16.9	75.4^{abcdi}±15.1
	PR-H	77.4^{cdhjk} ± 7.4	101^{ab} ± 5	53.2^{ihj} ± 3.5
	PR-HB	127^{ei} ± 9	102^{ab} ± 6	70.3^{abdi} ± 4.7
	F-C	69.4^{bcdjk} ±12.4	105^{ab} ± 4	104^{e} ± 6
	F-E	115^{efgi} ± 6	118^{b} ± 5	215^{k} ±11
IV	PR-HB	121^{ei} ±12	66.7^{ab}±12.6	30.8^{g} ± 6.7

[i] Statistical analysis excluded treatments OP-con and OP-tri. Not normally distributed, dunn test.
[ii] Assumed survival.

5.1.3 Nutritional composition of feedstock substrate

Pre-treating with fungi and ammonia increased the amount of nitrogen in the substrate provided to the larvae on a TS basis by 34 and 340% (from 0.50 to 0.67 mg nitrogen per larva and 0.81 to 3.57 mg nitrogen per larva, Table 5). The initial nitrogen per larva was 3.31 mg nitrogen in the reference treatment (FW-C). However, the initial nitrogen per larva was higher in the treatments with plant residuals (9.18-13.1 mg nitrogen per larva). Also, the nitrogen content in the generated larvae was higher in the ones reared on plant residuals (6.18-7.87 %) compared to the reference treatment (5.49%).

Table 5. List of treatments with the total initial amount of nitrogen before pre-treatment and before BSFL composting, lignin, cellulose, hemicellulose, and lipids [mg/larva], and the nitrogen and lipid content [%] in larvae. Substrates used in treatments: food waste (FW), orange peels (OP), broccoli and cauliflower trimmings (BC), lettuce and cabbage (LC), cucumber plant residuals (PR). Pre-treatments used: no pre-treatment (C), *Trichoderma reesei* (T), ammonia (A), enzymes for 0d, 2d, and 4d (E0d), added enzymes (E) of different concentrations with frass (E/2, E2), frass (F), different larval densities with enzymes and frass (L2, L4), half substrate density with enzymes and frass (H), and cover (B). Different letters within columns indicate significant difference ($p<0.05$). Values are presented as mean ± SD (n=3), where LD (low deviation) means SD < 0.005.

	Before pre-treatment [mg TS/larva]	Before BSFL composting [mg TS/larva]					Larvae$_{TS}$ [%]	
	Nitrogen	Nitrogen	Lignin	Cellulose	Hemi-cellulose	Lipids	Nitrogen	Lipids
Paper I								
OP-C		0.92^{a} ±0.02					4.88^{a} ±0.31	
BC-C		1.22^{b} ±0.01					6.12^{bcd} ±0.37	
FW-C		3.31^{c} ±0.06					5.49^{ab} ±0.58	
OP-T								
BC-T	0.50^{a} ±0.06	0.67^{d} ±0.08					5.81^{abc} ±0.56	
OP-A	0.81^{b} ±0.01	3.57^{e} ±0.06					5.91^{abc} ±0.66	
BC-A								
Paper III								
PR-C		9.42^{f} ±LD	20.1^{a} ±0.01	46.9^{a} ±0.01	25.2^{a} ±0.01	8.21^{a} ±LD	7.87^{e} ±0.20	8.19^{abc} ±0.73
PR-F		9.85^{g} ±LD	23.1^{b} ±LD	45.1^{b} ±LD	36.7^{b} ±LD	7.76^{b} ±LD	7.83^{e} ±0.25	7.32^{abc} ±0.14
PR-E		9.42^{f} ±LD	20.1^{a} ±0.01	46.9^{a} ±0.01	25.2^{a} ±0.01	8.21^{a} ±LD	7.67^{e} ±0.25	8.27^{abc} ±0.55
PR-EF		9.86^{g} ±0.01	23.1^{b} ±0.02	45.1^{b} ±0.03	36.8^{b} ±0.04	7.76^{b} ±LD	7.74^{e} ±0.38	5.81^{ab} ±0.50
PR-H		9.18^{h} ±0.02	21.5^{c} ±0.04	41.9^{c} ±0.06	34.5^{c} ±0.08	7.21^{c} ±0.01	7.80^{e} ±0.13	4.93^{a} ±0.65
PR-HB		9.19^{h} ±0.01	21.6^{c} ±0.02	42.0^{c} ±0.03	34.5^{c} ±0.05	7.22^{c} ±LD	7.25^{de} ±0.06	8.83^{bc} ±1.99
F-C		11.3^{i} ±LD	30.8^{d} ±LD	43.3^{d} ±0.01	64.2^{d} ±0.01	7.13^{d} ±LD	7.21^{de} ±0.21	10.5^{cd} ±0.8
F-E		11.3^{i} ±LD	30.8^{d} ±0.01	43.3^{d} ±0.01	64.2^{d} ±0.01	7.13^{d} ±LD	6.85^{cde} ±0.29	13.8^{d} ±2.5
Paper IV								
PR-HB		13.1^{j} ±LD	32.8^{e} ±LD	57.4^{e} ±LD	41.0^{e} ±LD	15.2^{e} ±LD	6.18^{bcd} ±0.50	8.51^{abc} ±1.36

Most of the total initial nitrogen (around 75%) was lost in the ammonia pre-treated orange peels treatment (OP-A, Figure 2). The degree of nitrogen loss was in the same range for the reference treatment (FW-C) and across the treatments in Paper III (around 25% loss), where cucumber plant residuals were treated in a laboratory set-up. The frass control pre-treated with enzymes (F-E), the treatment control with orange peels, and the broccoli and cauliflower pre-treated with *Trichoderma reesei* had lower amounts of lost nitrogen.

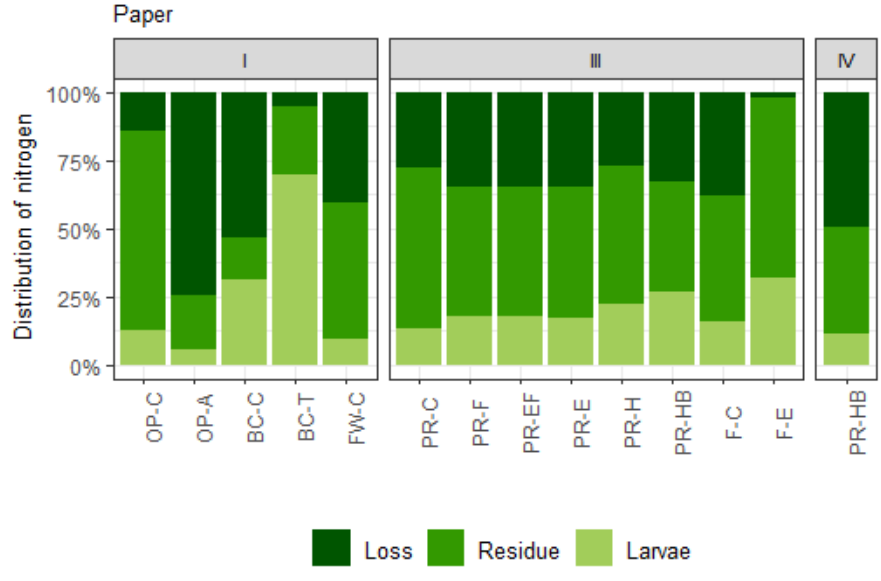

Fig 2. Mass balance (total solids (TS) basis) of nitrogen in black soldier fly larvae (BSFL) composting of orange peels (OP), broccoli and cauliflower (BC), food waste (FW), and plant residuals (PR) without pre-treatments (C) or with pre-treatments of ammonia (A), *Trichoderma reesei* (T), frass (F), enzymes (E), half amount of substrate (H) with a cover (HT), and only frass as a substrate with (F-E) or without addition of enzymes (F-C). Distribution of nitrogen is divided into three fractions: larvae (light green), residue (green), and loss of nitrogen (dark green).

5.1.4 Emissions of greenhouse gases and ammonia

In Paper I, the emissions of CH_4, N_2O, and NH_3 emitted from the reference treatment (FW-C) were between 0.3-9.1 mg/kg initial VS (Figure 3a-c). The total emissions of CH_4 and N_2O, expressed in CO_2-equivalents over a 100-year period (IPCC, 2013) on a VS basis, were found to be low compared with the direct CO_2 emissions (Figure 3d-e). The total emissions of CH_4 and N_2O

were generally higher for treatments with broccoli and cauliflower (6.1-22 g CO_2eq/kg initial VS, Figure 3e). The total emissions were similar across the treatments, with only the control treatments with orange peels and broccoli and cauliflower (OP-C, BC-C) being significantly different from each other (Figure 3f).

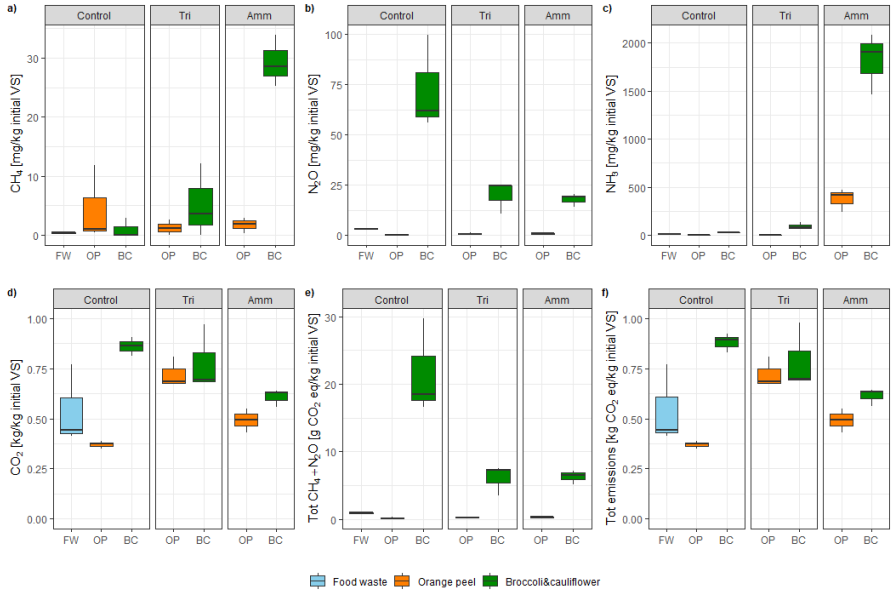

Fig 3. Total emissions, *i.e.*, emissions during black soldier fly larvae (BSFL) composting and during pre-treatment, calculated per kg of initial VS for food waste (FW, blue), orange peels (OP, orange), and broccoli and cauliflower trimmings (BC, green) without pre-treatment (control), pre-treated with *Trichoderma reesei* (tri), and ammonia solution (amm): a) CH_4 [mg/kg VS]; b) N_2O [mg/kg VS]; c) NH_3 [mg/kg VS]; d) total CO_2 [kg/kg VS]; e) CO_2 equivalents [g CO_2-eq/kg VS] of the CH_4 and N_2O emissions; and f) CO_2 equivalents [kg CO_2-eq/kg VS] of all emissions.

5.1.5 Degradation efficiency

In the laboratory set-up of cucumber plant residual treatments, the degradation of lignin was lowest in the treatment with cover (PR-HB) compared to the other treatments (Paper III). Similar lignin degradation was observed in the pilot-scale setting (PR-HB, Figure 4). The treatment most similar in composition to that treatment in Paper III, but without a cover (PR-H), demonstrated higher lignin degradation.

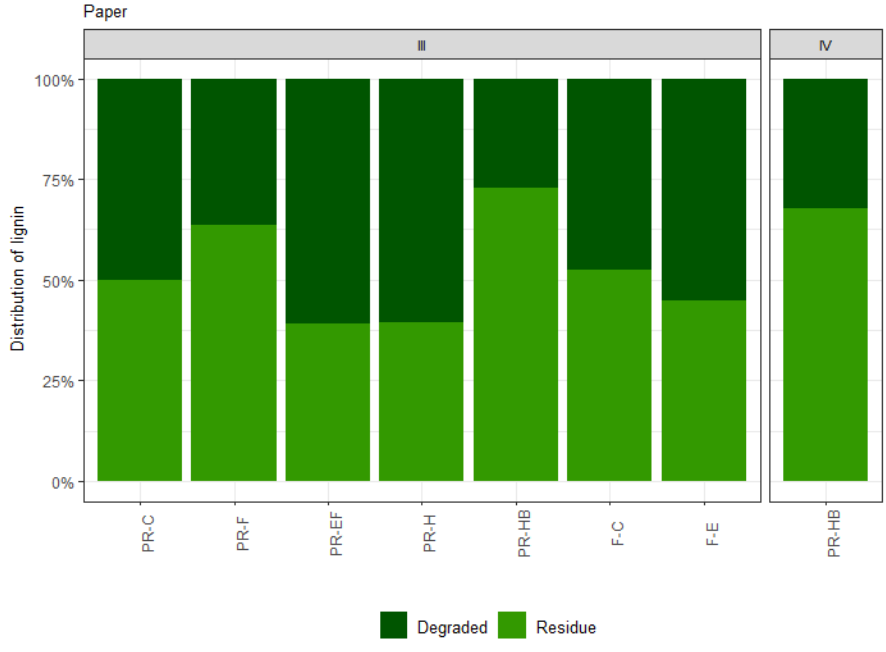

Fig 4. Mass balance (total solids (TS) basis) of lignin in black soldier fly larvae (BSFL) composting of plant residuals (PR) without additions (C) or with additions of ammonia (A), *Trichoderma reesei* (T), frass (F), enzymes (E), half amount of substrate (H) with a cover (HT), and only frass as a substrate with (F-E) or without addition of enzymes (F-C). Distribution of lignin is divided into two fractions: residue (green) and loss of lignin (dark green).

In Paper III, the addition of enzymes in frass (F-E) resulted in larger degradation of cellulose compared to the treatment with only frass (F-C, Figure 5). A larger cellulose degradation was observed in the pilot-scale setting (PR-HB, Paper IV) compared with the bench-scale setting (PR-HB).

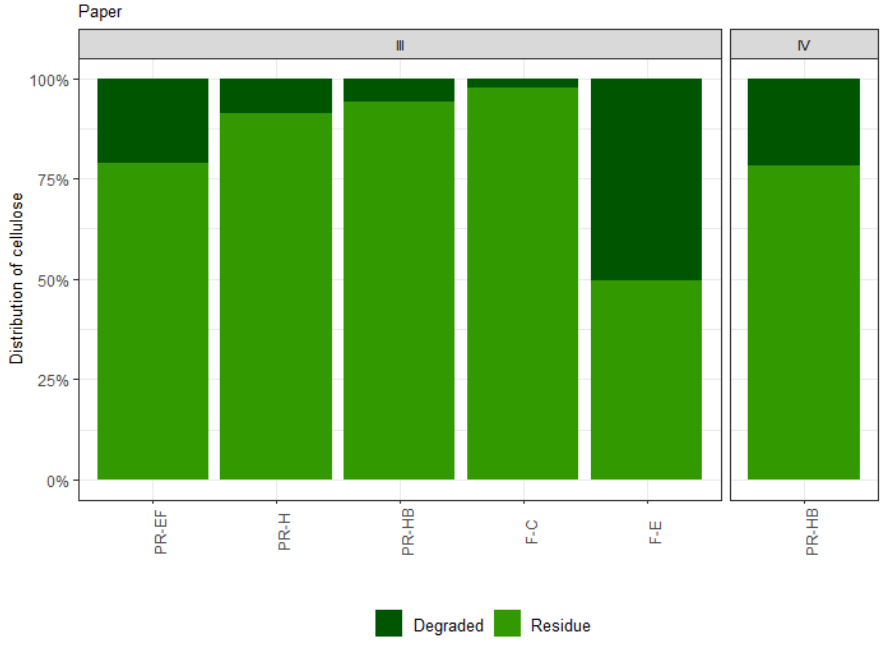

Fig 5. Mass balance (total solids (TS) basis) of cellulose in black soldier fly larvae (BSFL) composting of plant residuals (PR) without additions (C) or with additions of ammonia (A), *Trichoderma reesei* (T), frass (F), enzymes (E), half amount of substrate (H) with a cover (HT), and only frass as a substrate with (F-E) or without addition of enzymes (F-C). Distribution of lignin is divided into two fractions: residue (green) and loss of cellulose (dark green).

In Paper III, the degradation of hemicellulose was low in the treatment with the addition of frass (PR-F) and higher in the control (PR-C) and with added enzymes (PR-E, Figure 6). In Paper IV, the degradation of hemicellulose in the treatment (PR-HB) was similar to the small-scale treatment (PR-HB) in Paper III.

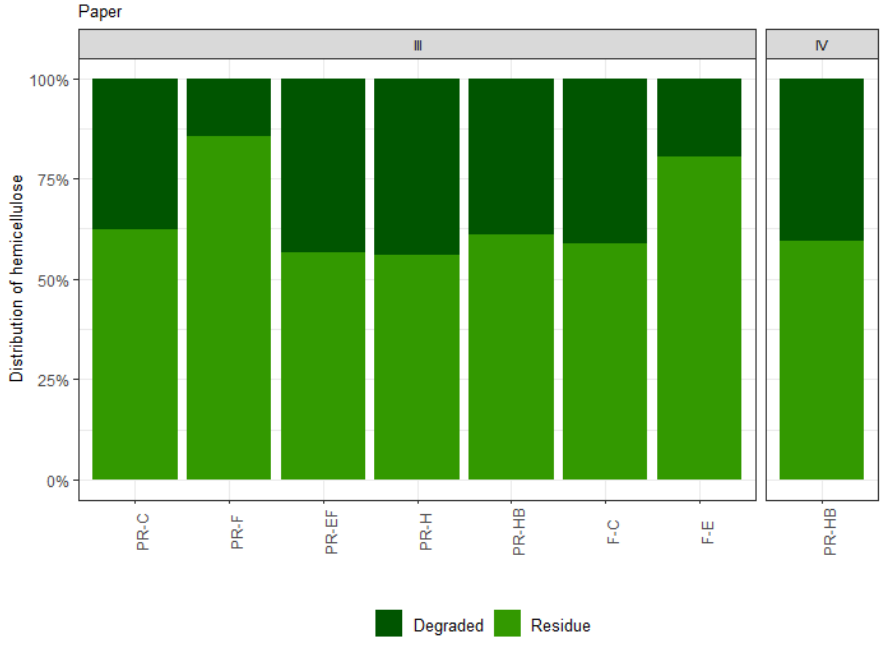

Fig 6. Mass balance (total solids (TS) basis) of hemicellulose in black soldier fly larvae (BSFL) composting of plant residuals (PR) without additions (C) or with additions of ammonia (A), *Trichoderma reesei* (T), frass (F), enzymes (E), half amount of substrate (H) with a cover (HT), and only frass as a substrate with (F-E) or without addition of enzymes (F-C). Distribution of lignin is divided into two fractions: residue (green) and loss of hemicellulose (dark green).

5.1.6 Pesticides

The degradation of pesticides was higher for all detected pesticides in the pilot-scale setting (Paper IV) compared to the degradation in the laboratory setting (Paper III, Figure 7). Diphenylamine and penconazole were not analysed in the laboratory setting. Bifenazate, propamocarb, and proquinazid were screened for in the pilot-scale setting but were not detected. Although the levels of pesticides in the larvae were small (scarcely visible in Figure 7), their concentrations in the larval biomass exceeded the maximum residue limits permitted for human consumption in cattle meat (for seven pesticides) and cucumbers (for two pesticides, Figure 8).

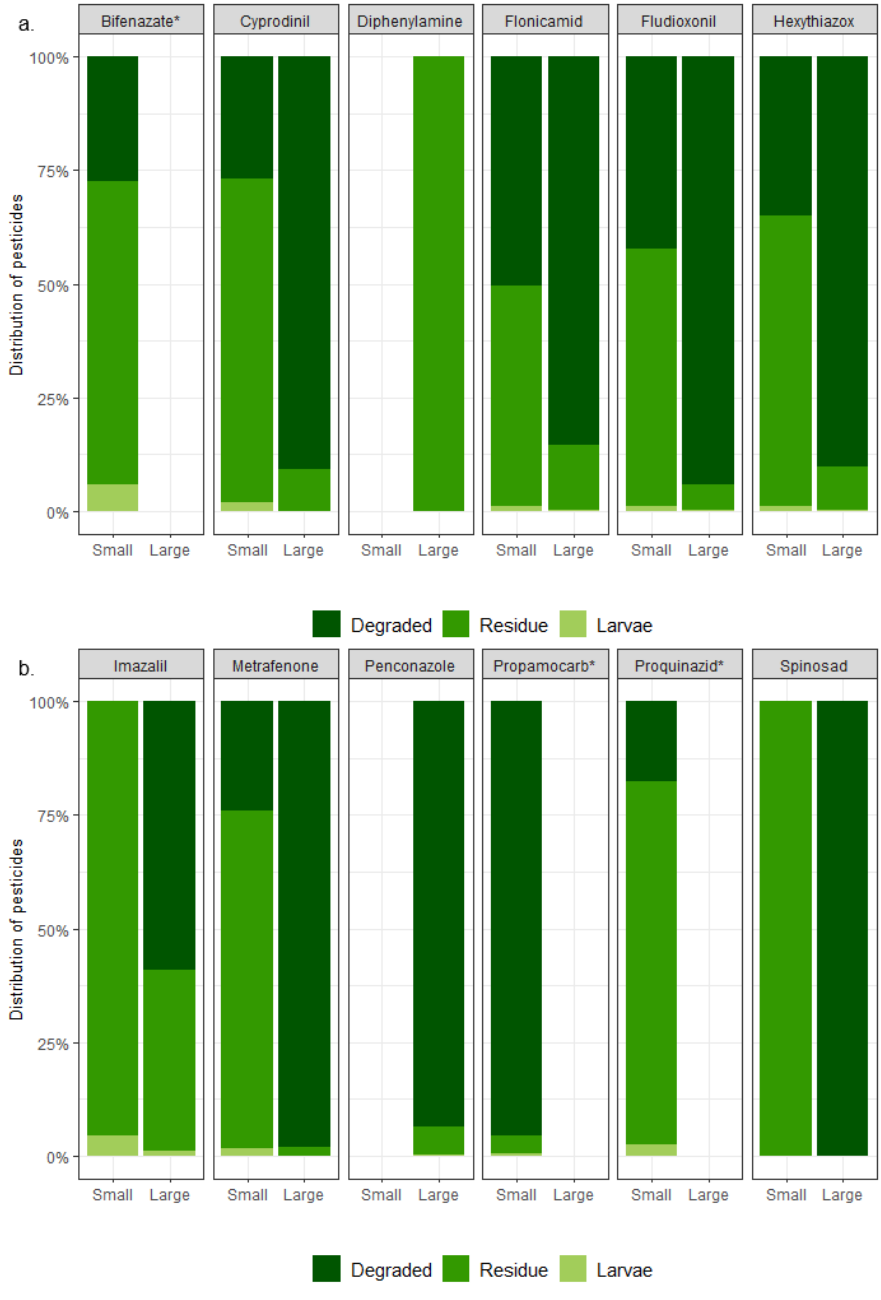

Fig 7. Mass balance of pesticides from large- and small-scale trial with cucumber plant residuals. Pesticides with an * have been tested for both trials.

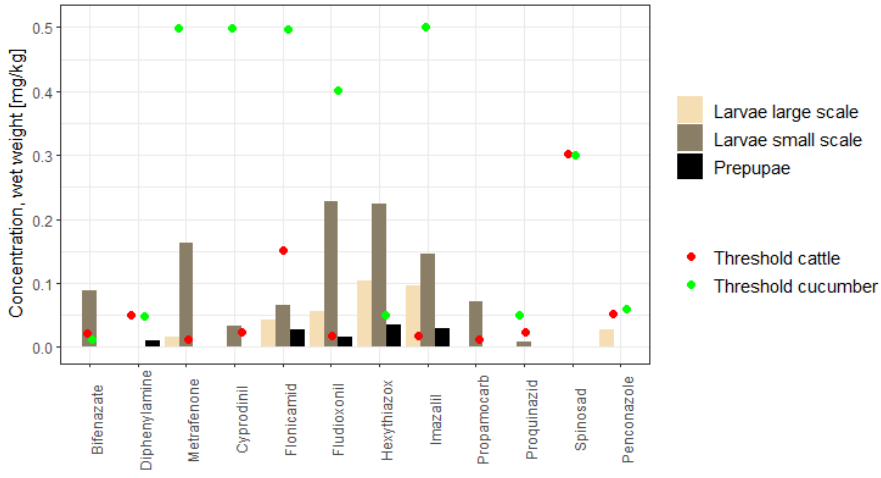

Fig 8. Concentrations of pesticides in larvae and prepupae from the large-scale setting and larvae from the small-scale setting together with the threshold values for meat from cattle and cucumber.

6. Discussion

The use of single-source substrates, such as vegetable and fruit waste, presents notable challenges for BSFL composting systems. Whilst BSFL are known for their broad substrate tolerance, the nutritional and structural characteristics of homogenous plant-based waste streams can significantly constrain process efficiency (Papers I-IV). Unlike heterogeneous food waste, which often contains a more balanced ratio of protein and carbohydrates, single-source fruit and vegetable wastes tend to be nutritionally imbalanced, with a predominance of carbohydrates and low protein content (Gold et al., 2020a). This imbalance can negatively affect larval growth, development, and larval yield (Paper I-IV).

Furthermore, vegetable and fruit wastes that do not originate from post-consumer food waste streams often contain higher proportions of complex polysaccharides and structural carbohydrates—such as cellulose, hemi-cellulose, and lignin (Amin et al., 2017; Zahir et al., 2020). These long-chain carbon compounds are inherently more resistant to degradation, limiting their bioavailability to both larvae and associated microorganisms (Gao et al., 2025; Liu et al., 2024). Consequently, these substrates exhibit slower degradation, leading to reduced BCE and lower material reduction (Papers I-IV).

6.1 Pre-treatment

6.1.1 Impact of pre-treatment on larval VS-load

As outlined in the background (section 3.7.1), it has been found that the optimal VS dose BSF larvae reared on dog food (a proxy for food waste) is 0.2 gram of volatile solids (VS) per larva (Lopes et al., 2023). In the studies described in this thesis, larvae were provided with larval VS doses as low as 0.03 (Paper I) and up to 0.3 (Paper IV) grams of VS per larva. It was found that a too low VS larval dose did not improve the BCE but impaired the growth potential of the larvae. However, it was also found that the digestibility of the VS influenced the quantity of substrate necessary to support optimal larval growth. Volatile solids content decreased significantly after pre-treatment by 0.5-16.6% units across all treatments, apart from the treatment of lettuce and cabbage pre-treated with enzymes for 2 d (LC-E2d,

Table 2), likely due to microbial respiration during this phase. The most substantial VS reduction occurred in the *Trichoderma reesei* treatment with broccoli and cauliflower (65%, BC-T), which also exhibited elevated CO_2 emissions, indicating that active fungal and bacterial respiration took place (Figure 3d). As the VS larval dose was not balanced after pre-treatment in Paper I, the enhanced pre-treatment degradation resulted in a reduced VS load per larva in both the ammonia (0.07 g VS/larva for BC-A) and fungal treatments (0.03 g VS/larva for BC-T) compared to the treatments pre-treated with enzymes (0.22-0.24 g VS/larva) in Paper II (Table 1).

Interestingly, such extensive material reduction during pre-treatment was not observed with orange peels (11-25%, Table 3) or in previous studies with banana peels (1.2-13%) (Isibika et al., 2019), suggesting that broccoli and cauliflower are more readily degradable by microbial communities than fibrous substrates such as orange and banana peels. Considering that lower substrate availability has been linked to reduced larval growth (Paz et al., 2015), this may partly explain performance differences in treatments with broccoli and cauliflower peels (5-14% BCE, 66-84% M.Red) and orange peels (2-6% BCE, 39-60% M.Red) in Paper I.

In the *T. reesei* treatment with broccoli and cauliflower (BC-T), the nitrogen retained in the VS seemed to be primarily assimilated by the larvae (Figure 2). This indicate that *T. reesei* enhanced nutrient accessibility to the larvae, which is consistent with studies showing that fungal pre-treatment can increase nitrogen and crude protein concentrations per unit of dry matter (Jalc et al., 1997; Zeng et al., 2011). It suggests that the remaining substrate was efficiently utilised by the larvae, but that the VS larval dose was not sufficient to support optimal growth.

6.1.2 Chemical pre-treatment with ammonia solution

Ammonia solution was used as a pre-treatment to increase the substrate's nitrogen content and to stimulate microbial degradation of complex compounds (see section 3.8.2). Whilst it did not enhance BCE or larval yield, it improved material reduction in the orange peel treatment (OP-A, Paper I, Tables 3 and 4) and increased total CO_2 emissions compared to the control (Figure 3d), indicating increased microbial respiration. Although orange and banana peels have similarly high C/N ratios (63, theoretically; (Livsmedelsverket)), the addition of nitrogen may have enhanced microbial protein synthesis in the orange peel and banana peel substrate, shifting the

protein-to-carbohydrate ratio towards, which should be closer to the optimal ratio of 1 for BSFL performance (Gold et al., 2020a).

No improvements regarding BCE, material reduction or larval yield were observed for the broccoli and cauliflower treatment (BC-A), which is in accordance with the findings by Isibika et al. (2019) who used banana peels. The emissions of N_2O were reduced, whereas CH_4 increased with ammonia pre-treatment compared to the control (BC-C). The ammonia pre-treatment turned the substrate into a slurry with even high moisture content (TS 7.3%) than the original substrate (TS 8.6%, Table 2), which may have contributed to the higher CH_4 emissions, as high-moisture substrates tend to promote CH_4 emissions due to enhanced anaerobic conditions (Chen, J. et al., 2019). The reduced N_2O emissions may be attributed to altered nitrogen dynamics under alkaline conditions, which potentially suppressed the nitrification and denitrification pathways typically responsible for N_2O formation (Lin et al., 2022). The ammonia-treated broccoli and cauliflower was left to enable effective separation of the larvae at harvesting, which likely contributed to unrecorded emissions. This highlights the challenges associated with BSFL composting of wet substrates, as excessive moisture not only promotes anaerobic conditions and methane formation but also complicates the harvesting of larvae from the treatment residue.

In contrast to what was found for BSFL composting—where ammonia pre-treatment showed no significant effect on process efficiency—applying ammonia for 24 h prior to anaerobic digestion has been shown to significantly increased methane yield after 39 d of anaerobic digestion (Wang et al., 2018). However, in that study, the addition of ammonia alone did not improve methane yield unless pH was adjusted and maintained above 9 during treatment. The increased yield was attributed to the release of soluble organic matter and the presence of free nitrogen ammonia above 0.1 g/L wastewater, which enhanced biodegradability (Wang et al., 2018). This concentration is substantially lower than the 7 g/kg orange peels (4% dry mass) used in Paper I. Peguero et al. (2023), observed a decreased process efficiency when applying three days pre-treatment with 5% ammonia (5% dry mass) on spent grain, oat pulp and grass clippings, which the authors attributed to ammonia toxicity. Although maintaining a high pH throughout the ammonia pre-treatment resulted in an increased methane yield, it may not be the case for BSFL composting. Even though initial composting pH values of up to 10 have not been shown to significantly affect larval weight or

development time (Ma et al., 2018; Meneguz et al., 2018a), a high pH combined with elevated ammonia concentrations can be toxic to many biological organisms, including invertebrates, as increased pH raises the proportion of toxic unionised ammonia relative to the ammonium ion (Zhang et al., 2023). Invertebrates such as BSFL may therefore be more sensitive to ammonia than bacterial communities. In Paper I and Peguero et al. (2023), the substrate pH was neutralised before BSFL composting. In trials where this was not done, high larval mortality was reported (Almqvist, 2020). In contrast, the microorganisms did not appear to be adversely affected by a pH above 9 together with a lower ammonia concentration during anaerobic digestion (Wang et al., 2018). This aligns with the findings of the present study, in which ammonia pre-treatment appeared to stimulate microbial degradation—evidenced by increased CO_2 emissions and material reduction—rather than BSFL growth, even at high ammonia concentrations.

6.1.3 Biological pre-treatment with fungi

Pre-treatment with *Trichoderma reesei* was intended to facilitate fibre degradation into simpler carbohydrates through fungal enzymatic activity, thereby improving substrate digestibility for the larvae. Although BCE or larval yield did not improve compared with the control (OP-C, BC-C), material reduction increased for both the orange peel and the broccoli and cauliflower treatments (OP-T, BC-T, Paper I, Tables 3 and 4). This was accompanied by elevated CO_2 emissions (Figure 3d), indicating that fungi—and possibly associated bacteria—contributed to substrate degradation and respiration during both the pre-treatment and composting phases.

The reduced BCE may be attributed to fungal utilisation of easily degradable nutrients prior to larval introduction, resulting in a very low VS per larva (0.03 g VS/larva, as discussed in 6.1.1). In contrast, Isibika et al. (2019) reported a 61–100% increase in BCE when applying fungi pre-treatment on banana peels. This improvement may be attributed to the greater fibre content of the substrate, which may have amplified the effect of the pre-treatment, and to the larger VS dose provided to the larvae (0.8-1.2 g VS/larva). This provided a more substantial resource for fungal activity, leaving more nutrients accessible for larval uptake.

The fungal pre-treatment of orange peels (OP-T) resulted in a product that was difficult to separate due to the formation of a hard crust overlaying the moist substrate. This could have possibly been prevented through

intermittent mixing during both the pre-treatment and BSFL composting phases, or in combination with extending the pre-treatment duration to allow drying of the substrate.

Reported fungal pre-treatment times vary greatly across studies, ranging from 4 h to 21 d (Pennacchio et al., 2021; Szűcs et al., 2021; Wang et al., 2021). For instance, Pennacchio et al. (2021) applied a 4 h fungal treatment followed by 72 h of enzymatic hydrolysis, concluding that longer fungal exposure did not significantly improve outcomes. However, they observed that *Trichoderma harzianum* enhanced cellulose availability, likely due to its biosurfactant properties which facilitate altering cellulose and increase surface area for improved hydrolysis (Pitocchi et al., 2020).

In the present study, enzyme pre-treatment was conducted for 0 to 4 d and fungal pre-treatment for 14 d. These durations are within the lower to mid-range of those previously reported. The outcomes suggest that even brief enzymatic or fungal action can impact substrate characteristics, but optimisation of treatment duration and conditions remains essential to maximise digestibility and overall process efficiency in BSFL composting. Combining fungal and enzymatic treatments may therefore reduce the required pre-treatment duration, particularly as enzyme activity tends to decline over time during fungal pre-treatment (Szűcs et al., 2021). Whilst Szűcs et al. (2021) suggest a 10 d fungal incubation to improve biogas yields from cellulose-rich substrates, a combined approach may be more suitable for BSFL systems. Even a brief fungal pre-treatment can initiate partial degradation of lignocellulosic structures, thereby increasing the accessibility of embedded nutrients for further hydrolysis (Pitocchi et al., 2020). For recalcitrant substrates such as orange peels, a shorter, integrated fungal–enzymatic pre-treatment may therefore facilitate improved degradation of carbohydrates and fibres, increasing digestibility for the larvae without compromising operational costs or process efficiency with broader (fungi) and targeted amount of enzymes. However, the studies mentioning shorter pre-treatment durations did not assess the effectiveness of separating larvae from the residue, which was a notable challenge observed in the fungal pre-treatment of orange peels (OP-T).

6.1.4 Effect of enzyme pre-treatment duration on substrates with varying lignocellulose contents

When evaluating different durations of enzyme pre-treatments for a mixture of lettuce and cabbage, direct application of enzymes resulted in a significantly increased BCE compared to the control (LC-E0d, LC-C, Table 3). In contrast, applying the same direct enzyme treatment to plant residuals (PR-E) did not yield a significant improvement in BCE or larval yield, but instead resulted in significantly higher material reduction.

When comparing substrates with differing lignocellulosic content but similar moisture levels (86-91%, Table 2), pre-treatment that reduces moisture content may affect BCE and material reduction in different ways. High moisture content can limit aeration, promote compaction and nutrient dilution, reducing larval access to nutrients (Lalander et al., 2020). Dewatering can alleviate these physical constraints, improving oxygen diffusion and larval mobility (Yakti et al., 2023). For the low-lignocellulose substrate, where nutrients are more readily bioavailable, dewatering may enhance BCE by concentrating digestible organics and reducing dilution effects (Lalander et al., 2020). Conversely, for the high-lignocellulose substrate, the impact of dewatering may be less pronounced in terms of BCE, as the structural complexity and low digestibility of fibrous components remain the primary bottlenecks (Yakti et al., 2023).

The treatments differed markedly in substrate structure, as the lettuce and cabbage mix had a slurry-like consistency and the plant residuals were cut up into pieces of 10 cm and less. Smaller particle sizes generally offer a greater surface area-to-volume ratio, facilitating microbial colonisation and enzymatic action, which can accelerate the breakdown of organic matter and further increase BCE (Čičková et al., 2015; Seyedalmoosavi et al., 2022). However, excessively fine particles may also lead to compaction of the substrate bed, reducing porosity and oxygen diffusion (Seyedalmoosavi et al., 2022). This can result in anaerobic zones that hinder microbial activity and create unfavourable conditions for larval development, potentially leading to increased mortality or reduced waste degradation (Lopes et al., 2023). A lower survival rate was observed in the treatments with lettuce and cabbage; however, it did not result in less material reduction or BCE in comparison to plant residuals (Tables 3 and 4).

Conversely, substrates with very large or coarse particles may retain structural integrity for longer, limiting the accessibility of nutrients and

slowing down decomposition. Larvae may also struggle to physically manipulate or ingest larger particles (Bruno et al., 2020), which could contribute to a reduced BCE. However, the significantly lower BCE observed in the control treatment with plant residuals (PR-C), compared to the one with lettuce and cabbage (LC-C), is more likely attributed to differences in the nutrient profile rather than particle size alone. None the less, maintaining an optimal particle size range—fine enough to ensure accessibility but coarse enough to preserve aeration and moisture balance—is essential for maximising the efficiency of BSFL composting systems (Peguero et al., 2024).

The other main difference was the physicochemical characteristics of the substrate, particularly its lignocellulosic content. Substrates with a low lignocellulose content—such as leafy greens, *e.g.*, lettuce and cabbage—typically consist of soft tissues with minimal structural carbohydrates (cellulose, hemicellulose) and lignin (Livsmedelsverket, 2025). These substrates already provide a relatively high bioavailability of nutrients to both BSFL and associated microbial communities. In such cases, the direct addition of enzymes during feeding can accelerate the breakdown of readily degradable fractions, such as proteins and starches, thus improving nutrient assimilation by the larvae (Dzepe et al., 2025). Since the substrate is already physically and chemically accessible, a prolonged pre-treatment phase may offer limited additional benefits and could even result in nutrient losses via microbial respiration prior to larval introduction, which was observed when the enzyme pre-treatment was ongoing for 2 and 4 d (Table 3).

In contrast, substrates with a high lignocellulose content, such as plant residuals and banana peels, are structurally complex and resistant to degradation (Isibika et al., 2019). Lignocellulosic structures pose a significant barrier to microbial colonisation and enzymatic hydrolysis due to the protective lignin surrounding cellulose and hemicellulose fibres (Rehman et al., 2025). In the study by Isibika et al. (2019), 14 d fungal pre-treatments using *Rhizopus oligosporus* and *Trichoderma reesei* significantly improved BCE, likely because of substantial fibre reduction—up to 42% in the *T. reesei* treatment. These findings suggest that pre-treatments, particularly enzymatic, may yield more pronounced effects on BCE when applied to lignocellulose-rich substrates.

In such cases, extended enzymatic pre-treatment involving cellulases, hemicellulases, and lignin-modifying enzymes may be essential. Prolonged

enzyme exposure allows for a progressive breakdown of cell wall structures, improving substrate porosity and releasing fermentable sugars and other nutrients that become more accessible to BSFL and microbial communities during the composting process (Dzepe et al., 2025; Theron et al., 2023).

In practice, the decision to apply direct enzymatic supplementation as opposed to a longer pre-treatment should be based on a compositional analysis of the substrate. It appears that low fibre substrates benefit from immediate enzymatic activity to boost larval assimilation rates and reduce processing time, whilst high-fibre, recalcitrant substrates may require more intensive preparation to overcome structural barriers prior to larval feeding. Although extended pre-treatment durations provide biochemical advantages, these must be weighed against practical limitations. Prolonged incubation increases the operational time and may necessitate additional measures to control microbial contamination, pH fluctuations, or enzyme deactivation.

Furthermore, the economic costs associated with enzyme use and the energy required to maintain optimal conditions must be justified by substantial improvements in substrate degradation and BSFL productivity. For example, Theron et al. (2023) demonstrated that steam pre-treatment of sugarcane bagasse and wheat straw significantly improved nutrient availability and enhancing larval growth. However, they also highlighted limitations including high energy demands, incomplete lignin breakdown and elevated inhibitory compounds, which constrained the overall efficiency. Pre-treatment resulted in greater investment in time, space, and resources, which was not justified by the achieved improvement in larval production (Theron et al., 2023).

6.2 Assessment

6.2.1 Process efficiency

The treatments with orange peels, broccoli and cauliflower, and lettuce and cabbage had significantly lower BCE_{VS} and larval yield or were similar to their respective control (Tables 3 and 4). This does not align with the hypothesis that the evaluated pre-treatments would improve the process efficiencies.

When lettuce and cabbage were used as substrate, only the direct addition of enzymes (LC-E0d) resulted in a significant increase in BCE_{VS} and

material reduction and no significant difference of larval yield compared to the lettuce and cabbage control (LC-C). When using plant residuals, almost all treatments had significantly increased BCE_{VS}, material reduction, and larval yield compared to the control (PR-C), which supports the initial hypothesis that the enzymes and frass improved BCE and material reduction.

The BCE and material reduction achieved with BSFL reared on orange peels and plant residuals were comparable to values reported for similarly fibrous substrates (lignocellulosic biomass 47-69% dry mass) such as banana peels and grass clippings (Isibika et al., 2019; Peguero et al., 2023). Vegetables such as broccoli, cauliflower, lettuce, and cabbage generally contain lower lignocellulosic biomass (0–34%) and a higher soluble sugar content (20–66%) than plant residuals or grass clippings (Livsmedelsverket, 2025).

Gold et al. (2020a) reported a BCE of 23% and material reduction of 53% using a mixed vegetable substrate (lignocellulose 31.5%; soluble sugars 15.5%), whilst in the present study, broccoli and cauliflower yielded a BCE of 5–14% and material reduction of 66–84%, and lettuce and cabbage showed 18–24% BCE and 74–89% material reduction (Table 3). These differences can be attributed to substrate composition, physical structure, and digestibility, as well as to microbial activity during the process (Gold et al., 2020a; Lu et al., 2021; Yakti et al., 2023). BSFL performance is strongly influenced by both the larvae and their associated microbial communities, whose contributions to organic matter degradation vary depending on the substrate type (Gold et al., 2020a; Liu et al., 2020). Mixed vegetable canteen waste—often comprised of leafy greens, starchy residues, and minor amounts of fats and proteins—tends to offer a more balanced nutrient profile than single-vegetable substrates (Carmona-Cabello et al., 2020; Gold et al., 2020a; Isibika et al., 2021). This diversity improves digestibility and nutrient availability for both larvae and microorganisms. Additionally, such waste is often thermally processed (*e.g.*, cooked or steamed), which disrupts cell walls and increases substrate porosity, enhancing microbial colonisation and enzymatic hydrolysis (Amin et al., 2017; Zahir et al., 2020). These factors can explain the higher BCE observed with canteen waste in previous studies compared to the single or dual-vegetable substrates used here. In contrast, diets limited to oily waste or raw fruit and vegetables have shown low BCE or high larval mortality (Klammsteiner et al., 2021; Lalander et al., 2019).

Improved physical conditions may still support greater material reduction, as larvae can more efficiently fragment and partially degrade the substrate. Reducing excess water lowers the overall mass of the input material which has been shown to increase the BCE when comparing identical substrates at different moisture levels (Lalander et al., 2020). Instead of employing mechanical dewatering, wet substrates could be blended with drier materials such as rice brand, to regulate the moisture level of the substrates (Laksanawimol et al., 2024). Another option is to use frass as a drying agent, which offers additional benefits, as frass re-circulation has been shown to not only aid in moisture reduction but also increase larval yield per unit of VS from food waste, indicating more efficient nutrient utilisation (Lopes et al., 2024). For wet substrates—such as broccoli and cauliflower (Paper I) and lettuce and cabbage (Paper II)—blending with a drier substrate and one high in nitrogen to balance the larvae's nutritional needs may be a more effective strategy than applying a pre-treatment. For substrates rich in lignocellulosic biomass—such as plant residues (Papers III and IV)—pre-treatment is likely to have a more pronounced effect.

6.2.2 Greenhouse gas emissions

Greenhouse gas (GHG) emissions from BSFL composting ranges between 26-147 kg CO_2 per tonne wet weight, 0.1-3 g CH_4 per tonne wet weight, 0.1-9 g N_2O per tonne wet weight, 0-342 g NH_3 per tonne wet weight from Paper I and others, and for composting it ranges between 16-270 kg CO_2 per tonne wet weight, 23-520 g CH_4 per tonne wet weight, 4-400 g N_2O per tonne wet weight, 0-1540 g NH_3 per tonne wet weight (Ermolaev et al., 2015; Mertenat et al., 2019; Pang et al., 2020; Zhang et al., 2016). If not properly managed, emissions of CH_4, N_2O, and NH_3 can increase up to 100-fold in both conventional and BSFL composting (Ermolaev et al., 2015; Mertenat et al., 2019; Pang et al., 2020; Zhang et al., 2016). However, residues from BSFL composting are typically not chemically and biologically stable, and N_2O emissions may arise during their subsequent maturation, posing a risk of further emissions during storage (Chen, Jiangshan et al., 2019). If anaerobic zones develop, increased CH_4 emissions are anticipated in both BSFL composting and conventional composting processes (Ermolaev et al., 2015; Ermolaev et al., 2019).

6.2.3 Lignin degradation

The addition of frass to plant residuals (PR-F) in Paper III resulted in a reduced lignin degradation, although this decrease was not statistically significant when compared with the control (PR-C, Figure 5). However, when enzymes as well as frass were added (PR-EF), lignin degradation increased significantly.

The significantly lower lignin degradation observed under a plastic foam cover (PR-HB) may be attributed to altered physical and biological conditions that inhibit aerobic degradation processes. Unlike cellulose and hemicellulose, lignin breakdown relies on oxygen-dependent oxidative enzymes such as laccases and peroxidases (Mattila et al., 2020; Xiang et al., 2024b). Foam covers likely restrict gas exchange, reducing oxygen diffusion and promoting hypoxic or anaerobic microenvironments that suppress aerobic lignin-degrading microbes.

Increased heat and moisture retention under the cover may further favour anaerobic bacteria, which are generally not efficient in degrading lignin (Benner et al., 1984; Odier and Monties, 1983). Such conditions may shift the microbial community away from aerobic taxa such as *Actinobacteria* and *Bacilli*, thereby reducing microbial diversity (Ventorino et al., 2015). Since BSFL activity enhances aeration through movement and mixing (Gorrens et al., 2022; Nayak et al., 2024), the foam cover may also limit larval-induced oxygenation, leading to more stratified and compacted substrates.

Although larval survival was not affected by the cover (Table 4), the reduced lignin degradation suggests that key microbial lignin degraders were inhibited. As lignin breakdown primarily occurs in the substrate, whereas cellulose and hemicellulose are largely degraded by the larvae (Xiang et al., 2024b), the latter processes seemed to be unaffected by the cover-induced microenvironments (Figures 4–6).

6.2.4 Pesticide degradation

Microbial degradation is a key mechanism in pesticide degradation, with bacteria and fungi transforming the material via enzymatic activity and assimilation into biomass (Parte, 2017). Currently approved pesticides are generally designed to have minimal negative environmental impact as they exhibit greater efficacy at lower doses, reduced environmental persistence, and lower toxicity to non-target organisms compared to banned organochlorine compounds, which, despite being prohibited for several

decades, continue to persist and contaminate the environment (Meijer et al., 2025; UNEP, 2016).

In broader contexts, ultraviolet (UV) exposure has been demonstrated to enhance pesticide degradation primarily through direct photolysis, whereby the chemical structure of the pesticide is broken down by UV radiation (EL-Saeid et al., 2022; Yang, Y. et al., 2022). In addition to photodegradation, soil-mediated microbial processes also play a critical role, as diverse microbial communities can metabolise pesticide compounds, thereby contributing to their breakdown. Furthermore, interactions between pesticides and organic matter in the soil—such as adsorption, complexation, and co-metabolism—can influence both the rate and extent of degradation. These combined mechanisms highlight the multifactorial nature of pesticide attenuation in environmental systems (Yang, Y. et al., 2022).

In this study, all analysed pesticides were degraded to a larger extend on a total solids (TS) basis in the large-scale trial compared to the small-scale trial (Figure 7). Imazalil, which remained undegraded in the small-scale trial, was reduced by approximately 58% in the large-scale setting (Paper IV). The enhanced pesticide degradation observed in the pilot-scale set-up, in which the substrate was provided in a single feeding event, with half the substrate volume (Paper IV), likely resulted from improved aeration, microbial activity, and environmental stability.

A reduced substrate volume created a thinner layer, allowing for better oxygen diffusion, whilst larval movement further enhanced mixing and oxygenation (Nayak et al., 2024). Aerobic conditions are critical for the breakdown of many pesticides (Arias-Estévez et al., 2008), and improved oxygen availability likely supported more efficient microbial degradation.

However, as discussed in section 6.2.2, the foam cover used in the pilot-scale treatment—but not used in the small-scale treatment—likely restricted gas exchange and reduced oxygen diffusion during the initial phase. As the foam covers were removed after a week, subsequent differences in degradation may be attributed more to the differences in box volume than to previous oxygen limitations. A larger box may have favoured improved degradation: Yakti et al. 2022 observed a significant increase in larval growth and substrate temperature in larger rearing scales, which likely enhanced substrate breakdown and material reduction through improved physical and biological conditions. It is thus plausible that the bioconversion process fostered a microbial-rich environment capable of pesticide

degradation, especially in the large-scale trial (Paper IV), despite its lower larval bioconversion efficiency (5% BCE, 47% M.Red, 31 mg TS/cm^2 larval yield) compared to the small-scale setup (8% BCE, 46% M.Red, 95 mg TS/cm^2 larval yield, Paper III).

Another difference in the large and small-scale set-up was the number of feeding occasions. Although multi-feeding treatments reduce compaction and enhance aeration, the brief post-feeding interval (9 d) may be insufficient to achieve complete pesticide mineralisation. This limitation likely accounts for the comparatively lower degradation rates observed in the small-scale treatment (PR-HB, Figure 7, Paper IV). Lalander et al. (2016) demonstrated that BSFL composting accelerates the degradation of several pesticides compared to treatments without larvae, and similar trends were observed here. Degradation in small-scale trials was lower than in large-scale setups but remained negligible in the absence of larvae, underscoring the importance of larval-associated microbial communities as well as choice of box size (Gold et al., 2020b; Yakti et al., 2022).

6.3 BSFL composting of high-lignocellulose substrates in a pilot scale setting

Compared to high-lignocellulose substrates such as cucumber plant residuals, the treatment of food waste using BSFL composting presents a significantly more straightforward and efficient process. Food waste, with its balanced nutrient profile, higher moisture content, and lower structural complexity, requires no pre-treatment. In the present study, the food waste treatment yielded high BCE (23%), material reduction (64%) and larval yield (370 mg TS/cm^2), whilst also allowing for relatively straight forward separation of larvae from frass. In contrast, the treatment of cucumber plant residuals yielded lower BCE (6%), material reduction (54%) and larval yield (31 mg TS/cm^2), when conducted within a comparable timeframe, but posed several operational challenges. Pre-treatment was required to improve nutrient accessibility, yet the outcome remained suboptimal. Separation of larvae from the undegraded residue was particularly problematic and only a minimal number of larvae could be harvested. Furthermore, larvae were observed escaping from the boxes, which is further discussed below. These observations underscore the importance of substrate selection and quality in

the design of BSFL systems, as well as the need for tailored process strategies when working with structurally complex agricultural residues.

Despite comparable larval densities and similar VS being provided to the larvae, the small-scale BSFL composting system demonstrated significantly higher BCE, material reduction, and larval yield compared to the large-scale setup (Tables 1 and 3). These inconsistencies are likely because of scale-dependent environmental, variations that likely influenced larval behaviour and microbial activity (Yakti et al., 2022). In the large-scale trial, the reduced substrate volume and depth together with the larger box may have contributed to an increased passive aeration, promoting aerobic microbial processes that support the breakdown of complex organic matter (Nayak et al., 2024; Yakti et al., 2022), as well as allowing time for pesticide degradation. At the same time, the one-time feeding may have resulted in the physical inaccessibility to deeper substrate layers, limiting degradation and nutrient uptake by the larvae.

Moreover, metabolic heat accumulation in large volumes can result in temperature stratification, with central regions experiencing elevated temperatures that may inhibit both larvae and microbial consortia (Benner et al., 1984; Odier and Monties, 1983). However, this was not observed in the large-scale trial, due to the mass larval escape which resulted in a perceived lower survival rate (and thus BCE). High substrate evaporation could also have caused substrate cooling, and together these factors may have impaired larval activity and substrate degradation.

Substrate heterogeneity likely contributed to poor heat retention, as heterogeneous, porous matrices disrupt conductive heat flow due to air-filled voids and increased thermal resistance (Skibinski et al., 2019). Consequently, substrate temperatures remained around 20°C, below the larvae's optimal range of 30–35°C, slowing development and promoting larvae escape (Chia et al., 2018). The combined metabolic heat from larvae and microorganisms was insufficient to counter evaporative cooling, limiting feeding, digestion, and bioconversion. The reduced active larval biomass further decreased heat production, linking enhanced escape and BCE to unfavourable thermal conditions, which is consistent with previous findings (Chia et al., 2018).

The treatment of substrates with a nutritional profile (*e.g.*, high lignocellulose content, low protein content) that is poorly suited to BSFL composting, presents several practical and economic challenges. Whilst such

suboptimal substrates often yield lower BCE due to their recalcitrant nature, low bioavailability, or mismatched C/N and protein to carbohydrate ratios (Dzepe et al., 2025; Isibika et al., 2021), the process may still offer important environmental and waste management benefits that merit consideration, particularly in large-scale operations.

6.4 Feasibility of BSFL treatment of high-lignocellulose substrates

The limitations discussed above must be weighed against the broader cost-saving and environmental advantages of the process. From an economic perspective, the loss of larval biomass value—resulting from reduced biomass yields and the lack of efficient separation technologies when residues are insufficiently degraded—can significantly diminish the financial viability of BSFL composting. This is particularly relevant when treating nutritionally imbalanced substrates, such as those with low protein content or high structural recalcitrance, which compromise larval growth and development (Lalander et al., 2018; Surendra et al., 2020). At the same time, one of the key benefits of BSFL treatment is its capacity to significantly reduce the volume and mass of biowaste, thereby lowering the logistical and financial burdens associated with downstream handling, transport, and disposal. In regions with high disposal fees or stringent waste management regulations, this volume reduction alone may justify the implementation of BSFL systems.

Even when larval biomass yields are suboptimal, BSFL treatment can still deliver important environmental services, such as organic matter stabilisation, partial detoxification (*e.g.*, pesticide reduction), and contributions to circular economy frameworks. As discussed in section 6.2.3, treatment of cucumber plant residual waste can reduce pesticide concentrations, although not always to levels that meet regulatory thresholds (Figure 8). Nevertheless, this partial degradation represents a clear improvement over uncontrolled dumping, which poses a risk of pesticide leaching into soil and groundwater (Löfkvist et al., 2009).

Compared to conventional alternatives—such as composting, anaerobic digestion, or incineration at centralised facilities—BSFL treatment offers a lower-energy and decentralised solution. These alternatives often involve

costly disposal fees (*e.g.*, 660 SEK per tonne[2] at designated recycling stations), require extensive infrastructure, and cause significant logistical and carbon costs (Eisted et al., 2009; SRV, 2025).

In this context, the application of BSFL treatment to high lignocellulose wastes like cucumber plant residues emerges as a viable alternative, even without the economic return of larval biomass. The approach would be more attractive if financial compensation were available for waste reduction services. Ultimately, BSFL treatment offers considerable benefits through volume reduction, partial detoxification, and compatibility with resource recovery systems—particularly relevant given the limited treatment options currently available for such challenging substrates (Cattaneo et al., 2024).

Substrates with high lignocellulose content is poorly suited to anaerobic digestion due to several constraints: it is generated intermittently as a point-source stream, it possesses high lignin content that resists microbial degradation under anaerobic conditions, and it has a low protein content that fails to meet the nutritional demands of the anaerobic microbial community (Carlsson and Uldal, 2009; Hansson and Christensen, 2005). Thus, extended residence times within the digester are required to achieve satisfactory degradation, reducing process efficiency and economic feasibility (Carlsson and Uldal, 2009; Hansson and Christensen, 2005).

For BSFL treatment to work effectively as an option for high-lignocellulosic substrates, certain adaptations must be made and a suggestion that could work is substrate blending to achieve a more fitting nutritional profile (Isibika et al., 2021). However, this could increase the workload in the case of cucumber plant residuals, as large flows are generated at specific times, and coordinating their treatment with other waste types would add logistical complexity.

An alternative strategy to mitigate this challenge could involve adopting harvesting methods utilised in early BSFL composting systems, in which the larvae were allowed to develop into the prepupal stage within the substrate and exit the material autonomously, making use of their innate migratory behaviour. This approach, although associated with a lower BCE (19%), yielded a higher material reduction (69%) compared to the food waste reference treatment (23% BCE, 64% M.Red), likely due to the presence of

[2] https://nsr.se/foretag/lamna-verksamhetsavfall/priser/

larvae at multiple developmental stages throughout the process (Dortmans, 2015). This approach could also contribute to added benefits such as lower pesticide concentration in prepupae, as it was found to be lower in the prepupae than in the larvae (Figure 8).

If time and space constraints were not limiting factors, pre-treatment of plant residuals prior to larval feeding could prevent uncontrolled degradation during storage (as demonstrated in Paper IV) and optimise co-treatment timing with other waste arrivals included to improve process efficiency.

Grau et al. (2022) analysed three BSFL composting models within Surabaya's municipal waste management system (Indonesia), each representing a different level of centralisation. The fully centralised model (Scenario 1), where all processing stages were conducted at a single facility, demonstrated the highest financial viability due to economies of scale. Scenario 2 involved decentralised waste treatment and post-processing in smaller local units, whilst maintaining the fly colony centralised, was found to lower transport costs but made profitability more dependent on scale and product value. Scenario 3 involved decentralised waste treatment only, with centralised fly colony and post-processing, offering a compromise between reduced transport needs and central oversight, though with slightly lower financial returns. The study illustrates the trade-offs between economic performance, logistical efficiency, and potential environmental and social benefits, such as reduced emissions and enhanced local employment (Grau et al., 2022). However, the economic assessments in these models assume that larval biomass constitutes a valuable product stream—providing a significant source of revenue that underpins the financial viability of each scenario.

In the case of BSFL treatment using substrates such as cucumber plant residuals or orange peels, this assumption does not hold, as larval growth is significantly limited due to the poor nutritional quality and structural complexity of these materials. Consequently, the absence of marketable larval biomass necessitates a different approach to evaluating the feasibility of BSFL systems for such feedstocks. Instead, the focus should shift towards assessing the process primarily as a waste reduction strategy, with economic justification potentially tied to avoided disposal costs, reduced waste volumes, and environmental co-benefits rather than direct product revenues. Accordingly, the centralised and decentralised configurations must be reinterpreted considering these altered value dynamics. Their feasibility

depends not only on financial metrics, but also on contextual factors such as available space, access to technical expertise, and the capacity to accommodate longer processing times required for more recalcitrant substrates. Under these conditions, the viability of BSFL treatment may rely less on direct returns from biomass sales and more on its contribution to sustainable waste management, regulatory compliance, and circular economy goals.

In conclusion, whilst BSFL composting of high-lignocellulose substrates may not generate profitable biomass yields if not blended with other substrates, it can offer a valuable waste reduction and detoxification service that supports sustainable large-scale waste management.

7. Conclusions

BSFL composting of food and agro-industry wastes presents several biological and operational challenges, particularly due to the substrates' structural recalcitrance, low protein content, and intermittent availability. Whilst pre-treatment methods—chemical, fungal, and enzymatic—can enhance substrate degradability and material reduction, their impact on BCE and larval yield is variable and substrate-dependent.

Noteworthily, the most effective improvements were observed for plant residuals when they were pre-treated with enzymes and frass at a lower substrate density (PR-HB) at small scale. Direct addition of enzymes to lettuce and cabbage improved BCE, material reduction and larval yield. Although the BCE was at a similar level as for the reference substrate food waste, the larval yield achieved was only 20% to that of food waste, due to its high moisture content. The other treatments across all studies, achieved high material reduction but yielded lower or similar BCE when pre-treated. However, none of the plant-based waste treatments attained BCE or larval yields comparable to those achieved with food waste. Substrates with high moisture content likely benefit more from dewatering or the use of drying agents, whilst fibrous or structurally complex materials typically demand targeted pre-treatments to break down resistant components and increase nutrient availability.

Direct emissions of CH_4 and N_2O were low relative to CO_2 in all treatments. Both fungi and ammonia pre-treated broccoli and cauliflower emitted less N_2O than the control, however, ammonia pre-treatment increased NH_3 emissions.

Large-scale BSFL composting of fibrous, pesticide-contaminated plant residuals can facilitate partial pesticide degradation, primarily through enhanced microbial activity. Whilst larval BCE was lower in large-scale systems compared to small-scale trials, degradation of several pesticides—including imazalil—was more pronounced, likely due to favourable physical conditions and a one-time feeding regime that allowed prolonged microbial action. Although pesticide levels in the residual material did not consistently fall below EU regulatory thresholds, it highlights BSFL composting's potential as an environmentally beneficial waste management strategy.

Large-scale trials revealed significant performance reductions compared to small-scale systems, likely due to environmental factors such as

inadequate aeration, thermal instability, and substrate heterogeneity. Nonetheless, BSFL composting consistently contributed to significant waste volume reduction and partial pesticide degradation, demonstrating its potential as a sustainable alternative to conventional waste management, especially for fibrous agricultural residues with limited existing treatment options. For successful deployment at larger scales, system adaptations are necessary. These may include optimising pre-treatment strategies, adjusting the entire composting set-up for non-optimal substrates, and improving environmental controls to support microbial and larval performance.

In summary, different types of substrates require different management strategies and whilst BSFL composting may not be economically profitable for nutritional suboptimal substrates, it remains a viable strategy for environmentally sustainable waste management—supporting broader goals of circular economy development and resource recovery.

8. Future research

Future research should focus on optimising BSFL treatment systems for nutritionally suboptimal substrates through substrate blending or tailored pre-treatment strategies and improved process control at scale. Further studies are also needed to better understand the interactions between substrate composition, microbial communities, and larval performance, especially under the variable environmental conditions typical of large-scale operations.

Research should explore combinations of chemical, fungal, and enzymatic pre-treatments that balance nutrient preservation with fibre degradation to improve BSFL composting outcomes for homogeneous nutritionally challenging substrates.

Developing low labour harvesting methods, such as self-harvesting by prepupae, may increase feasibility for substrates with low bioconversion potential. Economic and life cycle assessments of decentralised systems compared with centralised systems—particularly in regions lacking established waste infrastructure—will be essential to identify the most sustainable and cost-effective implementation models. Lastly, investigations into the long-term environmental impacts of BSFL-treated residues, including greenhouse gas emissions during maturation and the fate of residual pesticides, will help to ensure the safe and responsible integration of BSFL composting into circular waste management systems.

References

Afedzi, A.E.K., Afrakomah, G.S., Gyan, K., Khan, J., Seidu, R., Baidoo, T., Sultan, I.N., Tareen, A.K., Parakulsuksatid, P., 2025. Enhancing Economic and Environmental Sustainability in Lignocellulosic Bioethanol Production: Key Factors, Innovative Technologies, Policy Frameworks, and Social Considerations. Sustainability 17(2), 499.

Alagappan, S., Hong, H., Mikkelsen, D., Olarte Mantilla, S., James, P., Yarger, O., Hoffman, L., Cozzolino, D., 2025. Investigating the effect of larval instar, post-harvest treatments, and substrate on the nutritional profile of black soldier fly larvae (Hermetia illucens). Animal Production Science 65(8), -.

Alagappan, S., Rowland, D., Barwell, R., Cozzolino, D., Mikkelsen, D., Olarte Mantilla, S.M., James, P., Yarger, O., Hoffman, L., 2022. Organic side streams (bioproducts) as substrate for black soldier fly (Hermetia illucens) intended as animal feed: chemical safety issues. Animal Production Science 62(17), 1639-1651.

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., Wang, M.-Q., 2021. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics 9(3), 42.

Almqvist, R., 2020. Fly larvae composting of fibrous food industry waste - impact of pre-treatment, Department of Molecular Sciences. SLU.

Amin, F.R., Khalid, H., Zhang, H., Rahman, S.u., Zhang, R., Liu, G., Chen, C., 2017. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express 7(1), 72.

Amrul, N.F., Kabir Ahmad, I., Ahmad Basri, N.E., Suja, F., Abdul Jalil, N.A., Azman, N.A., 2022. A Review of Organic Waste Treatment Using Black Soldier Fly (Hermetia illucens). Sustainability 14(8), 4565.

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R., Šantek, B., 2018. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng Life Sci 18(11), 768-778.

Andraskar, J., Yadav, S., Khan, D., Kapley, A., 2023. Treatment Options for Municipal Solid Waste by Composting and Its Challenges. Indian J Microbiol 63(3), 235-243.

Angulo, J., Mahecha, L., Yepes, S.A., Yepes, A.M., Bustamante, G., Jaramillo, H., Valencia, E., Villamil, T., Gallo, J., 2012. Quantitative and nutritional characterization of fruit and vegetable waste from marketplace: a potential use as bovine feedstuff? J Environ Manage 95 Suppl, S203-209.

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C., García-Río, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture, Ecosystems & Environment 123(4), 247-260.

Au - Chundawat, S.P.S., Au - Pal, R.K., Au - Zhao, C., Au - Campbell, T., Au - Teymouri, F., Au - Videto, J., Au - Nielson, C., Au - Wieferich, B., Au - Sousa, L.,

Au - Dale, B.E., Au - Balan, V., Au - Chipkar, S., Au - Aguado, J., Au - Burke, E., Au - Ong, R.G., 2020. JoVE(158), e57488.
Bajwa, U., Sandhu, K.S., 2014. Effect of handling and processing on pesticide residues in food- a review. Journal of Food Science and Technology 51(2), 201-220.
Balan, V., Mohammadi, M., Dale, B.E., 2025. Advancements in ammonia-based pretreatment: key benefits and industry applications. RSC Sustainability.
Banks, I.J., Gibson, W.T., Cameron, M.M., 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop Med Int Health 19(1), 14-22.
Barragán-Fonseca, K., Dicke, M., van Loon, J., 2018a. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). Entomologia Experimentalis et Applicata 166.
Barragán-Fonseca, K., Pineda-Mejia, J., Dicke, M., van Loon, J.J.A., 2018b. Performance of the Black Soldier Fly (Diptera: Stratiomyidae) on Vegetable Residue-Based Diets Formulated Based on Protein and Carbohydrate Contents. J Econ Entomol 111(6), 2676-2683.
Barragán-Fonseca, K.Y., Nurfikari, A., van de Zande, E.M., Wantulla, M., van Loon, J.J.A., de Boer, W., Dicke, M., 2022. Insect frass and exuviae to promote plant growth and health. Trends in Plant Science 27(7), 646-654.
Beasley, J., Kuehny, J., Gentimis, T., Fields, J., 2023. Black Soldier Fly Frass Supports Plant Growth and Reduces Nitrogen Leaching during Coleus Production. HortTechnology 33(3), 305-312.
Beesigamukama, D., Tanga, C.M., Sevgan, S., Ekesi, S., Kelemu, S., 2023. Waste to value: Global perspective on the impact of entomocomposting on environmental health, greenhouse gas mitigation and soil bioremediation. Science of The Total Environment 902, 166067.
Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 57(1), 289-300.
Benner, R., Maccubbin, A.E., Hodson, R.E., 1984. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora. Appl Environ Microbiol 47(5), 998-1004.
Bessa, L.W., Pieterse, E., Marais, J., Hoffman, L.C., 2020. Why for feed and not for human consumption? The black soldier fly larvae. Compr Rev Food Sci Food Saf 19(5), 2747-2763.
Bhandari, S., Pandey, S., Giri, K., Wagle, P., Bhattarai, S., Neupane, R.B., 2019. Effects of different fertilizers on the growth and yield of okra (Abelmoschus esculentus L.) in summer season in Chitwan, Nepal. Archives of Agriculture and Environmental Science 4, 396-403.
Bohm, K., Hatley, G.A., Robinson, B.H., Gutiérrez-Ginés, M.J., 2023. Analysis of Chemical and Phytotoxic Properties of Frass Derived from Black Soldier Fly-Based Bioconversion of Biosolids. Sustainability 15(15), 11526.

Bonelli, M., Bruno, D., Caccia, S., Sgambetterra, G., Cappellozza, S., Jucker, C., Tettamanti, G., Casartelli, M., 2019. Structural and Functional Characterization of Hermetia illucens Larval Midgut. Front Physiol 10.
Book, A.J., Lewin, G.R., McDonald, B.R., Takasuka, T.E., Wendt-Pienkowski, E., Doering, D.T., Suh, S., Raffa, K.F., Fox, B.G., Currie, C.R., 2016. Evolution of High Cellulolytic Activity in Symbiotic Streptomyces through Selection of Expanded Gene Content and Coordinated Gene Expression. PLoS Biol 14(6), e1002475.
Borel, P., Hammaz, F., Morand-Laffargue, L., Creton, B., Halimi, C., Sabatier, D., Desmarchelier, C., 2021. Using black soldier fly larvae reared on fruits and vegetables waste as a sustainable dietary source of provitamin a carotenoids. Food Chemistry 359, 129911.
Bruno, D., Bonacci, T., Reguzzoni, M., Casartelli, M., Grimaldi, A., Tettamanti, G., Brandmayr, P., 2020. An in-depth description of head morphology and mouthparts in larvae of the black soldier fly Hermetia illucens. Arthropod Structure & Development 58, 100969.
Carlsson, M., Uldal, M., 2009. Substrathandbok för biogasproduktion, RAPPORT U2009:14. Avfall Sverige, Malmö.
Carmona-Cabello, M., García, I.L., Sáez-Bastante, J., Pinzi, S., Koutinas, A.A., Dorado, M.P., 2020. Food waste from restaurant sector - Characterization for biorefinery approach. Bioresour Technol 301, 122779.
Castro-Herrera, D., Prost, K., Kim, D.-G., Yimer, F., Tadesse, M., Gebrehiwot, M., Brüggemann, N., 2023. Biochar addition reduces non-CO2 greenhouse gas emissions during composting of human excreta and cattle manure. Journal of Environmental Quality 52(4), 814-828.
Cattaneo, A., Meneguz, M., Dabbou, S., Tambone, F., Scaglia, B., 2024. Local circular economy: BSF insect rearing in the Italian Agri-Food Industry. Waste Manag 179, 234-244.
Chen, J., Hou, D., Pang, W., Nowar, E.E., Tomberlin, J.K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z., Li, Q., 2019. Effect of moisture content on greenhouse gas and NH_3 emissions from pig manure converted by black soldier fly. Science of The Total Environment 697, 133840.
Chen, J., Hou, D., Pang, W., Nowar, E.E., Tomberlin, J.K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z., Li, Q., 2019. Effect of moisture content on greenhouse gas and NH(3) emissions from pig manure converted by black soldier fly. Sci Total Environ 697, 133840.
Chia, S.Y., Tanga, C.M., Khamis, F.M., Mohamed, S.A., Salifu, D., Sevgan, S., Fiaboe, K.K.M., Niassy, S., van Loon, J.J.A., Dicke, M., Ekesi, S., 2018. Threshold temperatures and thermal requirements of black soldier fly Hermetia illucens: Implications for mass production. PLOS ONE 13(11), e0206097.
Choi, S., Hassanzadeh, N., 2019. BSFL Frass: A Novel Biofertilizer for Improving Plant Health Whilst Minimizing Environmental Impact. THE CANADIAN SCIENCE FAIR JOURNAL 2(2).
Čičková, H., Newton, G.L., Lacy, R.C., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Management 35(0), 68-80.

Coman, V., Teleky, B.E., Mitrea, L., Martău, G.A., Szabo, K., Călinoiu, L.F., Vodnar, D.C., 2020. Bioactive potential of fruit and vegetable wastes. Adv Food Nutr Res 91, 157-225.
Cornish-Bowden, A., 2014. Current IUBMB recommendations on enzyme nomenclature and kinetics. Perspectives in Science 1(1), 74-87.
Dabbou, S., Cattaneo, A., Cattapan, F., Malinverni, R., Macchi, E., Manenti, I., Todesco, S., Poloni, E., Rosà, R., Santori, D., Meneguz, M., Pertot, I., Sezzi, E., 2025. Impact of live black soldier fly larvae supplementation on laying hen performance, stress levels and excreta microbiota. Poultry Science 104(9), 105418.
Diener, S., Studt Solano, N.M., Roa Gutiérrez, F., Zurbrügg, C., Tockner, K., 2011a. Biological Treatment of Municipal Organic Waste using Black Soldier Fly Larvae. Waste and Biomass Valorization 2(4), 357-363.
Diener, S., Zurbrügg, C., Roa-Gutiérrez, F., Hong Dang, N., Morel, A., Koottatep, T., Tockner, K., 2011b. Black soldier fly larvae for organic waste treatment - prospects and constraints.
Do, S., Koutsos, L., Utterback, P.L., Parsons, C.M., de Godoy, M.R.C., Swanson, K.S., 2020. Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecectomized rooster assay1. J Anim Sci 98(1).
Dortmans, B., 2015. Valorisation of organic waste : effect of the feeding regime on process parameters in a continuous black soldier fly larvae composting system, Energy and Technology. SLU, Uppsala.
Dzepe, D., Nana, P., Fotso, A., Tchuinkam, T., Djouaka, R., 2020. Influence of larval density, substrate moisture content and feedstock ratio on life history traits of black soldier fly larvae. Journal of Insects as Food and Feed 6(2), 133-140.
Dzepe, D., Ndindeng, S.A., Ogbon, E.A., Riggi, L., Lalander, C., Djouaka, R., 2025. Enzymatic pretreatment optimizes rice husk decomposition by black soldier fly, Hermetia illucens L. (Diptera: Stratiomyidae) larvae. BMC Environmental Science 2(1), 10.
Edwards, M.C., Williams, T., Pattathil, S., Hahn, M.G., Doran-Peterson, J., 2014. Replacing a suite of commercial pectinases with a single enzyme, pectate lyase B, in Saccharomyces cerevisiae fermentations of cull peaches. Journal of Industrial Microbiology and Biotechnology 41(4), 679-686.
EEA, E.E.A., 2024. Emissions of the main air pollutants in Europe.
Eiland, F., Klamer, M., Lind, A.M., Leth, M., Bååth, E., 2001. Influence of Initial C/N Ratio on Chemical and Microbial Composition during Long Term Composting of Straw. Microbial ecology 41, 272-280.
Eisted, R., Larsen, A.W., Christensen, T.H., 2009. Collection, transfer and transport of waste: accounting of greenhouse gases and global warming contribution. Waste Manag Res 27(8), 738-745.
EL-Saeid, M.H., BaQais, A., Alshabanat, M., 2022. Study of the Photocatalytic Degradation of Highly Abundant Pesticides in Agricultural Soils. Molecules 27(3), 634.
Ercoli, L., Rossetto, R., Di Giorgi, S., Raffaelli, A., Nuti, M., Pellegrino, E., 2023. Effective bioremediation of clarithromycin and diclofenac in wastewater by

microbes and Arundo donax L. Environmental Science and Pollution Research 30(31), 77193-77209.
Erickson, M.C., Islam, M., Sheppard, C., Liao, J., Doyle, M.P., 2004. Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. J Food Prot 67(4), 685-690.
Ermolaev, E., Jarvis, A., Sundberg, C., Smars, S., Pell, M., Jonsson, H., 2015. Nitrous oxide and methane emissions from food waste composting at different temperatures. Waste Management 46, 113-119.
Ermolaev, E., Lalander, C., Vinneras, B., 2019. Greenhouse gas emissions from small-scale fly larvae composting with *Hermetia illucens*. Waste Management 96, 65-74.
Ermolaev, E., Pell, M., Smårs, S., Sundberg, C., Jönsson, H., 2012. Greenhouse gas emission from covered windrow composting with controlled ventilation. Waste Management & Research 30(2), 155-160.
European Commission, 2021. Commission Regulation (EU) 2021/1925 of 5 November 2021 Amending Certain Annexes to Regulation (EU) No 142/2011 as Regards the Requirements for Placing on the Market of Certain Insect Products and the Adaptation of a Containment Method, L393. Official Journal of the European Union, pp. 4-8.
Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (Hermetia illucens) - Possibilities and limitations for modification through diet. Waste Management 102, 40-47.
FAO, 1998. Fruit and vegetable processing: Nutritional value, in: Nations., F.a.A.O.o.t.U. (Ed.).
FAO, 2022. The State of World Fisheries and Aquaculture 2022: Towards blue transformation, in: Nations, F.a.A.O.o.t.U. (Ed.).
FAO, 2024. The State of World Fisheries and Aquaculture 2024.
FAO, F.a.A.O.o.t.U.N., 2023. The State of Food Security and Nutrition in the World 2023, in: FAO (Ed.).
Fatima, S., Afzal, A., Rashid, H., Iqbal, S., Zafar, R., Khalid, K., Rauf, A., Majeed, M., Malik, A., Carter, C.G., 2023. Dietary replacement of soybean meal with black soldier fly larvae meal in juvenile Labeo rohita and Catla catla: Effects on growth, nutritional quality, oxidative stress biomarkers and disease resistance. PLoS One 18(11), e0294452.
Ferronato, N., Torretta, V., 2019. Waste Mismanagement in Developing Countries: A Review of Global Issues. International Journal of Environmental Research and Public Health 16(6), 1060.
Fogg, P., Boxall, A.B., Walker, A., Jukes, A.A., 2003. Pesticide degradation in a 'biobed' composting substrate. Pest Management Science 59(5), 527-537.
Gage, E., Terry, L.A., Falagán, N., 2025. Biological factors and production challenges drive significant UK fruit and vegetable loss. J Sci Food Agr 105(4), 2109-2117.

Gage, E., Wang, X., Xu, B., Foster, A., Evans, J., Terry, L.A., Falagán, N., 2024. Reducing food loss and waste contributes to energy, economic and environmental sustainability. Journal of Cleaner Production 451, 142068.
Gao, Q., Guo, L.-n., Li, S.-h., Wu, W., Ding, J.w., Xu, H.-j., Luo, C.-b., Li, J., Li, D.-q., Liu, Z.-q., 2025. Biodegradation mechanism of cellulose, hemicellulose, and lignin in bacteria-dominant aerobic composting from agricultural biomass waste:A review. Carbohydrate Polymer Technologies and Applications 11, 100879.
Gold, M., Cassar, C.M., Zurbrugg, C., Kreuzer, M., Boulos, S., Diener, S., Mathys, A., 2020a. Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. Waste Management 102, 319-329.
Gold, M., Fowles, T., Fernandez-Bayo, J.D., Miner, L.P., Zurbrügg, C., Nansen, C., Bischel, H.N., Mathys, A., 2022. Effects of rearing system and microbial inoculation on black soldier fly larvae growth and microbiota when reared on agri-food by-products. Journal of Insects as Food and Feed 8(2), 113-128.
Gold, M., Tomberlin, J.K., Diener, S., Zurbrugg, C., Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. Waste Management 82, 302-318.
Gold, M., von Allmen, F., Zurbrügg, C., Zhang, J., Mathys, A., 2020b. Identification of Bacteria in Two Food Waste Black Soldier Fly Larvae Rearing Residues. Frontiers in Microbiology 11.
Gorrens, E., De Smet, J., Vandeweyer, D., Bossaert, S., Crauwels, S., Lievens, B., Van Campenhout, L., 2022. The bacterial communities of black soldier fly larvae (Hermetia illucens) during consecutive, industrial rearing cycles. Journal of Insects as Food and Feed 8(10), 1061-1076.
Grau, M., Dortmans, B.M.A., Egger, J., Virard, G., Zurbrügg, C., 2022. Modelling the financial viability of centralised and decentralised black soldier fly larvae waste processing units in Surabaya, Indonesia. Journal of Insects as Food and Feed 9, 1-14.
Green, J.M.H., Croft, S.A., Durán, A.P., Balmford, A.P., Burgess, N.D., Fick, S., Gardner, T.A., Godar, J., Suavet, C., Virah-Sawmy, M., Young, L.E., West, C.D., 2019. Linking global drivers of agricultural trade to on-the-ground impacts on biodiversity. Proceedings of the National Academy of Sciences 116(46), 23202-23208.
Gunther, F.A., Whitacre, D., Albert, L.A., Hutzinger, O., Knaak, J., Mayer, F., Morgan, D.P., Park, D.L., Tjeerdema, R., Voogt, P., Yang, R.S.H., Giesy, J., Stevens, J., Ware, G., 2010. Reviews of Environmental Contamination and Toxicology. 202.
Halloran, A., Roos, N., Eilenberg, J., Cerutti, A., Bruun, S., 2016. Life cycle assessment of edible insects for food protein: a review. Agronomy for Sustainable Development 36(4), 57.
Hansson, A., Christensen, K., 2005. Biogas ger energi till ekologiskt lantbruk. Jordbruksverket.

Hender, A., Siddik, M.A.B., Howieson, J., Fotedar, R., 2021. Black Soldier Fly, Hermetia illucens as an Alternative to Fishmeal Protein and Fish Oil: Impact on Growth, Immune Response, Mucosal Barrier Status, and Flesh Quality of Juvenile Barramundi, Lates calcarifer (Bloch, 1790). Biology 10(6), 505.
Holmes, L.A., Vanlaerhoven, S.L., Tomberlin, J.K., 2013. Substrate Effects on Pupation and Adult Emergence of Hermetia illucens (Diptera: Stratiomyidae). Environmental Entomology 42(2), 370-374.
IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change., in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.). Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press, p. 1553.
IPCC, 2019. Climate Change and Land: An IPCC Special Report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.
IPCC, 2023. Climate Change 2023: Synthesis Report, in: Core Writing Team, H.L.a.J.R.e. (Ed.). Geneva, Switzerland, pp. 35-115.
Isibika, A., Vinneras, B., Kibazohi, O., Zurbrugg, C., Lalander, C., 2019. Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. Waste Management 100, 151-160.
Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C., Lalander, C., 2021. Co-composting of banana peel and orange peel waste with fish waste to improve conversion by black soldier fly (Hermetia illucens (L.), Diptera: Stratiomyidae) larvae. Journal of Cleaner Production 318, 128570.
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). Bioresource Technology 290, 121785.
Jalc, D., scaron, an, Siroka, P., Cere, scaron, aacute, kov, aacute, Zuzana, 1997. Effect of six species of white-rot basidiomycetes on the chemical composition and rumen degradability of wheat straw. The Journal of General and Applied Microbiology 43(3), 133-137.
Janssen, R.H., Vincken, J.-P., van den Broek, L.A.M., Fogliano, V., Lakemond, C.M.M., 2017. Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. Journal of Agricultural and Food Chemistry 65(11), 2275-2278.
Jones, B., Tomberlin, J., 2019. Impact of Larval Competition on Life-History Traits of the Black Soldier Fly (Diptera: Stratiomyidae). Annals of the Entomological Society of America 112, 505-510.
Kariuki, E.G., Kibet, C., Paredes, J.C., Mboowa, G., Mwaura, O., Njogu, J., Masiga, D., Bugg, T.D.H., Tanga, C.M., 2023. Metatranscriptomic analysis of the gut microbiome of black soldier fly larvae reared on lignocellulose-rich fiber diets unveils key lignocellulolytic enzymes. Frontiers in Microbiology Volume 14 - 2023.
Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S.-i., Hashizume, A., Ido, A., Miura, C., Miura, T., Nakamura, S., Seyama, T.,

Matsumoto, Y., Kasai, K., Fujitani, Y., 2019. Evaluation of Black Soldier Fly (Hermetia illucens) Larvae and Pre-Pupae Raised on Household Organic Waste, as Potential Ingredients for Poultry Feed. Animals 9(3), 98.
Kenis, M., Bouwassi, B., Boafo, H., Devic, E., Han, R., Koko, G., Koné, N.G., Maciel-Vergara, G., Nacambo, S., Pomalegni, S.C.B., Roffeis, M., Wakefield, M., Zhu, F., Fitches, E., 2018. Small-Scale Fly Larvae Production for Animal Feed, in: Halloran, A., Flore, R., Vantomme, P., Roos, N. (Eds.), Edible Insects in Sustainable Food Systems. Springer International Publishing, Cham, pp. 239-261.
Khodadoust, A., Khoshkholgh, M., Noveirian, H.A., Mohseni, M., Khara, H., 2024. Effects of fermented soybean meal with methionine and lysine supplementation on hemato-biochemical parameters, digestive enzyme activities and health status in Beluga, Huso huso. Aquaculture Reports 39, 102478.
Kisaakye, J., Beesigamukama, D., Haukeland, S., Subramanian, S., Thiongo, P., Kelemu, S., Tanga, C., 2024. Chitin-enriched insect frass fertilizer as a biorational alternative for root-knot nematode (Meloidogyne incognita) management. Frontiers in Plant Science 15, 1361739.
Klammsteiner, T., Heussler, C.D., Insam, H., Schlick-Steiner, B.C., Steiner, F.M., 2025. Larval density drives thermogenesis and affects microbiota and substrate properties in black soldier fly trials. iScience 28(7), 112794.
Klammsteiner, T., Walter, A., Bogataj, T., Heussler, C.D., Stres, B., Steiner, F.M., Schlick-Steiner, B.C., Insam, H., 2021. Impact of Processed Food (Canteen and Oil Wastes) on the Development of Black Soldier Fly (Hermetia illucens) Larvae and Their Gut Microbiome Functions. Frontiers in Microbiology Volume 12 - 2021.
Kuschnig, N., Cuaresma, J.C., Krisztin, T., Giljum, S., 2021. Spatial spillover effects from agriculture drive deforestation in Mato Grosso, Brazil. Scientific Reports 11(1), 21804.
Kuschnig, N., Giljum, S., 2019. Soy production indirectly drives Brazilian deforestation. A case for considering spatial dependence, FINEPRINT Brief.
Laksanawimol, P., Anukun, P., Thancharoen, A., 2024. Use of different dry materials to control the moisture in a black soldier fly (Hermetia illucens) rearing substrate. PeerJ 12, e17129.
Lalander, C., Diener, S., Magri, M.E., Zurbrügg, C., Lindström, A., Vinnerås, B., 2013. Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)--from a hygiene aspect. Sci Total Environ 458-460, 312-318.
Lalander, C., Diener, S., Zurbrugg, C., Vinneras, B., 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). Journal of Cleaner Production 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. Science of The Total Environment 729(138968).
Lalander, C., Nordberg, A., Vinneras, B., 2018. A comparison in product-value potential in four treatment strategies for food waste and faeces - assessing composting, fly larvae composting and anaerobic digestion. Gcb Bioenergy 10(2), 84-91.

Lalander, C., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K., Vinnerås, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of The Total Environment 565, 279-286.
Lalander, C.H., Fidjeland, J., Diener, S., Eriksson, S., Vinneras, B., 2015. High waste-to-biomass conversion and efficient *Salmonella spp.* reduction using black soldier fly for waste recycling. Agronomy for Sustainable Development 35(1), 261-271.
Laursen, S.F., Flint, C.A., Bahrndorff, S., Tomberlin, J.K., Kristensen, T.N., 2024. Reproductive output and other adult life-history traits of black soldier flies grown on different organic waste and by-products. Waste Management 181, 136-144.
Lemme, A., Klüber, P., 2024. Rethinking Amino Acid Nutrition of Black Soldier Fly Larvae (Hermetia illucens) Based on Insights from an Amino Acid Reduction Trial. Insects 15(11), 862.
Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E., Lock, E.-J., 2017. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media. PLOS ONE 12(8), e0183188.
Lin, Y.-P., Ansari, A., Wunderlich, R.F., Lur, H.-S., Ngoc-Dan Cao, T., Mukhtar, H., 2022. Assessing the influence of environmental niche segregation in ammonia oxidizers on N2O fluxes from soil and sediments. Chemosphere 289, 133049.
Liu, C., Wang, C., Yao, H., Chapman, S., 2020. Pretreatment is an important method for increasing the conversion efficiency of rice straw by black soldier fly larvae based on the function of gut microorganisms. Science of The Total Environment 762, 144118.
Liu, Y., Xi, Y., Li, Q., Dzakpasu, M., Chen, R., Li, Y.-Y., 2024. Biokinetic and microbial insights into regulatory mechanisms of long-chain fatty acid degradation during food waste-lipid co-digestion within anaerobic membrane bioreactor. Bioresource Technology 408, 131223.
Livsmedelsverket, Livsmedelsverkets livsmedelsdatabas.
Livsmedelsverket, 2025. Livsmedelsverkets livsmedelsdatabas. https://soknaringsinnehall.livsmedelsverket.se/.
Lohri, C.R., Diener, S., Zabaleta, I., Mertenat, A., Zurbrügg, C., 2017. Treatment technologies for urban solid biowaste to create value products: a review with focus on low- and middle-income settings. Reviews in Environmental Science and Bio/Technology 16(1), 81-130.
Lopes, I.G., Lalander, C., Vidotti, R.M., Vinneras, B., 2020. Using Hermetia illucens larvae to process biowaste from aquaculture production. Journal of Cleaner Production 251.
Lopes, I.G., Wiklicky, V., Ermolaev, E., Lalander, C., 2023. Dynamics of black soldier fly larvae composting – Impact of substrate properties and rearing conditions on process efficiency. Waste Management 172, 25-32.
Lopes, I.G., Wiklicky, V., Vinnerås, B., Yong, J.W.H., Lalander, C., 2024. Recirculating frass from food waste bioconversion using black soldier fly larvae:

Impacts on process efficiency and product quality. Journal of Environmental Management 366, 121869.
Lopes, I.G., Yong, J.W.H., Lalander, C., 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. Waste Management 142, 65-76.
Lu, Y., Zhang, S., Sun, S., Wu, M., Bao, Y., Tong, H., Ren, M., Jin, N., Xu, J., Zhou, H., Xu, W., 2021. Effects of Different Nitrogen Sources and Ratios to Carbon on Larval Development and Bioconversion Efficiency in Food Waste Treatment by Black Soldier Fly Larvae (Hermetia illucens). Insects 12(6).
Löfkvist, K., Hansson, T., Svensson, S.A., 2009. Förluster av växtskyddsmedel till omgivande mark och vatten vid användning i svenska växthus - en genomgång av möjliga riskmoment, Rapport 2009:6. Faculty of Landscape Architecture, Horticulture and Crop Production Sciences.
Ma, J., Lei, Y., Rehman, K.u., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J.K., Zheng, L., 2018. Dynamic Effects of Initial pH of Substrate on Biological Growth and Metamorphosis of Black Soldier Fly (Diptera: Stratiomyidae). Environmental Entomology 47(1), 159-165.
Machado Tahamtani, F., Ivarsson, E., Wiklicky, V., Lalander, C., Wall, H., Rodenburg, B., Tuyttens, F., Hernandez, C., 2021. Feeding live Black Soldier Fly larvae (Hermetia illucens) to laying hens: effects on feed consumption, hen health, hen behavior, and egg quality. Poultry Science 100, 101400.
Magri, M.E., Fidjeland, J., Jönsson, H., Albihn, A., Vinnerås, B., 2015. Inactivation of adenovirus, reovirus and bacteriophages in fecal sludge by pH and ammonia. Science of The Total Environment 520, 213-221.
Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197, 1-33.
Mattila, H.K., Mäkinen, M., Lundell, T., 2020. Hypoxia is regulating enzymatic wood decomposition and intracellular carbohydrate metabolism in filamentous white rot fungus. Biotechnology for Biofuels 13(1), 26.
Mbokou Foukmeniok, S., Ogbon, A., Bougna Tchoumi, H.H., Dzepe, D., Santos, J.C.C., Riggi, L., Tonle Kenfack, I., Djouaka, R., 2024. Effect of the Rearing Substrate Contamination with λ-Cyhalothrin Pesticide on the Growth Performance and Survival of Black Soldier Fly (Hermetia illucens) Larvae: A Study of Biodegradation Kinetics. Chemistry Africa 7(5), 2833-2844.
Mehta, A., Yadav, A., Kumar, A., Kumari, K., 2024. Waste Utilization in Horticulture: An Overview. Journal of Experimental Agriculture International 46(5), 742–749.
Meijer, N., de Rijk, T., van Loon, J.J.A., Zoet, L., van der Fels-Klerx, H.J., 2021. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (Hermetia illucens) larvae. PLOS ONE 16(4), e0249362.
Meijer, N., Safitri, R.A., Tao, W., Hoek-Van den Hil, E.F., 2025. Review: European Union legislation and regulatory framework for edible insect production – Safety issues. animal 19, 101468.

Meneguz, M., Gasco, L., Tomberlin, J.K., 2018a. Impact of pH and feeding system on black soldier fly (Hermetia illucens, L; Diptera: Stratiomyidae) larval development. Plos One 13(8).
Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., Gasco, L., 2018b. 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.
Mertenat, A., Diener, S., Zurbrugg, C., 2019. Black Soldier Fly biowaste treatment - Assessment of global warming potential. Waste Management 84, 173-181.
Mohanram, S., Amat, D., Choudhary, J., Arora, A., Nain, L., 2013. Novel perspectives for evolving enzyme cocktails for lignocellulose hydrolysis in biorefineries. Sustainable Chemical Processes 1(1), 15.
Moll, L.V., Smet, J.D., Paas, A., Tegtmeier, D., Vilcinskas, A., Cos, P., Campenhout, L.V., 2022. In Vitro Evaluation of Antimicrobial Peptides from the Black Soldier Fly (Hermetia Illucens) against a Selection of Human Pathogens. Microbiology Spectrum 10(1), e01664-01621.
Mondello, G., Salomone, R., Ioppolo, G., Saija, G., Sparacia, S., Lucchetti, M.C., 2017. Comparative LCA of Alternative Scenarios for Waste Treatment: The Case of Food Waste Production by the Mass-Retail Sector. Sustainability 9(5), 827.
Mufungwe, J., Namukonde, N., Mwaanga, P., Johnson, T., Siamujompa, M., Mwango, N.C., Ngoma, J., Hang'ombe, B.M., 2025. Critical safety concerns in the production of black soldier Fly (Hermetia illucens) larvae in Africa. Discover Food 5(1), 74.
Myers, H.M., Tomberlin, J.K., Lambert, B.D., Kattes, D., 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37(1), 11-15.
Müller, A., Wiedmer, S., Kurth, M., 2019. Risk Evaluation of Passive Transmission of Animal Parasites by Feeding of Black Soldier Fly (Hermetia illucens) Larvae and Prepupae. Journal of Food Protection 82.
Nayak, A., Rühl, M., Klüber, P., 2024. Hermetia illucens (Diptera: Stratiomyidae): Need, Potentiality, and Performance Measures. Agriculture 14(1), 8.
Newton, L., Sheppard, D., Watson, D., Burtle, G., Dove, C., Tomberlin, J., Thelen, E., 2005. The black soldier fly, Hermetia illucens, as a manure management/resource recovery tool.
Nguyen, T.T.X., Tomberlin, J.K., Vanlaerhoven, S., 2015. Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. Environmental Entomology 44(2), 406-410.
Nyakeri, E.M., Ayieko, M.A., Amimo, F.A., Salum, H., Ogola, H.J.O., 2019. An optimal feeding strategy for black soldier fly larvae biomass production and faecal sludge reduction. Journal of Insects as Food and Feed 5(3), 201-214.
Odier, E., Monties, B., 1983. Absence of microbial mineralization of lignin in anaerobic enrichment cultures. Appl Environ Microbiol 46(3), 661-665.
Ogbon, E.A., Dzepe, D., Lalander, C., Wiklicky, V., Sinda, P.V.K., Adéoti, R., Mignouna, D., Gbaguidi, B., Behanzin, J.G., Riggi, L., Djouaka, R., 2025. Risk

assessment of black soldier fly (Hermetia illucens (L.), Diptera: Stratiomyidae) larvae composting for circular waste management in southern Benin. Journal of Insects as Food and Feed 11(8), 1483-1498.
Oliveira, F., Doelle, K., List, R., O'Reilly, J.R., 2015. Assessment of Diptera: Stratiomyidae, genus *Hermetia illucens* (L., 1758) using electron microscopy. Journal of Entomology and Zoology Studies 5(3), 147-152.
Pang, W., Hou, D., Chen, J., Nowar, E.E., Li, Z., Hu, R., Tomberlin, J.K., Yu, Z., Li, Q., Wang, S., 2020. Reducing greenhouse gas emissions and enhancing carbon and nitrogen conversion in food wastes by the black soldier fly. Journal of Environmental Management 260.
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. Journal of Cleaner Production 271.
Parodi, A., Gerrits, W.J.J., Van Loon, J.J.A., De Boer, I.J.M., Aarnink, A.J.A., Van Zanten, H.H.E., 2021. Black soldier fly reared on pig manure: Bioconversion efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions. Waste Management 126, 674-683.
Parte, S.G., Mohekar, A. D., & Kharat, A. S., 2017. Microbial degradation of pesticide: A review. African Journal of Microbiology Research 11(24), 992-1012.
Paz, A.S.P., Carrejo, N.S., Rodriguez, C.H.G., 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 and Biomass Valorization 6(6), 1059-1065.
Peguero, D.A., Gold, M., Endara, A., Niu, M., Zurbrügg, C., Mathys, A., 2023. Evaluation of ammonia pretreatment of four fibrous biowastes and its effect on black soldier fly larvae rearing performance. Waste Management 160, 123-134.
Peguero, D.A., Gold, M., Velasquez, L., Niu, M., Zurbrügg, C., Mathys, A., 2024. Physical pretreatment of three biowastes to improve black soldier fly larvae bioconversion efficiency. Waste Management 178, 280-291.
Pennacchio, A., Pitocchi, R., Varese, G.C., Giardina, P., Piscitelli, A., 2021. Trichoderma harzianum cerato-platanin enhances hydrolysis of lignocellulosic materials. Microb Biotechnol.
Pishgar-Komleh, S.H., Vernooij, A., Straub, P., 2022. Carbon footprint of processing city market waste for animal feed with Black Soldier Flies in Kampala, Uganda. Wageningen Livestock Research, Wageningen.
Pitocchi, R., Cicatiello, P., Birolo, L., Piscitelli, A., Bovio, E., Varese, G.C., Giardina, P., 2020. Cerato-Platanins from Marine Fungi as Effective Protein Biosurfactants and Bioemulsifiers. International Journal of Molecular Sciences 21(8), 2913.
Purkayastha, D., Sarkar, S., 2021. Sustainable waste management using black soldier fly larva: a review. International journal of Environmental Science and Technology.

R Core Team, 2024. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.
Rehman, K.u., Schwennen, C., Visscher, C., Plötz, M., Grabowski, N.T., Sultana, M.U.C., Wiesotzki, K., Hollah, C., Aganovic, K., Heinz, V., 2025. Closing the loop with pretreatment and black soldier fly technology for recycling lignocellulose-rich organic by-products: A progressive review. Carbohydrate Polymer Technologies and Applications 9, 100630.
Roberts, S., Jacquet, J., Majluf, P., Hayek, M.N., 2024. Feeding global aquaculture. Science Advances 10(42), eadn9698.
Saidani, M., Dabbou, S., Ben Larbi, M., Belhadj Slimen, I., Fraihi, W., Arbi, T., Amraoui, M., M'Hamdi, N., 2025. Effect of black soldier fly (Hermetia illucens L.) larvae meal on growth performance, carcass characteristics, meat quality, and cecal microbiota in broiler chickens. Frontiers in Animal Science Volume 6 - 2025.
Salomone, R., Saija, G., Mondello, G., Giannetto, A., Fasulo, S., Savastano, D., 2017. Environmental impact of food waste bioconversion by insects: Application of Life Cycle Assessment to process using Hermetia illucens. Journal of Cleaner Production 140, 890-905.
Schreven, S.J.J., Vries, H.d., Hermes, G.D.A., Zeni, G., Smidt, H., Dicke, M., Loon, J.J.A.v., 2022. Black Soldier Fly Larvae Influence Internal and Substrate Bacterial Community Composition Depending on Substrate Type and Larval Density. Applied and Environmental Microbiology 88(10), e00084-00022.
Seyedalmoosavi, M.M., Mielenz, M., Veldkamp, T., Daş, G., Metges, C.C., 2022. Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (Hermetia illucens) larvae compared to monogastric livestock species: a review. J Anim Sci Biotechnol 13(1), 31.
Sharma, R., Lamsal, B.P., Colonna, W.J., 2016. Pretreatment of fibrous biomass and growth of biosurfactant-producing Bacillus subtilis on biomass-derived fermentable sugars. Bioprocess Biosyst Eng 39(1), 105-113.
Sheppard, D., Newton, L., Thompson, S.A., Savage, S., 1994. A value added manure management system using the black soldier fly. Bioresource Technology 50(3), 275-279.
Skawińska, E., Zalewski, R.I., 2022. Combining the Water–Energy–Food and Food Waste–Food Loss–Food Security Nexuses to Reduce Resource Waste. Energies 15(16), 5866.
Skibinski, J., Cwieka, K., Haj Ibrahim, S., Wejrzanowski, T., 2019. Influence of Pore Size Variation on Thermal Conductivity of Open-Porous Foams. Materials 12(12), 2017.
Smetana, S., Palanisamy, M., Mathys, A., Heinz, V., 2016. Sustainability of insect use for feed and food: Life Cycle Assessment perspective. Journal of Cleaner Production 137.
Song, K.H., Woo, J.S., Kim, J.R., Ryu, G.L., Baek, Y.C., Oh, Y.K., Kwak, W.S., Park, K.K., 2020. Nutritional value and in situ degradability of fruit-vegetable byproducts and their feeding effects on performance of growing Hanwoo steers. Asian-Australas J Anim Sci 33(6), 973-980.

SRV, 2025. Trädgårdsavfall. https://www.srvatervinning.se/sorteringsguide/tradgardsavfall.
Su, Y., Yu, X., Sun, Y., Wang, G., Chen, H., Chen, G., 2018. Evaluation of Screened Lignin-degrading Fungi for the Biological Pretreatment of Corn Stover. Sci Rep 8(1), 5385.
Suckling, J., Druckman, A., Small, R., Cecelja, F., Bussemaker, M., 2021. Supply chain optimization and analysis of Hermetia illucens (black soldier fly) bioconversion of surplus foodstuffs. Journal of Cleaner Production 321, 128711.
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. Renew Energ 98, 197-202.
Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.-H.L., Khanal, S.K., 2020. Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.)) (Diptera: Stratiomyidae) (BSF). Waste Management 117, 58-80.
Suryadi, H., Judono, J.J., Putri, M.R., Eclessia, A.D., Ulhaq, J.M., Agustina, D.N., Sumiati, T., 2022. Biodelignification of lignocellulose using ligninolytic enzymes from white-rot fungi. Heliyon 8(2), e08865.
Szűcs, C., Kovács, E., Bagi, Z., Rákhely, G., Kovács, K.L., 2021. Enhancing biogas production from agroindustrial waste pre-treated with filamentous fungi. Biologia Futura.
Theron, W., Teke, G.M., Diedericks, D., Van Rensburg, E., Görgens, J.F., 2023. Steam pre-treatment of sugarcane bagasse and wheat straw as a cleaner feedstock for black soldier fly larvae rearing. Journal of Insects as Food and Feed 10(4), 585-599.
Tomberlin, J., Sheppard, D., 2002. Factors Influencing Mating and Oviposition of Black Soldier Flies (Diptera: Stratiomyidae) in a Colony. Journal of Entomological Science 37, 345-352.
Tomberlin, J.K., Adler, P.H., Myers, H.M., 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environ Entomol 38(3), 930-934.
Tomberlin, J.K., Huis, A.v., 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., Emhart, C., Epstein, M., Finke, M., Fischer, C.H., Gatlin, D., Grabowski, N.T., He, C., Heckman, L., Hubert, A., Jacobs, J., Joseph, J., Khanal, S.K., Kleinfinger, J.K., 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., 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, 307-309.
UNEP, 2016. Stockholm Convention on Persistent Organic Pollutants (POPs). https://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx.

United Nations Environment Programme, 2024. Food Waste Index Report 2024. Think Eat Save: Tracking Progress to Halve Global Food Waste.
University of Gothenburg, 2025. Varför matas odlad fisk med fisk? https://www.gu.se/swemarc-marint-vattenbruk/18-varfor-matas-odlad-fisk-med-fisk.
van Heugten, E., Martinez, G., McComb, A., Koutsos, L., 2022. Improvements in Performance of Nursery Pigs Provided with Supplemental Oil Derived from Black Soldier Fly (Hermetia illucens) Larvae. Animals 12(23), 3251.
van Kessel, K., Castelijn, G., van der Voort, M., Meijer, N., 2024. Investigation of Bacillus cereus growth and sporulation during Hermetia illucens larval rearing. Heliyon 10(24), e40912.
Ventorino, V., Aliberti, A., Faraco, V., Robertiello, A., Giacobbe, S., Ercolini, D., Amore, A., Fagnano, M., Pepe, O., 2015. Exploring the microbiota dynamics related to vegetable biomasses degradation and study of lignocellulose-degrading bacteria for industrial biotechnological application. Scientific Reports 5(1), 8161.
Waithaka, M.K., Osuga, I.M., Kabuage, L.W., Subramanian, S., Muriithi, B., Wachira, A.M., Tanga, C.M., 2022. Evaluating the growth and cost-benefit analysis of feeding improved indigenous chicken with diets containing black soldier fly larva meal. Front Insect Sci 2, 933571.
Wang, D., Liu, B., Liu, X., Xu, Q., Yang, Q., Liu, Y., Zeng, G., Li, X., Ni, B.-J., 2018. How does free ammonia-based sludge pretreatment improve methane production from anaerobic digestion of waste activated sludge. Chemosphere 206, 491-501.
Wang, Y., Luo, Y., Luo, L., Zhang, H., Liao, Y., Gou, C., 2021. Enhancement of the nutritional value of fermented corn stover as ruminant feed using the fungi Pleurotus spp. Scientific Reports 11(1), 11961.
Weththasinghe, P., Hansen, J.Ø., Mydland, L.T., Øverland, M., 2022. A systematic meta-analysis based review on black soldier fly (Hermetia illucens) as a novel protein source for salmonids. Reviews in Aquaculture 14(2), 938-956.
Wu, J., Xiong, L., Yang, Y., Li, C., Mao, P., Zhou, Q., Yu, G., Huang, X., Tyagi, R.D., He, X., Wong, J.W.C., 2026. Greenhouse gas emissions from black soldier fly composting of silver and silver sulfide nanoparticle-enriched sludge. Water Research 288, 124608.
Xiang, F., Han, L., Jiang, S., Xu, X., Zhang, Z., 2024a. Black soldier fly larvae mitigate greenhouse gas emissions from domestic biodegradable waste by recycling carbon and nitrogen and reconstructing microbial communities. Environmental Science and Pollution Research 31(23), 33347-33359.
Xiang, F., Zhang, Q., Xu, X., Zhang, Z., 2024b. Black soldier fly larvae recruit functional microbiota into the intestines and residues to promote lignocellulosic degradation in domestic biodegradable waste. Environmental Pollution 340, 122676.
Xu, L., Lin, Q., Wang, S., Chen, S., Yang, R., Liu, C., Hu, Q., Zhao, Z., Cao, Z., 2025. Efficacy of black soldier fly larvae in converting kitchen waste and the dynamic alterations of their gut microbiome. Journal of Environmental Management 377, 124613.

Yakti, W., Müller, M., Klost, M., Mewis, I., Dannehl, D., Ulrichs, C., 2023. Physical Properties of Substrates as a Driver for Hermetia illucens (L.) (Diptera: Stratiomyidae) Larvae Growth. Insects 14(3), 266.
Yakti, W., Schulz, S., Marten, V., Mewis, I., Padmanabha, M., Hempel, A.-J., Kobelski, A., Streif, S., Ulrichs, C., 2022. The Effect of Rearing Scale and Density on the Growth and Nutrient Composition of Hermetia illucens (L.) (Diptera: Stratiomyidae) Larvae. Sustainability 14(3), 1772.
Yang, C., Ma, S., Li, F., Zheng, L., Tomberlin, J.K., Yu, Z., Zhang, J., Yu, C., Fan, M., Cai, M., 2022. Characteristics and mechanisms of ciprofloxacin degradation by black soldier fly larvae combined with associated intestinal microorganisms. Science of The Total Environment 811, 151371.
Yang, Y., Ran, L., Pan, T., Yuan, F., Hu, D., Lu, P., 2022. Degradation of Sulfoxaflor in Water and Soil: Kinetics, Degradation Pathways, Transformation Product Identification, and Toxicity. Journal of Agricultural and Food Chemistry 70(11), 3400-3408.
Yu, M., Li, Z., Chen, W., Rong, T., Wang, G., Ma, X., 2019. Hermetia illucens larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. Journal of Animal Science and Biotechnology 10(1), 50.
Yu, Y., Zhang, J., Zhu, F., Fan, M., Zheng, J., Cai, M., Zheng, L., Huang, F., Yu, Z., Zhang, J., 2023. Enhanced protein degradation by black soldier fly larvae (Hermetia illucens L.) and its gut microbes. Frontiers in Microbiology Volume 13 - 2022.
Zahir, M., Fogliano, V., Capuano, E., 2020. Effect of soybean processing on cell wall porosity and protein digestibility. Food & Function 11(1), 285-296.
Zeng, J., Singh, D., Chen, S., 2011. Biological pretreatment of wheat straw by Phanerochaete chrysosporium supplemented with inorganic salts. Bioresource Technology 102(3), 3206-3214.
Zhang, H.Y., Li, G.X., Gu, J., Wang, G.Q., Li, Y.Y., Zhang, D.F., 2016. Influence of aeration on volatile sulfur compounds (VSCs) and NH_3 emissions during aerobic composting of kitchen waste. Waste Management 58, 369-375.
Zhang, T.-X., Li, M.-R., Liu, C., Wang, S.-P., Yan, Z.-G., 2023. A review of the toxic effects of ammonia on invertebrates in aquatic environments. Environmental Pollution 336, 122374.
Zhao, C., Shao, Q., Chundawat, S.P.S., 2020. Recent advances on ammonia-based pretreatments of lignocellulosic biomass. Bioresource Technology 298, 122446.
Zunzunegui, I., Martín-García, J., Santamaría, Ó., Poveda, J., 2025. Insect Frass as an Agricultural Resource Against Abiotic and Biotic Crop Stresses: Mechanisms of Action and Possible Negative Effects. Applied Sciences 15(7), 3606.

Popular science summary

Black soldier fly larvae (BSFL) are small insects with a big potential: they can turn food and plant waste into valuable products such as protein-rich animal feed and compost-like fertiliser. This makes BSFL composting an exciting option for sustainable waste management and part of the growing circular economy. But not all waste is equal — and some types, such as cucumber plant leftovers or orange peels, are difficult for the larvae to digest.

This study explored the extent to which BSFL composting works on plant-based waste with a low nutritional value. Various ways were tested to improve the process, including pre-treating the waste with fungi, enzymes, or ammonia, and adding insect frass (a dry, soil-like residue). It was assessed how much waste the larvae could convert into biomass, how much the total waste was reduced, if pesticides were being broken down, and what gases (such as methane or ammonia) were released during the process.

The results showed that whilst some treatments helped to reduce the amount of waste — especially fungal and enzyme pre-treatments — the larvae did not grow as much, meaning the process did not produce much usable insect biomass. At larger scales, the composting process worked even less efficiently due to challenges such as poor air flow and uneven temperatures. However, BSFL composting greatly reduced its volume and still helped to lower pesticide levels in the waste even though their levels did not decrease sufficiently to fall below EU regulatory thresholds. Additionally, emissions of greenhouse gases and ammonia were found to be low during the BSFL composting process.

Even if BSFL composting is not always profitable from a business perspective, especially with nutritionally suboptimal plant waste, it can still offer large environmental benefits. With the right system design and management, it could be a practical and sustainable way to handle tricky agricultural waste that would otherwise go to a landfill or require costly disposal.

Populärvetenskaplig sammanfattning

Amerikanska vapenflugor är små insekter med stor potential: de kan omvandla mat- och växtavfall till värdefulla produkter såsom proteinrikt djurfoder och kompostliknande gödningsmedel. Detta gör fluglarvskompostering till ett lovande alternativ för hållbar avfallshantering och en del av den växande cirkulära ekonomin. Dock är inte allt avfall likvärdigt – vissa typer, såsom gurkplantrester eller apelsinskal, är svåra för larverna att bryta ner.

Denna studie undersökte hur effektivt fluglarvskompostering fungerar på växtbaserat avfall med lågt näringsvärde. Olika sätt testades för att förbättra processen, bland annat förbehandling med svampar, enzymer eller ammoniak, samt tillsats av insektsfrass, en torr, jordliknande restprodukt. Det gjordes en bedömning av larvernas biomassaomvandling, materialreduktion och utsläpp av gaser såsom metan och ammoniak under processen.

Resultaten visade att vissa behandlingar, särskilt svamp- och enzymförbehandlingar, förbättrade avfallsminskningen, men larvtillväxten var begränsad, vilket minskade produktionen av användbar biomassa. På större skala fungerade fluglarvskomposteringen sämre, på grund av utmaningar som bristande luftflöde och ojämna temperaturer. Trots detta bidrog fluglarvskomposteringen till betydande minskning av avfallsvolymen och bekämpningsmedelsnivåerna i avfallet även om deras nivåer inte minskade tillräckligt för att falla under EU:s gränsvärden. Dessutom visade sig utsläppen av växthusgaser och ammoniak vara låga under fluglarvskomposteringen.

Även om fluglarvskompostering inte alltid är ekonomiskt lönsam, särskilt vid behandling av näringsfattigt växtavfall, kan den erbjuda betydande miljöfördelar. Med rätt systemdesign och hantering kan det vara en hållbar och praktisk lösning för att ta hand om svårhanterligt jordbruksavfall som annars riskerar att hamna på deponi eller kräva kostsam bortskaffning.

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Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment

L. Lindberg, E. Ermolaev, B. Vinnerås, C. Lalander *

Department of Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, 75007, Uppsala, Sweden

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ABSTRACT

One technology that implements circular economy principles is black soldier fly larvae (BSFL) composting. To assess the environmental impact of BSFL technology, more data on emissions of greenhouse gases (GHG) and ammonia are needed. This study investigated process efficiency and GHG emissions from BSFL composting of orange peels and a mix of broccoli and cauliflower trimmings, with and without pre-treatment. Two weeks of substrate pre-treatment with ammonia or fungi (*Trichoderma reesei*) were investigated, and direct emissions of GHG and ammonia from the process were evaluated. Process efficiency was evaluated by waste-to-biomass conversion efficiency (BCE) and material reduction. In BSFL composting of trimmings, BCE was not significantly improved by pre-treatment, However, larval volatile solids (VS) load in the fungi pre-treated treatment was very low, likely contributing to low BCE. BCE was low (6%) in the peel control and even lower in the pre-treatments, indicating that this substrate is unsuitable for BSFL composting. Material reduction was largest for trimmings (84%) and peels (60%) pre-treated with fungi. Emissions of methane (CH_4) and nitrous oxide (N_2O), expressed in CO_2-eq, were very low (0.04–1.57 g/kg initial wet weight (ww)) compared with direct CO_2 emissions (47–147 g/kg initial ww). Fungi pre-treatment appeared to make the trimmings more available to the larvae, while also drying out the substrate and removing a large proportion of available VS. Thus fungi pre-treatment could be used to increase waste treatment capacity. Ammonia pre-treatment reduced emissions of CH_4 and N_2O without affecting overall BCE, but significantly increased NH_3 emissions.

1. Introduction

Worldwide, municipal waste generation is expected to increase from 2 billion tonnes per year (average in 2016) to 3.4 billion tonnes by 2050 (Kaza et al., 2018). Globally, 70% of biodegradable waste is either dumped in the open or landfilled (Kaza et al., 2018). There, it degrades in an uncontrolled environment and can generate greenhouse gases (GHG), which contribute to climate change, and ammonia (NH_3), which contributes to acidification and eutrophication (Bernstad and Jansen, 2012; IPCC, 2014). Methane (CH_4) has a global warming potential of 34 carbon dioxide (CO_2)-equivalents with climate-carbon feedback over a 100-year period (GWP_{100}), while nitrous oxide (N_2O) has a GWP_{100v} of 298 CO_2-equivalents (IPCC, 2013). Globally, landfills are estimated to contribute 12% of total anthropogenic methane emissions (Saunois et al., 2020). In 2007, it was estimated that only 12% of methane emissions from landfills in that year were captured globally (Themelis and Ulloa, 2007).

The resources in biodegradable waste can be harvested if managed adequately (Slorach et al., 2019). Efficient reuse of resources is in line with the resource efficiency strategy established in the European Commission's action plan for a circular economy (European Commission, 2020). According to the European Union (EU) waste hierarchy, methods allowing material re-use should be implemented with the highest priority (European Union, 2016). One such method is fly larvae composting, in which proteins, carbohydrates and lipids from biodegradable wastes are concentrated in the larval biomass and indirectly reused (Suckling et al., 2021). The most commonly used fly species for this purpose is black soldier fly (*Hermetia illucens* L. (Diptera: Stratiomyidae)) (Tomberlin et al., 2015). The black soldier fly larvae (BSFL) biomass produced can be used in animal feed, due to its high protein and lipid content of around 25–40% and 20–60% on a dry matter (dm) basis, respectively (Ewald et al., 2020; Meneguz et al., 2018), while the treatment residues (frass) can be used as a fertiliser (Song et al., 2021). Biodegradable streams available for conversion in fly larvae composting are limited in the EU under Regulation (EC) No. 1774/2002, allowing only plant-based biodegradable streams for use as feed for larvae. The

* Corresponding author.
E-mail address: cecilia.lalander@slu.se (C. Lalander).

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processed larvae protein can be used as feed for fish, poultry, pigs and non-production animals (EU 2021/1372) (European Union, 2021)).

Process efficiency, in terms of biomass conversion efficiency (BCE) and material reduction, in BSFL composting of fruit and vegetable waste is lower than for mixed food waste (Lalander et al., 2019). This is possibly a result of low protein content in relation to high content of carbon with low digestibility (lignin and hemicellulose) in fruit and vegetable substrates (Gold et al., 2018; Kumar et al., 2018; Meneguz et al., 2018; Nguyen et al., 2015; Nyakeri et al., 2017).

One way to tackle the lower substrate availability in BSFL composting of these substrates could be pre-treatment to improve their digestibility. Isibika et al. (2019) found that pre-treatment with fungi or ammonia solution increased BCE in BSFL composting of banana peels. The underlying concept when using ammonia is for microorganisms in the BSFL treatment system to convert non-protein nitrogen (NH_3) into proteins through nitrogen assimilation to generate amino acids. Ammonia has also been shown to help microorganisms break down complex molecules (Tadele and Amha, 2015). The underlying concept with fungal pre-treatment is to degrade complex molecules into forms more available to the larvae.

Emissions of GHG have been found to be considerably lower in BSFL composting than in conventional windrow composting (Ermolaev et al., 2019; Mertenat et al., 2019). Previous small-scale evaluations have shown that combined CH_4 and N_2O emissions are 0.03–8.6 g/kg initial wet weight (ww) and that NH_3 emissions are 0.05–0.5 g/kg initial ww (Chen et al., 2019; Ermolaev et al., 2019; Guo et al., 2021; Pang et al., 2020; Parodi et al., 2020). However, most studies published so far have focused on assessment of emissions from BSFL composting of food waste, agricultural waste and manure. To our knowledge, studies investigating GHG emissions from fruit and vegetable wastes are currently lacking. Treatment of fruit and vegetable wastes in BSFL composting may require pre-treatment to achieve reasonable BCE (Isibika et al., 2019). The impact of bacterial pre-treatment on emissions during BSFL composting has been examined previously in a study on food waste by Ermolaev et al. (2019), but GHG emissions during the pre-treatment were not measured in that study. It is thus important to investigate the direct emissions associated with pre-treatment and with BSFL composting itself, to enable an overall assessment of the suitability and performance of BSFL composting of plant-based waste.

This study investigated the process efficiency of BSFL composting of orange peels and a mix of broccoli and cauliflower trimmings, with and without pre-treatment, and evaluated the associated direct emissions of GHG and ammonia during the two steps of the process.

2. Materials and methods

2.1. Materials

The plant-based waste used in the experiments was provided by a fruit and vegetable wholesaler (Grönsakshallen Sorunda, Stockholm, Sweden). The two plant-based waste fractions studied, peels from organically grown oranges (OP) and trimmings from broccoli and cauliflower (BC), were delivered separately. Upon arrival at the BSFL composting facility, the orange peels were stored for up to 6 d at 15 °C and then milled (Robot-Coupe, model Blixer 4 V.V, France) directly before use in experiments. The broccoli and cauliflower trimmings were stored for up to 9 d at 15 °C before milling and then stored for up to a further 3 d at −7±3 °C before use in experiments. The difference in storage time between orange peels and trimmings was caused by the delivery time of each substrate and the time when processing could occur. However, as the same within-substrate procedure was used, any difference in handling between substrates would not impact the results of the study.

Source-separated household food waste (FW) from Eskilstuna, Strängnäs and Örebro was collected and minced at the recycling plant Lilla Nyby in Eskilstuna (Eskilstuna & Strängnäs Energi och Miljö, Eskilstuna, Sweden) and transported to the BSFL composting facility at the Swedish University of Agricultural Sciences (SLU, Uppsala, Sweden). The minced food waste was used in the experiments directly upon arrival.

The larvae used in the study weighed around 1 mg each and were provided by the black solider fly colony at the Department of Energy and Technology, SLU, which has been run continuously since 2015 (Uppsala, Sweden).

2.2. Experimental set-up

The orange peels and mix of broccoli and cauliflower trimmings were used in three treatments: no pre-treatment control (-con), pre-treatment with the fungal species *Trichoderma reesei* (-tri) and pre-treatment with ammonia solution (-amm) (Table 1). The hypothesis in the *T. reesei* pre-treatment was that the fungi would assist in degradation of fibres unavailable to the larvae (Rehman et al., 2017) into shorter and more available carbohydrates, as *Trichoderma reesei* is known to produce cellulase and hemicellulase enzymes that break down high-fibre vegetable substrates (Kunamneni et al., 2014). The hypothesis for the ammonia pre-treatment was that it would improve the digestibility of fibres in the fruit and vegetable waste (Bals et al., 2010) and enhance the potential for ammonia assimilation by microorganisms (Wang et al., 2015).

2.2.1. Pre-treatments

Trichoderma reesei was pre-cultured on malt extract agar (MEA) at 28 °C for 7 d. The fungi were harvested by pouring sterile 0.9% NaCl solution onto the agar plate and scraping the fungi off with a 10-μL inoculation loop. The fungi solution was transferred to 50-mL centrifuge tubes, concentrated by centrifugation to a density of 10^7 g/mL and inoculated into the waste substrates to 1% (w/w) concentration. For ammonia pre-treatment, 24.5% ammonia solution (Nitor, Sweden) was added to the waste substrate to reach a total NH_3–N concentration of 1% (w/w), holding a pH of approximately 10. After the pre-treatment (Table 1), the pH of the pre-treated substrates was adjusted to pH 7.5 ± 0.5 using stepwise addition of concentrated sulphuric acid (Fisher Chemicals, Uppsala, Sweden). The pre-treated substrate was sealed in plastic bags. The amount of substrate at the beginning of the pre-treatment was 15 kg and that remaining after completion of pre-treatment was divided into three equal feeding portions for BSFL composting.

All pre-treatments were performed in triplicate, in standard plastic

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

		Pre-treatment time [d]	BSFL composting time [d]	Total substrate [kg ww]
Controls without pre-treatment				
OP-con	Orange peels	-	27	14.6 ± 0.1
BC-con	Broccoli and cauliflower trimmings	-	28	15.7 ± 0.1
FW-con	Food waste	-	17	15.4 ± 0.2
Trichoderma reesei *pre-treatment*				
OP-tri	Orange peels	16	31	15.4 ± 0.1
BC-tri	Broccoli and cauliflower trimmings	14	23	15.8 ± 0.5
Ammonia solution pre-treatment				
OP-amm	Orange peels	16	35	14.4 ± 0.1
BC-amm	Broccoli and cauliflower trimmings	16	30	15.9 ± 0.6

crates (treatment boxes) with inner dimensions 36.5 cm × 56.2 cm x 11.5 cm (width x length x height). The pre-treatments were performed at 30 ± 3 °C for 14–16 d (Table 1).

2.2.2. BSFL composting

All experiments were performed in a greenhouse with the temperature regulated to 30 ± 3 °C and each composting treatment was performed in triplicate. The treatment boxes were kept on a rack, with 3 cm between the boxes. The larval feeding rate was calculated based on the total amount of material prior to the pre-treatment (Table 1), and thus the rate differed for each treatment (Table 2). In each treatment, approximately 15×10^3 of 1-mg larvae were added, resulting in a density of 6.25 larvae/cm^2. The larvae were fed on days 0, 3 and 6 of the treatment, by adding new material without stirring. BSFL composting was stopped directly when the first prepupae were observed, but lasted for a minimum of 17 days (Table 1).

At the end of BSFL composting, larvae and residues were separated by sieving into two fractions, which were weighed separately. The average larval weight was estimated by weighing three sub-samples of 100 larvae each. The number of larvae was calculated by dividing the total weight of all larvae by the average larval weight and then estimating the larval survival. In two treatments, orange peel control (OP-con) and *T. reesei* pre-treated orange peels (OP-tri), larvae separation was not possible, as the moisture content of the treatment residues was too high. In these treatments, samples of 100 larvae were handpicked randomly from the treatment boxes to get the average larval weight, and the survival rate was assumed to be 100% to give an estimate of the total larval biomass produced.

2.3. Sampling

The total amount of substrate was weighed before pre-treatment and before BSFL composting. Material in each replicate was sampled in triplicate for analyses of total solids (TS), total volatile solids (VS), total nitrogen and pH (Level One pH meter, Inolab, with a Sentix electrode, PHM210, MeterLab®, Radiometer, Copenhagen). Samples for determination of GHG emissions were taken during the pre-treatment and BSFL composting.

2.3.1. Sampling for physical and chemical analyses

Samples for TS, VS, pH and nitrogen analysis were taken before the start of pre-treatment, before the start of BSFL composting and after BSFL composting. Three TS and VS samples were taken from each replicate and each sample contained substrate from five randomly selected areas in the box (10–15 g per sample). The same procedure was followed for pH measurements and sampling for nitrogen analysis, but with only one sample from each box (10 g per sample). The material removed for sampling was taken into account when calculating the larval feeding rate.

2.3.2. Gas emissions sampling

During pre-treatment, gas measurements were performed as soon as the substrate was placed in the treatment box, then after 1 w and 2 w of pre-treatment. The last gas emission measurement of the pre-treatment coincided with the first measurement of the BSFL composting, during which gas measurements were performed four times in total: before each of the three feeding occasions and at the end of BSFL composting, which was 7–25 d after the third feeding depending on the BSFL composting duration (Table 1).

The gas emissions were measured using a chamber technique in which gas samples were collected over a period of approximately 1 h to obtain the gas emission rates (Ermolaev et al., 2019). The pre-treatment and treatment boxes were placed inside a plastic chamber (gas box, 51, 800 cm^3) that was made airtight using a water lock with the container placed upside-down into a lid. The gas box was equipped with a rubber sampling port, from which gas samples were extracted with a syringe.

Table 2
Total solids (TS), total volatile solids (VS), pH and total nitrogen (N) content of all substrates measured at start (initial substrate), after pre-treatment, larvae and residue TS and VS content, and larval VS dose in the different treatments: OP (orange peels), BC (broccoli and cauliflower trimmings) and FW (food waste). Nitrogen content and pH values presented in brackets were measured after added ammonia solution at the start of pre-treatment. Values presented are mean ± SD (n = 3). Significant differences ($p < 0.05$) in TS, VS and pH of initial substrate, pre-treated substrate and BSFL-treated substrate are marked with *. Different superscript letters within columns indicate significant differences ($p < 0.05$) in TS and VS for larvae and in pH for residues.

	Initial substrate				After pre-treatment				Larval VS load[1]	Larvae		Residues	
	TS [%]	VS [%]	pH	N [g/kg TS]	TS [%]	VS [%]	pH	N [g/kg TS]	[g VS/larva]	TS [%]	VS [%]	TS [%]	VS [%]	pH
Controls without pre-treatment														
OP-con	21.5 ± 0.4*	96.7 ± 0.4*	3.4 ± 0.1*	4.4 ± 0.4	-	-	-	-	0.20 ± 0.003^{ab}	25.4 ± 1.2^{a}	90.0 ± 0.2^{a}	31.8 ± 1.4^{a*}	95.0 ± 0.1^{a*}	3.7 ± 0.1^{a*}
BC-con	8.2 ± 0.1*	87.4 ± 1.1*	5.7 ± 0.2*	14.1 ± 1.4	-	-	-	-	0.08 ± 0.002^{abc}	19.5 ± 1.4^{b}	83.0 ± 1.6^{b}	29.7 ± 5.6^{a*}	70.5 ± 0.6^{a*}	9.8 ± 0.1^{a*}
FW-con	24.7 ± 0.1*	89.3 ± 0.3*	4.1 ± 0.2*	25.5 ± 3.7	-	-	-	-	0.22 ± 0.004^{b}	40.6 ± 0.9^{c}	83.6 ± 0.1^{bc}	69.2 ± 3.0^{a*}	82.4 ± 0.2^{a*}	8.3 ± 0.1^{a*}
Trichoderma reesei *pre-treatment*														
OP-tri	21.1 ± 0.4*	96.6 ± 0.1*	4.3 ± 0.6*	-	29.8 ± 2.9*	94.0 ± 0.9*	3.5 ± 0.1*		0.14 ± 0.02^{abc}	23.6 ± 1.0^{ab}	87.7 ± 1.7^{a}	37.5 ± 3.8^{a}	92.5 ± 0.2^{d}	3.9 ± 0.1^{a}
BC-tri	8.2 ± 0.2	87.4 ± 1.5*	5.5 ± 0.1*	14.1 ± 1.4	11.3 ± 1.5*	70.8 ± 2.4*	8.1 ± 0.3*	19.0 ± 5.4	0.03 ± 0.004^{c}	35.3 ± 2.6^{d}	77.3 ± 2.1^{d}	86.9 ± 2.0^{a*c}	57.5 ± 1.2^{a*e}	10.1 ± 0.3^{a*d}
Ammonia solution pre-treatment														
OP-amm	21.6 ± 0.2*	96.9 ± 0.1*	3.7 ± 0.6*	4.4 ± 0.4	19.2 ± 0.2*	96.4 ± 0.1*	6.4 ± 1.6* (10 ± 0.2)	19.5 ± 2.4	0.18 ± 0.003^{abc}	24.3 ± 2.0^{ab}	88.0 ± 0.8^{a}	52.8 ± 4.2^{a*d}	94.2 ± 0.2^{a*d}	7.6 ± 0.1^{e}
BC-amm	8.6 ± 0.1*	89.5 ± 0.3*	5.8 ± 0.3*	-	7.3 ± 0.2*	88.0 ± 1.3*	8.1 ± 0.3* (10 ± 0.4)	-	0.07 ± 0.004^{ac}	23.9 ± 2.7^{ab}	86.8 ± 0.9^{a*c}	15.0 ± 5.1^{a}	76.9 ± 1.4^{a*f}	8.2 ± 0.1^{c}

The material reduction during pre-treatment of trimmings was significantly ($p < 0.05$) greater in *T. reesei* pre-treatment (65%) than in ammonia pre-treatment (15%) (Table 3). In general, the reduction was greater in the trimmings than in the peels in both pre-treatments.

[1] Not normally distributed, non-parametric test.

An air inlet consisting of a 1 m long and 3 mm diameter tube was connected, to avoid suppression. A portable fan (Rubicson mini fan, Kjell & Co Elektronik AB, Sweden) was placed in the gas box to disperse the gas concentrations evenly. For each emission measurement, gas samples were extracted on three occasions: directly upon sealing the box, after 20 min and after 1 h. Exact times and volumes removed from the chamber were recorded and later used together with measured concentrations to calculate the emission rates for each of the samples by linear regression. Dilution was accounted for following the method described in Ermolaev et al. (2019).

2.4. Physical and chemical analyses

The TS samples were weighed before drying and after drying for a minimum of 48 h, at 70 °C to avoid evaporation of volatile solids (Vahlberg et al., 2013). For VS analysis, the samples were combusted at 250 °C for 2 h and at 550 °C for 4 h (modified ISO 18122:2015). Total nitrogen was measured as described in Lalander et al. (2015). In brief, 0.5 g samples were boiled in 15 mL concentrated sulphuric acid in a 100 mL volumetric flask for 20 min and then cooled before being diluted 1:50 in deionised water. A Crack-test 10 (114544) was used to oxidise all forms of nitrogen to nitrate, after which the nitrate concentration was measured using Spectroquant© kit number 114764.

2.5. Gas analyses

Concentrations of CO_2 and NH_3 were measured during sampling by connecting a reagent tube from a pump (Gas Detector, Kitagawa, Japan) directly to the sampling port, withdrawing 50–300 mL of the gas and recording the concentration for each tube, as described in Ermolaev et al. (2019). For measurements of CH_4 and N_2O, the gas was extracted from the sampling port using a 60 mL syringe and directly flushed into a 20 mL evacuated injection flask (Perkin Elmer) pre-filled with N_2. Concentrations of CH_4 and N_2O were measured using a gas chromatograph (Perkin Elmer Clarus 500, USA) with flame ionisation detector (FID) and thermal conductivity detector (TCD), as described in Ermolaev et al. (2015).

2.6. Calculations

The percentage material reduction on a volatile solids basis (Red_{VS}) was calculated as:

$$Red_{VS} = \left(1 - \frac{m\mathrm{VS}_{res}}{m\mathrm{VS}_{initial}}\right) \times 100 \quad (1)$$

where $m\mathrm{VS}_{res}$ and $m\mathrm{VS}_{initial}$ is the total mass of volatile solids in residues and initial material, respectively. For material reduction after pre-treatment, $m\mathrm{VS}_{res}$ and $m\mathrm{VS}_{initial}$ is the total mass of volatile solids in pre-treatment residues and initial material, respectively. For material reduction after BSFL composting, $m\mathrm{VS}_{res}$ and $m\mathrm{VS}_{initial}$ is the total mass of volatile solids in residues and initial substrate, respectively.

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

$$BCE_{VS} = \left(\frac{m\mathrm{VS}_{larvae}}{m\mathrm{VS}_{initial}}\right) \times 100 \quad (2)$$

where $m\mathrm{VS}_{larvae}$ and $m\mathrm{VS}_{initial}$ is the total mass of volatile solids in larvae and initial substrate, respectively. The BCE on a wet weight basis (BCE_{WW}) was calculated in the same way, but using the total wet weight of larvae and initial substrate.

Total VS loss was calculated as:

$$\text{Tot loss}_{VS} = \left(\frac{m\mathrm{VS}_{initial} - m\mathrm{VS}_{larvae} - m\mathrm{VS}_{res}}{m\mathrm{VS}_{initial}}\right) \times 100 \quad (3)$$

where $m\mathrm{VS}_{initial}$, $m\mathrm{VS}_{larvae}$ and $m\mathrm{VS}_{res}$ is the total mass of volatile solids in initial substrate, larvae and residues, respectively.

Percentage larval survival was calculated as:

$$\text{Survival} = \frac{n_{lv.end}}{n_{lv.start}} \times 100 \quad (4)$$

where $n_{lv.end}$ and $n_{lv.start}$ is total number of larvae at the end and at the start, respectively.

The total amount of gas emitted from pre-treatment and BSFL composting was calculated using the trapezoidal rule (Holman, 2011), in which the emission rates were plotted against time to calculate total emissions as described in Ermolaev et al. (2019).

2.7. Statistical analysis

For calculating gas emission rates, the Linest function in Excel (Microsoft version 16, USA) was used. Two-sided ANOVA (with 95% confidence interval) was used to evaluate whether the treatments were significantly different during pre-treatment and BSFL composting in terms of material reduction, BCE and gas emissions. Normality was verified in the model residuals (Shapiro-Wilk test). Where it was not verified, the non-parametric Kruskal-Wallis test was performed, followed by the Dunn test for multiple comparison (95% confidence interval) using the Benjamini and Hochberg correction method (Benjamini and Hochberg, 1995). General linear regression with 95% confidence interval was used to assess correlations between response variables and substrate properties. ANOVA, regression analyses, non-parametric tests and graphical representations were made in R (R Core Team, 2019).

3. Results

3.1. Process efficiency

All treatments with the same substrate had similar initial content of TS and VS (Table 2). In the treatments with orange peels and broccoli and cauliflower trimmings, the VS was reduced most by the *T. reesei* pre-treatment, which also significantly reduced the substrate moisture content, increasing the TS content of trimmings from 8.3 to 11%. Ammonia pre-treatment increased the moisture content of both peels and trimmings.

Larvae and residue TS varied significantly between the different treatments, ranging from 19.5 to 40.6%. Larvae fed on orange peels had similar TS and VS, regardless of whether the peels had been pre-treated or not, while the TS and VS of larvae reared on broccoli and cauliflower trimmings varied more between treatments. For all treatments, BSFL composting led to a reduction in moisture content (Table 2).

Neither of the pre-treatments gave a significant improvement in BCE_{VS} in BSFL composting. Similar BCE_{VS} was obtained for the broccoli and cauliflower trimmings and the control, while BCE_{VS} was significantly reduced for orange peels pre-treated with *T. reesei* (treatment OP-tri). BCE_{WW} for the entire process was not significantly different between the substrates within the same pre-treatments, but the *T. reesei* pre-treatment resulted in significantly lower BCE_{WW} than the ammonia pre-treatment (Table 3). Furthermore, the *T. reesei* pre-treated substrates had significantly lower BCE_{VS} compared with corresponding control.

Larval survival was lowest for *T. reesei* pre-treated broccoli and cauliflower trimmings (treatment BC-tri) (28%) and highest in the corresponding control (100%). Generally, larvae in all orange peel treatments had lower final larval weight (18–50 mg/larva) than in the other substrates, while larvae grown on food waste had the largest final weight (141 mg/larva), followed by the broccoli and cauliflower trimmings control (treatment BC-con) (108 mg/larva).

The material reduction on a VS basis for the entire process differed significantly ($p < 0.05$) between the trimmings treatments, with the material reduction of ammonia pre-treated trimmings (BC-amm) being

Table 3
Material reduction (Red) after pre-treatment; Red and biomass conversion efficiency (BCE) after black soldier fly larvae (BSFL) composting on a volatile solids (VS) basis and for the entire process on a VS and wet weight (ww) basis for the different substrates. and survival rate and final larval weight of the larvae; OP (orange peels), BC (broccoli and cauliflower trimmings) and FW (food waste). Values presented are mean ± SD (n = 3). Different superscript letters within columns indicate significant differences ($p < 0.05$).

	Pre-treatment	BSFL composting				Entire process				
	Red_{VS} [%]	Red_{VS} [%]	BCE_{VS} [%]	Survival[i] [%]	Final larval weight [mg]	Red_{VS} [%]	BCE_{VS} [%]	Red_{WW}[ii] [%]	BCE_{WW} [%]	Total VS loss [%]
Controls without pre-treatment										
OP-con		39 ± 3^{a}	5.7 ± 2^{a}	100[ii]	50 ± 12^{ac}	39 ± 3^{a}	5.7 ± 1.7^{a}	58 ± 1^{a}	5.2 ± 1.2^{ab}	33 ± 5^{a}
BC-con		81 ± 0.5^{b}	14 ± 1^{b}	62.8 ± 4.5^{ab}	108 ± 8^{be}	81 ± 0.5^{b}	14 ± 1^{b}	93 ± 1^{b}	6.4 ± 0.06^{b}	67 ± 1^{b}
FW-con		64 ± 1^{c}	23 ± 1^{c}	100 ± 6.3^{c}	141 ± 5^{b}	64 ± 1^{cd}	23 ± 1^{c}	87 ± 1^{ab}	14 ± 1^{c}	41 ± 2^{a}
Trichoderma reesei *pre-treatment*										
OP-tri	24 ± 8^{a}	43 ± 7^{ad}	2.6 ± 0.7^{d}	100[ii]	18 ± 4^{a}	60 ± 4^{cd}	1.8 ± 0.4^{d}	82 ± 0.5^{ab}	1.3 ± 0.3^{d}	58 ± 4^{bc}
BC-tri	65 ± 5^{b}	52 ± 9^{cd}	16 ± 1^{b}	28.1 ± 14.6^{a}	61 ± 23^{cd}	84 ± 1^{b}	5.4 ± 0.8^{a}	98 ± 0.1^{b}	1.4 ± 0.3^{d}	79 ± 1^{d}
Ammonia solution pre-treatment										
OP-amm	10 ± 0.02^{c}	52 ± 3^{ac}	5.0 ± 0.6^{ad}	96.0 ± 4.5^{cb}	43 ± 0^{ac}	58 ± 3^{d}	4.3 ± 0.6^{ad}	83 ± 1^{ab}	3.9 ± 0.2^{a}	53 ± 3^{c}
BC-amm	15 ± 0.2^{ac}	59 ± 3^{c}	15 ± 0.5^{b}	55.7 ± 25.0^{ab}	95 ± 26^{de}	66 ± 3^{c}	12 ± 0.4^{b}	76 ± 7^{ab}	4.6 ± 0.5^{a}	53 ± 4^{c}

[i] Statistical analysis excluded treatments OP-con and OP-tri.
[ii] Assumed survival.
[iii] Not normally distributed, non-parametric test.

lower (66%) than that in the control and in trimmings pre-treated with *T. reesei* (BC-tri, 84%). Both peel pre-treatments resulted in higher Red_{VS} compared with the control over the entire process. Treatment BC-tri resulted in the highest total VS loss, while the orange peel control (treatment OP-con) resulted in the lowest.

In both pre-treatments with peels, BCE was lower than for pre-treated trimmings even though the larval VS load was greater, and hence no direct correlation between larval VS load and BCE was found (Fig. 1a). Removing the peel treatments from the model improved the fit from $R^2 < 0.1$ to an adjusted $R^2 = 0.74$ (Fig. 1b). An even stronger positive correlation (adjusted $R^2 = 0.93$), was found when the material reduction in pre-treatment was included as an explanatory variable (Fig. 1c). The final larval weight was found to correlate with the larval VS load that the surviving larvae would have received, assuming that any larvae which did not survive the treatment did not consume any VS (Fig. 1d).

3.2. Gas emissions

3.2.1. Emissions during pre-treatment

The CO_2 emissions were mainly associated with the type of pre-treatment. Emissions from ammonia pre-treatment of substrates reached 0.03 CO_2–C kg/kg initial VS, while *T. reesei* pre-treated substrates emitted 0.12 CO_2–C kg/kg initial VS (Fig. 2a). For CH_4 and N_2O emissions, the amount of emissions was associated with the substrate rather than the type of pre-treatment (Fig. 2b–c).

3.2.2. Emissions during BSFL composting

Concentrations of CO_2 increased linearly ($R^2 > 0.95$ n = 85, $0.95 > R^2 > 0.75$ n = 14) for all emission rate measurements in all replicates, confirming that the gas boxes remained airtight and that the gas measurements had high accuracy. The emission rates were calculated and plotted against time, and the cumulative emissions were calculated for all treatments (Fig. 3a). Cumulative CO_2 emissions in all treatments increased nearly linearly, except for the trimmings control (BC-con), and total emissions ranged from 0.10 to 0.17 kg CO_2–C/kg substrate VS during BSFL composting (Fig. 3a). The total cumulative CO_2 emissions in the trimmings control reached 0.23 kg CO_2–C/kg substrate VS. All the peel treatments except OP-tri had lower cumulative emissions than other treatments. The CO_2 accumulation rates were similar across the peel treatments.

In treatments OP-tri, ammonia pre-treated orange peels (OP-amm), BC-con, BC-tri and the food waste control (FW-con), cumulative CH_4 emissions were small and increased linearly, from 0.2 to 1.6 mg CH_4–C/kg substrate VS (Fig. 3b). Ammonia pre-treated broccoli and cauliflower trimmings showed a sharper increase in accumulation rate after the initial period and reached total emissions of 23 mg CH_4–C/kg substrate VS (Fig. 3b). Total emissions from the peels control (OP-con) did not increase linearly and were 3.3 mg CH_4–C/kg initial VS. The cumulative CH_4 emissions from food waste were consistently lowest, but not significantly lower than in other treatments except for trimmings pre-treated with ammonia (BC-amm).

Cumulative N_2O emissions were close to zero for most treatments, with the exception of the trimmings control (BC-con) (Fig. 3c). Cumulative emissions increased gradually, from 0.1 to 1.9 mg N_2O–N/kg substrate VS in the peels and food waste treatments to 3.4 mg, 6.5 mg and 46 mg N_2O–N/kg substrate VS in BC-amm, BC-tri and BC-con, respectively (Fig. 3c).

3.2.3. Emissions during the entire process

Total CH_4 emissions varied more within treatment for treatments that had higher emissions (Fig. 4a). Total emissions of N_2O were higher in treatments with trimmings than with peels (Fig. 4b). Pre-treatment of trimmings reduced the total amount of N_2O emitted. Total N_2O emissions were not significantly different from zero in any of the peel treatments. The NH_3 pre-treatment increased total NH_3 emissions (Fig. 4c). Total CO_2 emissions from the entire process were within the same range for all assessed treatments (Fig. 4d).

Total emissions of CH_4 and N_2O, expressed in CO_2-equivalents over a 100-year period (IPCC, 2013) on a VS basis, were low compared with the direct CO_2 emissions, and were generally higher for trimmings as compared to the other substrates (Fig. 4e). The total emissions were similar between the treatments, with only the control trimmings and peels (BC-con, OP-con) being significantly different from each other (Fig. 4f).

4. Discussion

4.1. Impact of pre-treatment on biomass conversion efficiency and larval survival

In broccoli and cauliflower trimmings pre-treated with *T. reesei*, a large proportion of the initial VS was consumed in the pre-treatment (Table 3). This high VS reduction resulted in that treatment receiving the lowest larval VS feeding load (0.03 g VS/larva) in this study (Table 2), which could have caused the low larval survival rate (28%). However, BCE in BSFL composting of broccoli and cauliflower trimmings pre-treated with *T. reesei* (14%) was within the same range as in

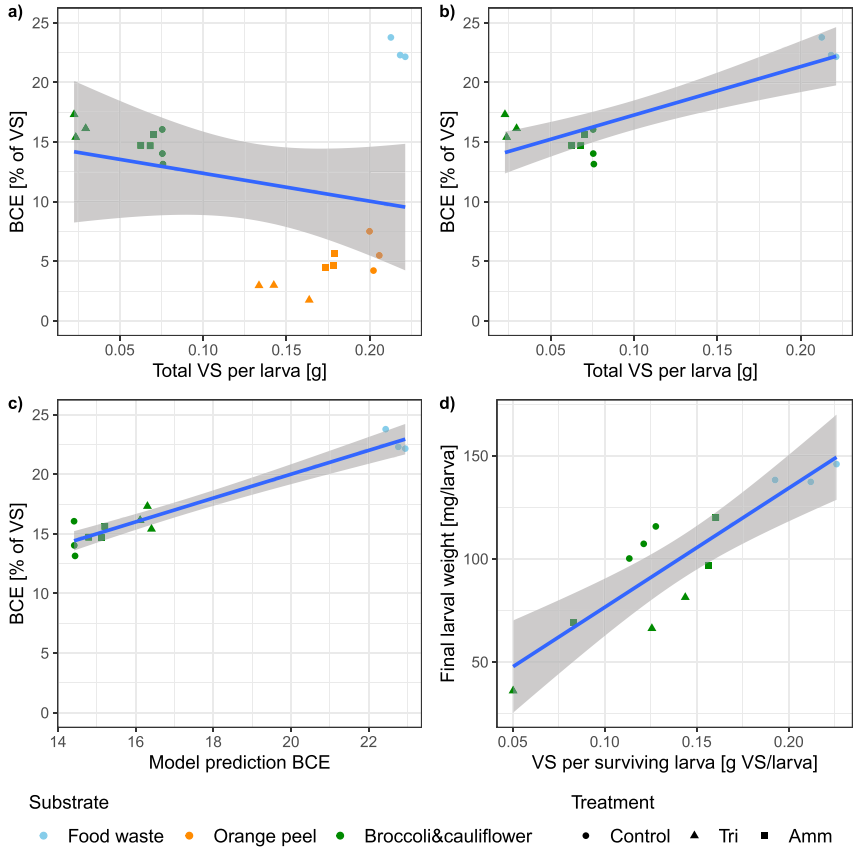

Fig. 1. Biomass conversion efficiency (BCE) in black soldier fly larvae (BSFL) composting in relation to a) total volatile solids (VS) per larva [g] ($R^2 < 0.1$), b) total VS per larva [g], when excluding the orange peel treatments (adjusted $R^2 = 0.74$; $p = 0.0003$), and c) predicted BCE for a model taking into account the total VS per larva and the reduction achieved in the pre-treatment (adjusted $R^2 = 0.93$, $p = 6 \times 10^{-6}$); and d) the final larval weight in relation to the total VS per surviving larva (adjusted $R^2 = 0.76$, $p = 0.0001$). Data shown for food waste (blue), orange peels (orange) and broccoli and cauliflower trimmings (green) without pre-treatment (control, ●) and pre-treated with *Trichoderma reesei* (tri, ▲) and ammonia solution (amm, ■). The grey areas represent the model fits with 95% confidence level.

the corresponding control (16%) (Table 3), suggesting that fungi pre-treatment made the trimmings more available to the larvae than in the control, which received a higher larval VS load (0.08 g VS/larva). A study by Lalander et al. (2019) demonstrated higher BCE with higher larval VS load, but in the present study no correlation between larval VS load and BCE was found when the full dataset was used (Fig. 1a). On excluding the orange peels, better model fit was found (Fig. 1b), suggesting that factors other than larval VS load played a role in the peel treatments. Including also the reduction during pre-treatment further improved the model fit (Fig. 1c), supporting the hypothesis that pre-treatment with *T. reesei* renders substrate more available to larvae, as this fungus has the ability to degrade complex molecules (Tadele and Amha, 2015). It is likely that a higher larval VS load for fungi pre-treated substrates would increase the BCE in the BSFL composting, however it would have to be investigated as to which point, since it has been suggested that there is a threshold at which increased larval feeding load no longer results in larger relative larval growth (Parra Paz et al., 2015).

It is likely that the low survival of larvae in broccoli and cauliflower trimmings pre-treated with *T. reesei* was due to the low larval VS feeding load (30 mg/larva), and not due to any potential mycotoxins produced by the fungi, as no known mycotoxin is produced by *T. reesei* (Frisvad et al., 2018). Furthermore, Isibika et al. (2019) observed no impact on larval survival during BSFL composting of *T. reesei* pre-treated banana peels. An alternative, or complementary, reason for the low survival could be the low TS content of the broccoli and cauliflower trimmings (7–11%) (Table 2). A previous study by Lalander et al. (2020) using a similar set-up as in the present study observed decreased survival with higher substrate moisture content (around 80% survival at 90% moisture content, 57% survival at 95% moisture content) and high survival variation in BSFL treatment of substrates with high moisture content.

Final larval weight in broccoli and cauliflower trimmings control (100 mg/larva) was considerably greater than after *T. reesei* pre-treatment (around 60 mg/larva), most likely due to the low larval VS load in the latter treatment (Fig. 1d). Larval survival was generally lower for broccoli and cauliflower trimmings than for the other substrates (Table 3).

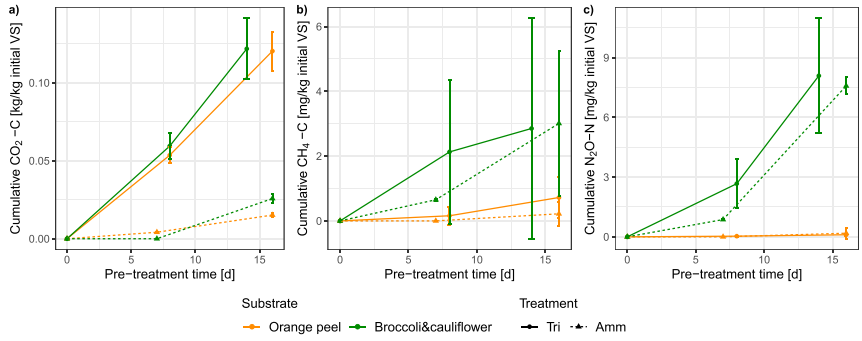

Fig. 2. Cumulative gaseous emissions of (a) CO_2, (b) CH_4 and (c) N_2O during pre-treatment of orange peels (orange) and broccoli and cauliflower trimmings (green) pre-treated with *Trichoderma reesei* (tri, ▲) or ammonia solution (amm, ■). The error bars represent the standard deviation between replicates (n = 3).

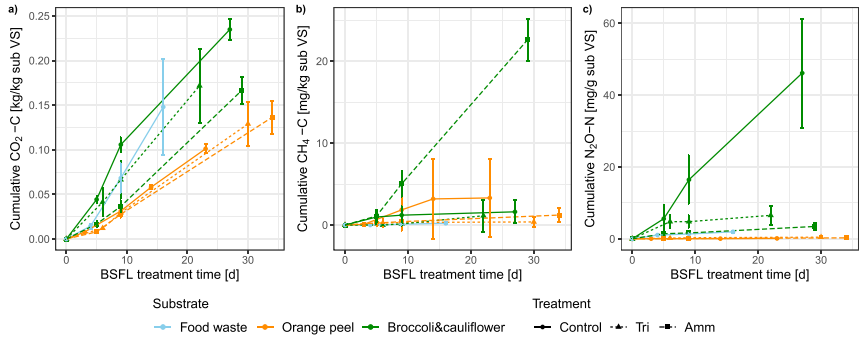

Fig. 3. Cumulative emissions of (a) CO_2, (b) CH_4 and (c) N_2O during black soldier fly larvae (BSFL) composting of food waste (blue), orange peels (orange) and broccoli and cauliflower trimmings (green) without pre-treatment (control, ●) and pre-treated with *Trichoderma reesei* (tri, ▲) and ammonia solution (amm, ■). The error bars represent the standard deviation between replicates (n = 3).

The orange peel control showed low BCE (<6%) and pre-treatment did not improve the BCE, in fact fungi pre-treatment (OC-tri) reduced it to >3. Orange peels have been found to have toxicity effects on insects (Amin et al., 2018; Bachi and Ahmed, 2017). Amin et al. (2018) found that orange peels caused 20–60% larval mortality among cotton leaf worms, while Bachi and Ahmed (2017) demonstrated toxicity in Mediterranean fruit flies, caused by the essential oils in the peels. The fungi pre-treatment decreased BCE, while the material reduction on a VS basis increased for both pre-treatments. This indicates that the pre-treatments did not result in complex molecules becoming more available to the fly larvae in the peel treatments, contradicting our initial hypothesis. However, the reduction during pre-treatment could have concentrated toxic compounds, resulting in decreased BCE compared with the control.

4.2. Impact of pre-treatment on material reduction

The VS reduction was lower during ammonia pre-treatment than in the *T. reesei* pre-treatment. The low reduction rate was correlated with low CO_2 emissions in the pre-treatments (Fig. 1a). In BSFL composting, the material reduction was lower for ammonia pre-treated broccoli and cauliflower trimmings compared with corresponding control, while BCE was not significantly different (Table 3). Furthermore, the total VS loss was lower for ammonia pre-treatment compared with the control and *T. reesei* pre-treatment, suggesting that the microbial community was less active in the ammonia pre-treated substrate, as the VS loss represents a combination of larval and microbial respiration. In contrast, ammonia pre-treatment of orange peels resulted in a greater material reduction and total VS loss compared with the control. The added ammonia likely acted as a nutrition source in the low-nitrogen peels, and thus stimulated microbial degradation (Tadele and Amha, 2015). The BCE for ammonia pre-treated orange peel was not significantly different from the control (Table 3), and it is thus likely that the added nitrogen favoured microbial growth, rather than larval growth. This could be because substances in the peels were more toxic to the larvae, as discussed above, than to the microorganisms. In the case of the broccoli and cauliflower trimmings, the nitrogen concentration was considerably higher than in orange peels, and thus addition of ammonia could have made the environment less favourable for microorganisms, rather than favouring their growth. High NH_3 concentrations are known to be toxic to most microorganisms (Vinnerås et al., 2008). However, the larvae did not seem to be impacted by the increased ammonia concentration in the trimmings.

4.2. Gas emissions

The material reduction and total VS loss during treatments was reflected in the total CO_2 emissions. On calculating the amount of carbon lost in CO_2 emissions and converting it to the amount of corresponding

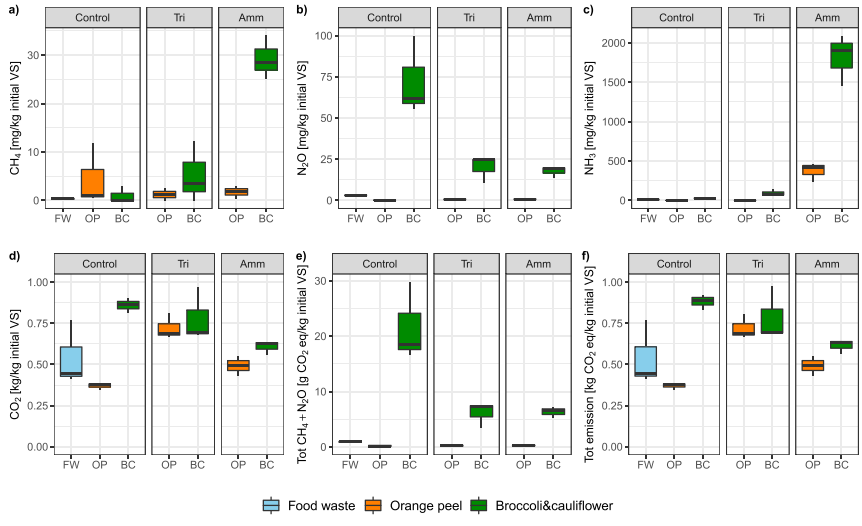

Fig. 4. Total emissions, i.e. emissions during black soldier fly larvae (BSFL) composting and during pre-treatment, calculated per kg of initial VS for food waste (FW, blue), orange peels (OP, orange) and broccoli and cauliflower trimmings (BC, green) without pre-treatment (control), pre-treated with *Trichoderma reesei* (tri) and ammonia solution (amm): a) CH_4 [mg/kg VS]; b) N_2O [mg/kg VS]; c) NH_3 [mg/kg VS]; d) total CO_2 [kg/kg VS]; e) CO_2 equivalents [g CO_2-eq/kg VS] of the CH_4 and N_2O emissions and f) CO_2 equivalents [kg CO_2-eq/kg VS] of all emissions.

VS, it was found that on average 48% of the degraded VS was lost via respiration, which closely matched the values calculated based on VS mass balance (Table 2). The remaining degraded VS were assumed to be transformed into larval biomass.

Total CO_2 emissions from the treatments ranged between 0.37 and 0.86 kg CO_2/kg VS (Fig. 3d). The trimmings control had significantly higher total emissions of CO_2 than the peels control, while the total emissions from food waste were not significantly different from either of the control treatments with peels and trimmings. There was a 67% loss of initial VS in the control treatment with trimmings, 41% in the food waste control and 33% in the peels control. This low biological activity in the peels control treatment corresponded to low BCE (Table 3). The VS loss did not correlate with high BCE, supporting the suggestion that a large proportion of material reduction was not due to biomass conversion into larvae; e.g. food waste had the highest BCE, while the emissions of CO_2 were the second lowest on average. This is in agreement with findings by Ermolaev et al. (2019). The CO_2 emissions generated per tonne of treated waste in BSFL composting were higher for trimmings than for food waste, which correlated well with the VS loss (Table 3, Fig. 4f).

The CH_4 emissions coincided with NH_3 emissions, while they correlated adversely with N_2O emissions in the trimmings treatments (Fig. 3a–c). Similarly, Chen et al. (2019) found that N_2O emissions were low while CH_4 emissions increased with moisture content in substrate and vice versa (Fig. 2b–c). In conventional composting, N_2O emissions can occur later in the process and can be associated with nitrate availability during the denitrification process (Feng et al., 2020). This is typically not seen in BSFL composting, as the process is considerably shorter. However, it has been reported that in substrates with initial nitrate availability, an early N_2O emissions peak can occur (Ermolaev et al., 2019), which declines as the nitrate is consumed and pH increases. This could explain the early N_2O emissions from treatments with trimmings and also the lower emissions in the ammonia pre-treatment, as pH would be higher in the presence of NH_3 (Pang et al., 2020).

Emissions of CH_4 per ton ww initial material in this study were at the lower end of the range reported for conventional composting (Table 4). The highest CH_4 emissions level for BSFL composting detected in our study and in other studies (Mertenat et al., 2019; Pang et al., 2020) was around 0.1% of the highest reported level in conventional composting (Beck-Friis et al., 2000; Ermolaev et al., 2015). The degradation of the incoming material in BSFL composting was generally higher than in conventional composting, but when adding the mass of the larvae produced the emissions of CO_2 were within the same order of magnitude (Table 4). The BSFL composting process produces residues that do not represent a stabilised compost, leading to a larger proportion of the initial carbon in the substrate being retained and creating the potential for emissions after completion of treatment (Song et al., 2021).

Reported values for emissions of N_2O and NH_3 in conventional composting are up to 100-fold higher (Table 4). Compared with the other treatments in this study, the total ammonia emissions from BSFL composting were 0.12–10.0% of those reported for conventional composting (Table 4), which shows that overall less nitrogen is lost as NH_3 during fly larvae composting. In ammonia pre-treatment of broccoli and cauliflower, the ammonia emissions were higher and up to 50% of the ammonia added in the pre-treatment was lost. Other studies have reported higher losses of nitrogen in BSFL composting, of between 30 and 40% of the inflow nitrogen (Lalander et al., 2015; Lopes et al., 2021). The lower nitrogen emissions seen in this study may be due to lower temperatures and pH, and higher moisture content (Chen et al., 2019). A higher nitrogen content in the substrate likely also favours higher ammonia volatilisation, in particular as the pH shifts to more alkaline conditions due to microbial breakdown of organic nitrogen in the ammonification step, further pushing the ammonium-ammonia equilibrium towards volatile ammonia (Emerson et al., 1975). Thus Parodi et al. (2020) suggested stopping BSFL composting at the peak of CO_2 production, immediately before ammonia starts to be produced in the

Table 4
Summary of emissions per tonne waste on a wet weight (ww) basis and valuable products generated during black soldier fly larvae (BSFL) composting in treatments with orange peels (OP), broccoli and cauliflower trimmings (BC) and food waste (FW) and in other studies with fly larvae composting and conventional composting.

	Larvae [kg]	Residues [kg]	CO_2 [kg]	CH_4 [g]	N_2O [g]	NH_3 [g]
Current study						
Orange peels	17–52	180–422	77–147	0.3–0.9	0.04–0.1	0.0–78
BC trimmings	14–64	23–243	47–62	0.07–2.2	1.4–5.2	5.4–342
FW control	142 ± 7	134 ± 7	115 ± 33	0.06 ± 0.01	0.6 ± 0.1	1.8 ± 1.3
BSFL composting						
Food waste[a]	120–190	570–640	92–100	1.0–3.0	0.19–1.0	Below detection limit
Biowaste[b]	220	320	-	0.4	8.6	-
Food waste[c]	19–136	-	26–48	0.06–0.79	0.06–0.50	0.05–0.50
Composting						
Food & garden waste[d]	-	330–750	16–270	23–520	4–400	Below detection limit
Household waste[e]	-	-	-	1700	78	-
Kitchen waste[f]	-	670–680	-	-	-	750–1540

[a] Ermolaev et al. (2019).
[b] Mertenat et al. (2019).
[c] Pang et al. (2020).
[d] Ermolaev et al. (2014).
[e] Beck-Friis et al. (2000).
[f] Zhang et al. (2016).

process. However, Guo et al. (2021) demonstrated that the largest contribution of emissions in BSFL treatment comes from post-treatment of the residues, indicating that more research is needed to determine how the treatment residues should be handled for lowest impact on the environment.

4.3. Feasibility of pre-treatment for BSFL composting

The moisture content of the broccoli and cauliflower trimmings was close to 92% at the start, but the residues were dry enough to allow larvae to be separated out at the end of the process. Cheng et al. (2017) concluded that the initial substrate moisture content should not exceed 80%, as dry separation would not be possible. However, Lalander et al. (2020) found that dry separation with initial moisture content 80–90% is possible if active ventilation is applied. The *T. reesei* and ammonia pre-treatments made a considerable impact on moisture content, with *T. reesei* pre-treatment decreasing the initial moisture content of trimmings in BSFL composting by 29%, while ammonia pre-treatment increased the moisture content of trimmings by 14%. The former led to a dry end product with a moisture content of 13%, compared with 70% in the control (Table 2). The peel treatments were considerably more difficult to separate, as they ended up with a moisture content of 68% and 63% for the control and fungi pre-treatment, respectively. Ammonia treatment of the peels resulted in a separable product with a moisture content of 47%. Comparing fungi pre-treatment of trimmings or not (assuming BCE as in the BSFL composting step, but a larval feeding load of 0.08 g VS in both treatments, and a total treatment time of 37 days for the fungi pre-treatment and 28 days for the control), the larvae production capacity was 0.4 and 2.2 g larvae/kg waste per day for the fungi pre-treatment and non-pre-treated substrate, respectively. The total treatment capacity, on the other hand, was 5.2 and 2.4 kg/m^2 and day, respectively, for the fungi pre-treatment and the non-pre-treated control. In cases where high waste throughput is of value due to waste fees, the fungi pre-treatment would add an extra benefit.

5. Conclusions

Pre-treatment of orange peels and broccoli and cauliflower trimmings with *T. reesei* or ammonia did not significantly improve biomass conversion efficiency during BSFL composting. However, both pre-treatments significantly increased the material reduction for the peels. BSFL composting of the orange peels resulted in low BCE and produced wet treatment residues (TS ~30%). Ammonia pre-treatment of peels resulted in drier (TS ~50%) treatment residues.

Both substrates performed less effectively than food waste in BSFL composting in terms of biomass conversion efficiency, total emissions of GHG and ammonia, and treatment time. However, higher waste treatment capacity was achieved on pre-treating the trimmings with *T. reesei*. Total CO_2-eq emissions (N_2O + CH_4) were almost four-fold higher for the control trimmings (no pre-treatment) than for ammonia pre-treated trimmings, with no negative impact on biomass conversion efficiency, but with increased NH3 emissions.

CRediT authorship contribution statement

L. Lindberg: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **E. Ermolaev:** Conceptualization, Investigation, Formal analysis, Supervision, Writing – review & editing. **B. Vinnerås:** Conceptualization, Resources, Supervision, Writing – review & editing. **C. Lalander:** Conceptualization, Investigation, Formal analysis, Resources, Supervision, Funding acquisition, Visualization, Writing – review & editing.

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

Amin, L.R., Abo-El-Enein, S.A., Abdel-Ghany, A.M., Samy, E.-B.S., Amin, T.R., 2018. Gas chromatography combined to mass spectrometry analyses of some citrus peels bioactive compounds against the cotton leafworm, *Spodoptera littoralis* (boisd.). Egypt.J. Agric. Res. 96 (1), 15–29.
Bachi, K., Ahmed, D.S.A., 2017. Effect of citrus essential oils on the larvae evolution of *Ceratitis capitata* Wied. 1824 (Diptera: Tephritidae). Biosci. Res. 14 (3), 686–693.
Bals, B., Murnen, H., Allen, M., Dale, B., 2010. Ammonia fiber expansion (AFEX) treatment of eleven different forages: improvements to fiber digestibility in vitro. Anim. Feed Sci. Technol. 155 (2), 147–155.
Beck-Friis, B., Pell, M., Sonesson, U., Jonsson, H., Kirchmann, H., 2000. Formation and emission of N_2O and CH_4 from compost heaps of organic household waster. Environ. Monit. Assess. 62 (3), 317–331.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57 (1), 289–300.
Bernstad, A., Jansen, J.L., 2012. Review of comparative LCAs of food waste management systems - current status and potential improvements. Waste Manag. 32 (12), 2439–2455.
Chen, J., Hou, D., Pang, W., Nowar, E.E., Tomberlin, J.K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z., Li, Q., 2019. Effect of moisture content on greenhouse gas and NH_3 emissions from pig manure converted by black soldier fly. Sci. Total Environ. 697, 133840.
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 Manag. 67, 315–323.
Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32 (12), 2379–2383.
Ermolaev, E., Jarvis, A., Sundberg, C., Smars, S., Pell, M., Jonsson, H., 2015. Nitrous oxide and methane emissions from food waste composting at different temperatures. Waste Manag. 46, 113–119.
Ermolaev, E., Lalander, C., Vinnerås, B., 2019. Greenhouse gas emissions from small-scale fly larvae composting with *Hermetia illucens*. Waste Manag. 96, 65–74.
Ermolaev, E., Sundberg, C., Pell, M., Jönsson, H., 2014. Greenhouse gas emissions from home composting in practice. Bioresour. Technol. 151, 174–182.
European Commission, 2020. Circular economy action plan - the European green deal. https://ec.europa.eu/environment/circular-economy/pdf/new_circular_economy_action_plan.pdf.
European Union, 2016. Combating Food Waste: an Opportunity for the EU to Improve the Resource-Efficiency of the Food Supply Chain, p. 74. Luxembourg.
European Union, 2021. In: Union, O.J.o.t.E. (Ed.), Legislation. Publications Office of the European Union, Luxembourg.
Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – possibilities and limitations for modification through diet. Waste Manag. 102, 40–47.
Feng, H.L., Wang, X.J., Cai, J.S., Chen, S.H., 2020. Discrepancies in N_2O emissions between household waste and its food waste and non-food waste components during the predisposal stage. J. Environ. Manag. 265.
Frisvad, J.C., Møller, L.L.H., Larsen, T.O., Kumar, R., Arnau, J., 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger, Aspergillus oryzae*, and *Trichoderma reesei*. Appl. Microbiol. Biotechnol. 102 (22), 9481–9515.
Gold, M., Tomberlin, J.K., Diener, S., Zurbrugg, C., Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. Waste Manag. 82, 302–318.
Guo, H., Jiang, C., Zhang, Z., Lu, W., Wang, H., 2021. Material flow analysis and life cycle assessment of food waste bioconversion by black soldier fly larvae (*Hermetia illucens* L.). Sci. Total Environ. 750, 141656.
Holman, J.P., 2011. Experimental methods for engineers. McGraw-Hill.
IPCC, 2013. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1553.
IPCC, 2014. Climate change 2014: mitigation of climate change. Contribution of working group III to the fifth assessment. Report of the intergovernmental panel on climate change. In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., Stechow, C.v., Zwickel, T., Minx, J.C. (Eds.), AR5. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
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 Manag. 100, 151–160.
Kaza, S., Yao, L., Perinaz, B.-T., Van Woerden, F., 2018. What A Waste 2.0: A Global Snapshot of Solid Waste Management to 2050. The World Bank.
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. Manag. 227, 189–199.
Kunamneni, A., Plou, F.J., Alcalde, M., Ballesteros, A., 2014. Chapter 24 - *Trichoderma* enzymes for food industries. In: Gupta, V.K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I., Tuohy, M.G. (Eds.), Biotechnology and Biology of Trichoderma. Elsevier, Amsterdam, pp. 339–344.
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, 138968.
Lalander, C., 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.
Lopes, I.G., Braos, L.B., Cruz, M.C.P., Vidotti, R.M., 2021. Valorization of animal waste from aquaculture through composting: nutrient recovery and nitrogen mineralization. Aquaculture 531, 735859.
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 Agric. 98 (15), 5776–5784.
Mertenat, A., Diener, S., Zurbrugg, C., 2019. Black Soldier Fly biowaste treatment - assessment of global warming potential. Waste Manag. 84, 173–181.
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.
Pang, W., Hou, D., Chen, J., Nowar, E.E., Li, Z., Hu, R., Tomberlin, J.K., Yu, Z., Li, Q., Wang, S., 2020. Reducing greenhouse gas emissions and enhancing carbon and nitrogen conversion in food wastes by the black soldier fly. J. Environ. Manag. 260.
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.
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 Valorization 6 (6), 1059–1065.
R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rehman, K.u., Cai, M., Xiao, X., Zheng, L., Wang, H., Soomro, A.A., Zhou, Y., Li, W., Yu, Z., Zhang, J., 2017. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). J. Environ. Manag. 196, 458–465.
Saunois, M., Stavert, A.R., Poulter, B., Bousquet, P., Canadell, J.G., Jackson, R.B., Raymond, P.A., Dlugokencky, E.J., Houweling, S., Patra, P.K., Ciais, P., Arora, V.K., Bastviken, D., Bergamaschi, P., Blake, D.R., Brailsford, G., Bruhwiler, L., Carlson, K. M., Carrol, M., Castaldi, S., Chandra, N., Crevoisier, C., Crill, P.M., Covey, K., Curry, C.L., Etiope, G., Frankenberg, C., Gedney, N., Hegglin, M.I., Höglund-Isaksson, L., Hugelius, G., Ishizawa, M., Ito, A., Janssens-Maenhout, G., Jensen, K.M., Joos, F., Kleinen, T., Krummel, P.B., Langenfelds, R.L., Laruelle, G.G., Liu, L., Machida, T., Maksyutov, S., McDonald, K.C., McNorton, J., Miller, P.A., Melton, J.R., Morino, I., Müller, J., Murguia-Flores, F., Naik, V., Niwa, Y., Noce, S., O'Doherty, S., Parker, R.J., Peng, C., Peng, S., Peters, G.P., Prigent, C., Prinn, R., Ramonet, M., Regnier, P., Riley, W.J., Rosentreter, J.A., Segers, A., Simpson, I.J., Shi, H., Smith, S. J., Steele, L.P., Thornton, B.F., Tian, H., Tohjima, Y., Tubiello, F.N., Tsuruta, A., Viovy, N., Voulgarakis, A., Weber, T.S., van Weele, M., van der Werf, G.R., Weiss, R. F., Worthy, D., Wunch, D., Yin, Y., Yoshida, Y., Zhang, W., Zhang, Z., Zhao, Y., Zheng, B., Zhu, Q., Zhu, Q., Zhuang, Q., 2020. The global methane budget 2000–2017. Earth Syst. Sci. Data 12 (3), 1561–1623.
Slorach, P.C., Jeswani, H.K., Cuéllar-Franca, R., Azapagic, A., 2019. Environmental and Economic Implications of Recovering Resources from Food Waste in a Circular Economy, vol. 693. Science of The Total Environment.
Song, S., Ee, A.W.L., Tan, J.K.N., Cheong, J.C., Chiam, Z., Arora, S., Lam, W.N., Tan, H.T. W., 2021. Upcycling food waste using black soldier fly larvae: effects of further composting on frass quality, fertilising effect and its global warming potential. J. Clean. Prod. 288, 125664.
Suckling, J., Druckman, A., Small, R., Cecelja, F., Bussemaker, M., 2021. Supply chain optimization and analysis of Hermetia illucens (black soldier fly) bioconversion of surplus foodstuffs. J. Clean. Prod. 321, 128711.
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.
Themelis, N.J., Ulloa, P.A., 2007. Methane generation in landfills. Renew. Energy 32 (7), 1243–1257.
Tomberlin, J.K., Huis, A.v., 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., Emhart, C., Epstein, M., Finke, M., Fischer, C.H., Gatlin, D., Grabowski, N.T., He, C., Heckman, L., Hubert, A., Jacobs, J., Joseph, J., Khanal, S.K., Kleinfinger, J.K., 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., 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, 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.
Vinnerås, B., Nordin, A., Niwagaba, C., Nyberg, K., 2008. Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. Water Res. 42 (15), 4067–4074.
Wang, P., Tan, Z., Guan, L., Tang, S., Zhou, C., Han, X., Kang, J., He, Z., 2015. Ammonia and amino acids modulates enzymes associated with ammonia assimilation pathway by ruminal microbiota in vitro. Livest. Sci. 178, 130–139.
Zhang, H.Y., Li, G.X., Gu, J., Wang, G.Q., Li, Y.Y., Zhang, D.F., 2016. Influence of aeration on volatile sulfur compounds (VSCs) and NH_3 emissions during aerobic composting of kitchen waste. Waste Manag. 58, 369–375.

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

L. Lindberg *, B. Vinnerås, C. Lalander

Department of Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, 75007 Uppsala, Sweden

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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

* Corresponding author.
E-mail address: lovisa.lindberg@slu.se (L. Lindberg).

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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}

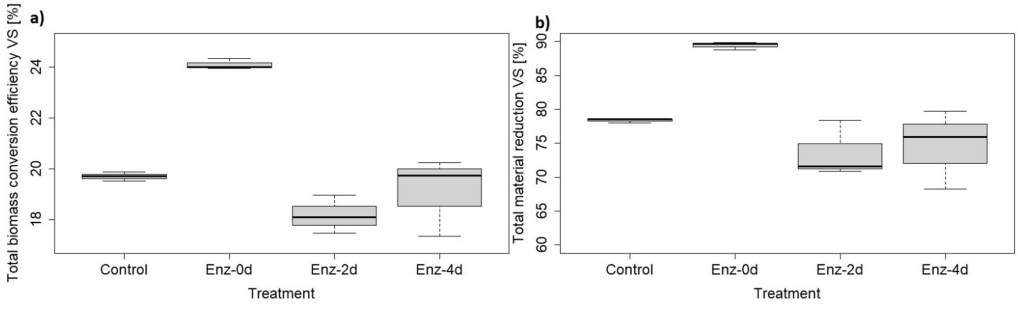

Fig. 1. Process efficiency parameters for the entire process on a total volatile solids (VS) basis in the control and after pre-treatment with directly added enzymes (Enz) for 0, 2 or 4 days: a) biomass conversion efficiency and b) material reduction.

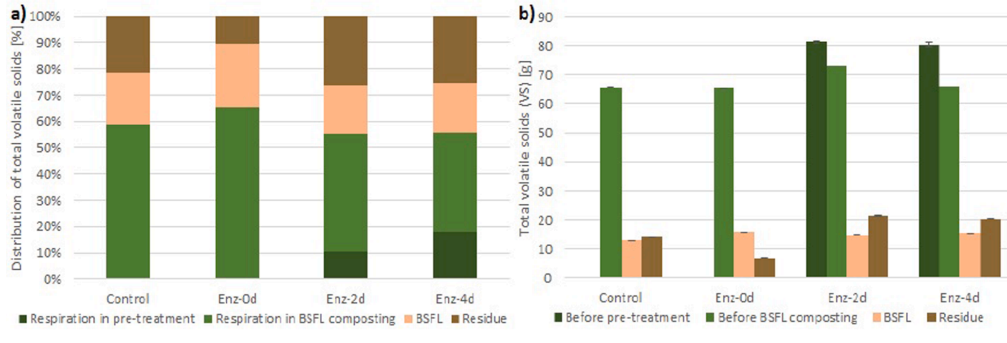

Fig. 2. Mass balance (volatile solids (VS) basis) during black soldier fly larvae (BSFL) composting of a mixture of cabbage and lettuce in the control and after pre-treatment with directly added enzymes (Enz) for 0, 2 or 4 days: a) distribution of initial VS during treatment divided into four fractions, 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.

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Process efficiency and the impact of enzyme and frass additions in black soldier fly larvae composting with fibrous plant-based waste

L. Lindberg *, B. Vinnerås, C. Lalander

Department of Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, Uppsala 75007, Sweden

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ABSTRACT

Biowaste treatment using the larvae of the black soldier fly (BSFL) continues to gain attention due to its ability to process a wide range of biodegradable waste sources and convert them into valuable products. Process efficiency depends on the substrate, with single-source vegetable waste generally yielding lower waste-to-biomass conversion efficiency (BCE) compared to multi-source blends. Enzyme addition can improve efficiency; however, enzymatic degradation releases water, resulting in a residue with a higher moisture content. This could be balanced by adding drier substrates, like insect frass. This study evaluated the impact of enzymes and/or frass additions to cucumber plant residuals on the process efficiency of BSFL composting, assessed in terms of BCE, material reduction, larval yield and the feasibility of dry sieving larvae. The study evaluated the most effective enzyme concentration for process and dry sieving efficiency, along with the impact of frass addition and varying larval and substrate densities. Adding both enzymes and frass was the only treatment that resulted in a significant increase in BCE, material reduction and larval yield compared to the control. The combination of enzymes and frass better facilitated the harvesting process in comparison to adding frass alone. In one treatment, where only half the substrate was added and a plastic foam cover was used, the harvesting process was simplified and the process efficiency increased; however, the larval yield did not differ from the control. The incorporation of enzymes and frass requires validation on an industrial scale to determine their suitability for treatments with BSFL composting.

1. Introduction

Fly larvae composting with black soldier fly larvae (BSFL, *Hermetia illucens* L. (Diptera: Stratiomyidae)) is a biological treatment that has been reported to be efficient for various organic waste types [32,45]. After treatment, the proteins, carbohydrates and lipids that are present in the waste are concentrated in the larval biomass, which can then be used as a protein source in animal feed [44]. Some of the nitrogen, phosphorus and potassium is concentrated in the treatment residue, known as frass, and is comparable to commercial organic fertilizer in terms of total nitrogen, total phosphorus and total potassium content, which typically ranges from 0.6 % to 4.8 %, 0.8–2.5 % and 0.2–2.1 %, respectively [1,43]. The biomass conversion efficiency (BCE) varies, however, depending on the type of biowaste treated, with lower efficiencies having been reported for mono-streams compared to food waste or multi-source blends, even when blending only vegetable waste (Gold, M. et al., 2020; [16,22]). The lower BCE observed for single-source vegetable waste is believed to result from its unbalanced nutritional composition for BSFL. This may be due to a low protein content combined with a high proportion of lignocellulosic biomass (*i.e.* lignin, cellulose and hemicellulose), which is not available to the larvae [11], or a suboptimal amino acid composition [26].

It has been demonstrated that a pre-treatment of biowaste with high lignocellulosic biomass (sugarcane bagasse and wheat straw), which combines steam pre-treatment and enzymatic hydrolysis, increased the BCE [48], while steam pre-treatment alone did not achieve the same improvement [47]. In that study, enzymatic hydrolysis alone was not investigated. A 100 % increase in BCE for volatile solids (VS) has been demonstrated for banana peels, another substrate with high lignocellulosic-rich substrates, following a pre-treatment with ammonia and/or fungi [15]. In contrast, Peguero et al. [38] demonstrated reductions in BCE following a 5 % ammonia pre-treatment of four fibrous biowaste types: brewers spent grain, cow manure, oat pulp, and grass clippings. The authors attributed this reduction to ammonia toxicity, which negatively affects both the microbial community in the substrate and the larvae themselves. The ammonia solution, and the enzymes

* Corresponding author.
E-mail address: lovisa.lindberg@slu.se (L. Lindberg).

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secreted by the fungi, likely improved substrate availability for the larvae by providing a nitrogen source and degrading otherwise indigestible compounds into easily available carbohydrates [10,46]. Lindberg et al. [29] found that the direct addition of enzymes to lettuce and cabbage in the presence of larvae increased BCE by 22 % compared to the control. The authors concluded that adding a separate enzymatic pre-treatment was unnecessary, as the enzymes could be effectively applied together with the larvae. The efficiency of enzymatic pre-treatment was demonstrated in a study on the cellulase pre-treatment of palm decanter cake, where the larvae increased their biomass 2.4-fold (though this was not statistically verified) when fed palm decanter cake that had been pre-treated for 72 h with 1 % *w/w* cellulase [19]. The enzyme activity unit per mL in Jing Lim et al. [19] was 60 times higher compared to that used in Lindberg et al. [29].

Both the microbial community in the larval intestines [53], as well as the microbial degradation of proteins, lipids and carbohydrates in the substrate [52], appear to be of vital importance to the larvae. Thus, the addition of microorganisms excreted by the BSFL has also been reported to increase the BCE and cause the material reduction of lignocellulosic-rich substrates (Gold, Moritz et al., 2020; [35]). Along the same lines, Lopes et al. [34] demonstrated that recirculating frass derived from the BSFL bioconversion of food waste as part of the feedstock increased larval yield per tonne of food waste by 95 %. This finding suggests that the frass retains nutrients that remain accessible for larval digestion. In addition, Lopes et al. [34] found that increased levels of frass correlated negatively with BCE and material reduction per treatment batch, even though the larval yield per tonne of treated food waste increased. Including an additive in the larval diet could shift the protein to carbohydrate ratio of the blend to better fit the nutritional needs of the larvae [7,39]. As demonstrated by Lopes et al. [34], frass could be added as an additive, as it has been shown to be rich in macronutrients, micronutrients and organic matter [2,41]. Another benefit of using frass alongside vegetable waste is that frass has a considerably lower moisture content and could thereby be used to lower the moisture content of the vegetable-frass blend. Vegetable wastes usually have a water content between 80 % and 92 % (Gold, M. et al., 2020; [28]). It has been shown that a water content of around 70 % results in higher BCE and material reduction, while keeping it below 80 % facilitates successful dry separation [4,23].

One particularly challenging biowaste to treat is cucumber plant residuals from horticulture cucumber production. These are low in protein and high in lignocellulosic biomass and are generated in large quantities at distinct intervals (personal communication, Biljana Vuckovic, 2023). Currently, this biowaste stream can either be treated at municipal recycling stations at a cost of 660 SEK per tonne[1] or spread on agricultural fields for degradation. In the latter case, pesticides used during production may leach out into the environment, polluting nearby water bodies [31]. Moreover, this biowaste is not an optimal feedstock for anaerobic digestion since it is generated intermittently as a point-source stream, contains high levels of lignin that are resistant to degradation by anaerobic microbial communities, and has a low protein content that does not meet the nutritional requirements of the microflora. As a consequence, it requires a longer residence time in the digester to be adequately degraded [3,14]. Fly larvae composting may serve as a viable alternative strategy for the treatment of this type of biowaste, as it may prevent pesticides leaching out into the environment, as it has been shown that selected pesticides are degraded in BSFL composting [25,37]. At the same time, it may offer farmers an opportunity to generate valuable products from the waste [24]. This technology is highly modular and can be readily adapted to handle large, one-time waste flows by adjusting batch capacity to match the waste quantities generated on-site [17].

The aim of this study was to investigate the potential methods for enhancing process efficiency in the fly larvae composting of cucumber plant residues from horticultural production. Efficiency was assessed through bioconversion efficiency (BCE), larval yield, larval survival, and material reduction. Specifically, the study evaluated whether the addition of enzymes, frass, or their combination could improve the overall treatment.

2. Materials and Methods

2.1. Materials

The cucumber plant residuals (PR) used in this study were provided by Tinas Grönsaksodling AB (Helsingborg, Sweden). The PR were minced at the greenhouse and, upon arrival at the lab, were stored for up to three months at −18°C before use.

The BSFL used in the study were sourced from a BSF colony maintained continuously since 2015 by the Environmental Engineering group at the Department of Energy and Technology, SLU (Uppsala, Sweden). The BSFL used for treatment were sieved through a 1 mm mesh sieve to ensure uniformity, and the average weight was 0.33 ± 0.14 g per 100 larvae.

Commercial cellulase and pectinase purchased from Enzyme Supplies (Oxford, United Kingdom) were used in the experiment.

2.2. Experimental set-up

The primary aim of this study was to evaluate whether supplementing a recalcitrant biowaste stream (cucumber plant residuals from horticulture production) with bio-chemical amendments could improve the process performance of fly larvae composting by increasing BCE, larval yield, larval survival and material reduction.

A total of twelve treatments were investigated (Fig. 1): a control with only plant residuals (PR-C); PR treated with enzymes (PR-E); PR with frass additive (PR-F); and PR treated with enzymes and with frass additive (PR-EF). To better understand the impact of the enzyme addition, several treatments were designed using PF-EF as a reference, with slight modifications. These included two treatments with a half and a double dose of enzymes relative to EF (PR-E/2 and PR-E2), one treatment combining double larval density and double the dose of enzymes (PR-L2), and one treatment with double larval density and four times the enzyme dose (PR-L4), all compared to PR-EF. Due to the bulky nature of the plant residuals, two additional treatments were established using half the volume of substrate, frass and larval density compared to PR-EF. One treatment (PR-H) had half the amount of PR-EF in a box of the same size, while the other treatment (PR-HT) used the same setup but with a foam cover, leaving small gaps at the edges. The cover was added based on previous observations that the top layer of the plant residuals tended to dry out and remain undegraded in previous treatments. To assess whether frass should be considered part of the degradable feedstock or regarded as inert, two control treatments were included: one using only frass as a substrate (F-C) and one using frass and enzyme treatment (F-E). These treatments were intended to evaluate the potential of frass to support further larval degradation activity.

2.2.1. Additives applied in treatments

2.2.1.1. Enzymes. The bio-chemical additives applied included the enzymes cellulase and pectinase, along with frass derived from the BSFL bioconversion of pig feed. It was hypothesised that the enzyme addition would hydrolyze complex polysaccharides (namely cellulose and pectin) in the plant residuals, yielding more readily available carbohydrates accessible to the larvae, thus increasing BCE and larval yield [30]. The temperature during the treatment was set to reflect standard BSFL composting conditions, despite being below the optimal range for cellulase and pectinase activity (40–60°C) [20,40]. The direct addition

[1] https://nsr.se/foretag/lamna-verksamhetsavfall/priser/

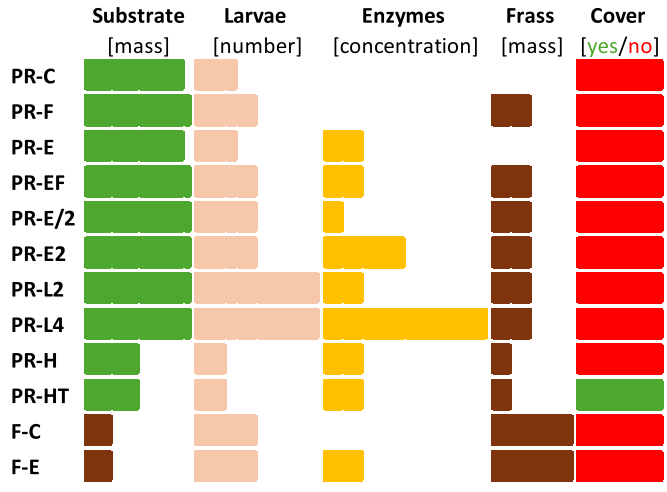

Fig. 1. A schematic overview of the twelve treatments. The size of the stacks correlates with the wet weight mass in each treatment.

of enzymes, rather than relying on fungal enzyme production, circumvents the risk of competition for the substrate between fungi and larvae, and eliminates the time required for fungal growth and enzyme synthesis. The enzymes were added to a concentration of 1 % *w/w*, following the procedure described in Lindberg et al. [29]. This addition corresponds to 150–300 enzyme units per g VS in the plant residuals. One unit (1 U) refers to the enzyme's catalytic activity, defined as the amount of enzyme that catalyses the conversion of 1 μmole of a specific substrate per minute under defined assay conditions. Enzyme catalytic activity is influenced by environmental conditions such as temperature, pH and the concentration of the chosen substrate. Different amounts of the enzyme were targeted: 300 units for cellulase per g PR and 150 units for pectinase per g PR in the reference treatment PR-EF (Table 1).

2.2.1.2. Frass. Due to the high moisture content of the vegetable and plant materials (TS: 13 %, VS: 70 %, Table 2), frass derived from the BSFL bioconversion of pig feed (TS: 85 %, VS: 83 %, Table 2) was added to reduce the moisture content of the plant residual-frass blend. The frass addition was hypothesised to facilitate larval harvesting at the end of the treatment [34] (*i.e.,* making it possible to harvest the larvae using a sieve rather than requiring more labour intensive methods, such as manual picking, for efficient larval harvesting). Moreover, the addition of frass was expected to improve the process in several ways. It was hypothesised that it would balance the nutrient composition in the PR through an increase in the protein to carbohydrate ratio [7,39], improve the physical structure of the plant residuals through the reduced formation of large air pockets and increased surface area, thereby enhancing larval access and microbial activity [35], and introduce beneficial microorganisms from the start of the treatment ([5]; Gold, Moritz et al., 2020).

2.2.2. BSFL composting

All treatments were performed in an insulated, temperature-controlled freight container set at 28°C. All treatments were performed in triplicate. The treatment boxes (16.6 cm × 14.7 cm × 10.3 cm) were placed inside larger boxes, which were arranged in a rack with 3 cm spacing between them. Every third day, the boxes were rotated in the rack to minimise the effect of a temperature gradient. The larval feeding load was 0.7 – 1.5 g ww per larva, while a VS feed dose per larva of 0.2 g was targeted; the exact amount provided was calculated after the completion of BSFL composting (Table 1).

The weight of the substrate was recorded at the start of each treatment. In the enzyme treatments, the weight was recorded prior to enzyme addition, after which the mixture was thoroughly stirred. In the frass treatments, 7 % *w/w* frass was added on top of the substrate, and the seed-larvae were placed onto the frass. In each treatment, 500–2000 larvae were added, yielding a larval density of 2.0 – 8.2 larvae/cm^2. The number of larvae added was based on the amount of VS provided per larva (Table 1). The larvae were fed by layering fresh substrate on top of the existing substrate without stirring (all treatments with PR). Due to the bulky nature of PR, feeding of the total feed load was distributed across seven feeding occasions over the first seven days (treatments PR-C, PR-F, PR-E, PR-EF, PR-E/2, PR-E2, PR-L2, PR-L4; Table 1). In the treatments with half the volume of PR, frass and enzymes (PR-H and PR-HT), feeding was distributed across three feeding occasions over the first seven days of treatment. The plastic foam cover was removed 7 days prior to harvesting (PR-HT). In the control treatments with only frass (F-C, F-E), all of the substrate was added at the start of the treatment. Water was added to reach a moisture content of 70 %. The BSFL composting lasted for 16 days for plant residuals and for 11 days in the frass controls.

The treatment boxes, including their contents, were weighed before and after BSFL composting. The larvae were then either picked out manually, separated from the treatment residue using a sieve with a mesh size of 2 mm, or a combination of both. The larvae were weighed, and the total amount of residue was calculated by subtracting the larval weight from the total weight before separation. The average larval weight was determined by weighing three independent samples, each consisting of at least 100 larvae, and dividing the total weight by the number of larvae in each sample. The total number of larvae remaining at the end of the treatment was estimated by dividing the total weight of all larvae by the average larval weight.

2.3. Sampling

Samples for TS, VS, pH, crude lipids, crude protein and fibre analysis were collected from the substrate before and after BSFL composting, with post-treatment samples including material from both the larvae and residues. One sample of 100 g in total per replicate was collected, contained substrate from a minimum of five areas in the treatment box at depths from 0 to 5 cm. Analysis for TS and VS was conducted directly

Table 1
A list of substrates and additions, the amount of cellulose and pectinase added, the number of larvae, the volatile solids (VS) load per larva and the total amount of substrate on a wet weight (ww) basis added in each treatment. Values are presented as mean ± SD (n = 3), where LD (low deviation) means SD < 0.0003.

	Explanation	Substrate	Addition	Cellulase [u/g VS]	Pectinase [u/g VS]	Number of larvae	BSFL total VS load [g VS/larva]	Plant residuals [g ww/treat.]	Frass [g ww/ treat.]
PR-C	Plant residuals	Plant residuals	None	-	-	680	0.20^{a}±LD	1 380 ± 0.3	
PR-F	Plant residuals with frass	Plant residuals	Frass	-	-	1000	0.21^{b}±LD	1 380 ± 0.2	102 ± 0.1
PR-E	Plant residuals with enzymes	Plant residuals	Enzymes	336	159	680	0.20^{a}±LD	1 380 ± 0.3	
PR-EF	Plant residuals with enzymes and frass	Plant residuals	Enzymes and frass	215	102	1000	0.21^{b}±0.01	1 380 ± 0.1	102 ± 0.2
PR-E/2	Plant residuals with frass and half conc. enzymes	Plant residuals	Enzymes and frass	107	51	1000	0.21^{b}±LD	1 380 ± 0.4	102 ± 0.2
PR-E2	Plant residuals with frass and double conc. enzymes	Plant residuals	Enzymes and frass	420	211	1000	0.20^{a}±0.02	1 380 ± 0.2	102 ± 0.3
PR-L2	Plant residuals with frass, enzymes, and double larval density	Plant residuals	Enzymes and frass	225	107	2000	0.10^{c}±LD	1 380 ± 0.6	102 ± 0.1
PR-L4	Plant residuals with frass, 4 times larger conc. enzymes and double larval density	Plant residuals	Enzymes and frass	818	420	2000	0.10^{c}±0.02	1 380 ± 0.3	102 ± 0.5
PR-H	Half volume of plant residuals, enzymes and frass	Plant residuals	Enzymes and frass	264	119	500	0.18^{d}±0.03	690 ± 0.3	50 ± 0.2
PR-HT	Half volume of plant residuals, enzymes, frass and cover	Plant residuals	Enzymes and frass	264	119	500	0.18^{d}±0.02	690 ± 0.2	50 ± 0.1
F-C	Frass control	Frass	None[i]	-	-	1000	0.23^{e}±LD		319 ± 0.1
F-E	Frass control with enzymes	Frass	Enzymes[i]	170	81	1000	0.23^{e}±LD		319 ± 0.1

[i]Water added to the treatment.

after sampling from the initial substrates and residue, and directly after freezing the larvae for samples of the larval biomass. Larval biomass, residue and initial substrate were kept frozen at −18°C, and selected samples were sent for proximate analysis (crude protein, crude fat, ash) and fibre composition analysis at Eurofins (EC reg 152/2009 mod. and NF V 18–122). The treatments selected for analysis were those with higher biomass conversion efficiency compared to the others, along with the control treatments, resulting in a total of eight of the twelve treatments being analysed.

2.3.1. Analysis at Eurofins

Crude lipid content was determined through the gravimetric lipids' determination principal using Gerhardt instruments. Total nitrogen was determined using the Kjeldahl method and the protein content was calculated using a conversion factor of 4.76 [18]. Determinations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were made using the Van Soest technology (NF V18–122) [50]. This method is based on successive treatments with detergents and applies to both simple feeds and compound feeds. It allows the estimation of total fibre, the lignocellulosic complex and lignin (although it is not suitable for determining a lignin content below 0.5 %). After solubilisation and degradation of starch, proteins, and lipids, the water-insoluble residue provides the plant cell wall the content of the food. This method makes it possible to better take pectin into account than in NDF. The NDF process makes it possible to obtain the insoluble fraction in the neutral detergent, hemicellulose, cellulose and lignin. The ADF process makes it possible to obtain the insoluble fraction in acid detergent at 72 %, which is cellulose and lignin. The process using ADL gives the insoluble fraction of lignin.

2.3.2. Physical and chemical analysis

For the determination of TS, samples were dried at 60°C for a minimum of 48 h, to avoid the evaporation of VS [49]. The VS content was determined through sequential combustion, first at 250°C for 2 h followed by combustion at 550°C for 4 h (modified ISO 18122:2015). The pH was determined using a solid-state pH electrode (InLab Solids Pro-ISM, Mettler Toledo), which is designed for direct measurements in solid and semi-solid materials. The probe was inserted into each replicate material, ensuring it did not come into contact with the bottom of the treatment box.

2.4. Calculations

The 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 the VS of larvae and the total mass of the initial substrate given to larvae, respectively.

The percentage material reduction on a VS basis (Red_{VS}) 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 the total mass of the initial material, respectively.

$$\text{Larval yield}_{TS} = \frac{mTS_{larvae}}{a_{box}} \quad (3)$$

where mTS_{larvae} is the total mass of the TS of the larvae and a_{box} is the area of the treatment box.

The mass balance on a TS basis was calculated as:

$$mTS_{initial} = mTS_{larvae} + mTS_{residue} + mTS_{loss} \quad (4)$$

Table 2
Total solids (TS) and total volatile solids (VS) content in the initial substrate, containing plant residuals (PR) and frass (F), larvae, and residues from black soldier fly larvae (BSFL) composting. Treatments with no additions (C), added enzymes (E) of different concentrations (E/2, E2), frass (F), different larval densities (L2, L4), half substrate density (H) and cover (T). Significant differences ($p < 0.05$) in TS and VS from the initial substrate are denoted *. Different letters within columns indicate significant difference ($p < 0.05$). Values are presented as mean ± SD ($n = 3$), where LD (low deviation) means SD < 0.001.

	Initial substrate						Products			
	Plant residuals		Frass		Combined *(calculated)*[i]		Larvae		Residue	
	TS [%]	VS [%]	TS [%]	VS [%]	TS [%]	VS [%]	TS[iii] [%]	VS [%]	TS [%]	VS [%]
PR-C	14.7 ± 2.8	71.2 ± 5.1			13.7 ±LD	71.1 ±LD	25.7^{*abc}±0.5	72.2^{abc}±0.5	40.9^{*ab}±4.7	57.1^{*ab}±0.4
PR-F	14.7 ± 2.8	71.2 ± 5.1	86.4 ± 1.6	85.3 ± 0.7	18.7 ±LD	75.7 ±LD	24.0^{*cd}±0.6	73.7^{*bd}±0.7	40.7^{*ab}±1.6	64.3^{*cd}±0.8
PR-E	14.7 ± 2.8	71.2 ± 5.1			13.7 ±LD	71.1 ±LD	25.3^{*b}±0.3	72.2^{abc}±0.7	29.5^{*c}±2.1	56.8^{*a}±2.6
PR-EF	14.7 ± 2.8	71.2 ± 5.1	86.4 ± 1.6	85.3 ± 0.7	18.7 ±LD	75.7 ±LD	24.4^{*cde}±1.1	73.4^{ad}±1.0	49.9^{*bd}±3.7	64.4^{*cd}±2.4
PR-E/2	14.7 ± 2.8	71.2 ± 5.1	86.4 ± 1.6	85.3 ± 0.7	18.7 ±LD	75.7 ±LD	24.0^{*cd}±1.0	70.7^{*ac}±0.6	60.0^{*de}±3.8	64.4^{*cd}±2.6
PR-E2	13.0 ± 1.5	71.1 ± 5.6	82.9 ± 5.3	86.0 ± 0.5	17.8 ±LD	75.8 ±LD	23.7^{*cd}±0.4	73.4^{*ad}±0.5	36.9^{*ac}±1.6	64.4^{*cd}±1.4
PR-L2	13.0 ± 1.5	71.1 ± 5.6	82.9 ± 5.3	86.0 ± 0.5	17.8 ±LD	75.8 ±LD	19.4^{*d}±0.2	70.0^{*c}±1.3	47.9^{*b}±2.5	62.7^{*bcd}±3.5
PR-L4	13.0 ± 1.5	71.1 ± 5.6	82.9 ± 5.3	86.0 ± 0.5	17.8 ±LD	75.8 ±LD	27.3^{bc}±5.3	74.5^{bd}±0.8	83.5^{*fg}±7.9	70.6^{e}±2.9
PR-H	12.4 ± 1.3	67.4 ± 7.0	84.7 ± 2.1	76.3 ± 0.9	17.4 ±LD	70.5 ±LD	33.3^{*ab}±1.0	72.1^{abc}±0.7	91.1^{*f}±0.2	59.3^{*ad}±0.6
PR-HT	12.4 ± 1.3	67.4 ± 7.0	84.7 ± 2.1	76.3 ± 0.9	17.4 ±LD	70.5 ±LD	26.6^{*abe}±0.4	72.1^{abc}±1.3	61.2^{*e}±4.9	54.6^{*a}±1.8
F-C[ii]			90.9 ± 0.1	78.6 ± 0.4	31.8 ± 0.1	78.6 ±LD	35.0^{ab}±3.3	75.7^{d}±1.6	84.6^{*fg}±1.0	67.0^{*ce}±0.4
F-E[ii]			90.9 ± 0.1	78.6 ± 0.4	31.8 ±LD	78.6 ±LD	38.7^{a}±2.9	81.3^{*}±0.6	80.1^{*g}±2.2	71.0^{*e}±1.0

[i] Calculated based on measured values from PR and frass separately.
[ii] Water added to the treatment.
[iii] Normality was not achieved, and rank transformation was used.

where *m*TS is the total mass of the TS of protein, nitrogen, lipids, lignin, cellulose or hemicellulose in either the initial amount substrate, larvae, residue or lost through degradation.

The cellulose content was estimated as the difference between acid detergent fibre (ADF) and acid detergent lignin (ADL), whereas the hemicellulose content was estimated as the difference between the neutral detergent fibre (NDF) and acid detergent fibre (ADF). The ADL corresponded to the lignin content.

2.5. Statistical analysis

Statistically significant differences were calculated for BCE, material reduction, larval yield, nutritional content, TS and VS, and the degradation of lignin, cellulose and hemicellulose, using the analysis of variance (ANOVA) with a 5 % significance level. Tukey's Honest Significant Difference (Tukey's HSD) with a 5 % significance level was used when a significant difference was found. Normality was verified in the model residuals using the Shapiro-Wilk test. When normality was not achieved, a Box-Cox test was used to determine what type of data transformation was necessary to achieve normality. A paired *t*-test with a 5 % significance level was used to compare TS and VS values at different stages in each treatment. All statistical tests and graphical representations were made in R (R [6]).

3. Results

The total solids (TS) and total volatile solids (VS) in the initial substrate consisting of plant residuals (TS: 13 ± 1, VS: 70 ± 2) and frass (TS: 85 ± 3, VS: 83 ± 5) did not vary greatly (Table 2). The larvae from the treatments with only frass (F-C and F-E) had a higher TS and VS content; however, this was not significantly different from the larvae in the other treatments. The TS and VS varied among the treatment residues. It was noted that it was easy to separate larvae using a sieve from residues with TS higher than 60 % and these were classified to have a high larval harvest efficiency. The treatments with a residue of 47 % < TS < 60 % were separated from the larvae with a combination of sieving and manually picking the larvae from the residue (Table 3). The residues were significantly different from the initial substrate, with a higher TS and lower VS content. The visual appearance of the harvested residues is shown in Fig. 2.

The survival in all treatments was > 74 % and larval size ranged between 44 and 127 mg, with the treatment with double larval density and four times the concentration of enzymes (PR-L4) resulting in the smallest larvae, while the covered treatment with a half volume of the substrate, frass and enzymes (PR-HT, Table 3) resulted in the largest larvae.

The material reduction (M.Red) was significantly lower in the

Table 3
The harvesting method used for each residue (sieving, manual picking or a combination of both).

	Harvesting method	
	Sieving	Manual picking
PR-C		x
PR-F	x	x
PR-E		x
PR-EF	x	x
PR-E/2	x	
PR-E2		x
PR-L2	x	x
PR-L4	x	
PR-H	x	
PR-HT	x	
F-C	x	
F-E	x	

Fig. 2. Pictures of the treatment residues after harvest from treatments a) PR-E/2, b) PR-F, and c) PR-E.

control (PR-C) compared to the treatments with the additions of enzymes, frass or their combination (PR-F, PR-E and PR-EF), as well as the treatment with double the amount of larvae (PR-L2) and the covered treatment (PR-HT, Table 4). Using only frass (F-C and F-E) as a substrate did not result in a significantly higher material reduction compared to the control; however, it did result in a significantly higher BCE_{VS} and larval $yield_{TS}$. The treatments that had a significantly higher BCE_{VS} compared to the control were the treatments with half volume of substrate, frass and enzymes (PR-H and PR-HT), the frass controls (F-C and F-E) and the treatment with added enzymes and frass (PR-EF). The mass of larvae generated per unit of space (larval yield, mg TS/cm^2) is an important factor from an industrial point of view, as it reflects the efficiency of space utilization. Several treatments had a significantly higher larval yield compared to the control. However, the only treatment that resulted in significantly higher values of all the parameters mentioned compared to the control was the PR-EF treatment.

The lignin content in the residue was highest in the treatments with only added enzymes (PR-E) and the treatment with a foam cover (PR-HT, Table 5). The treatments with a lower lignin content were the treatments with half volumes of substrate, frass and enzymes (PR-H) and with the additions of enzymes and frass (PR-EF); however, these were not significantly different from the frass controls (F-C and F-E). The cellulose content was significantly higher in the treatment with enzymes added. The hemicellulose content was highest in the frass control with added enzymes (F-E), but it was not significantly different from the other frass control (F-C) and the enzyme treatment (PR-E).

The lipid content in larvae was significantly higher in the treatment with cover (PR-HT) and the frass controls (F-C and F-E, Table 6). There was no significant difference in nitrogen content in larvae from the different treatments.

The distribution of nitrogen among larvae, treatment residue and losses was similar across all treatments with the exception of the frass control with enzymes (F-E), where a greater proportion of nitrogen remained in the residue and less was attributed to losses (Fig. 3a). In the covered treatment (PR-HT), nearly 75 % of the lignin was retained in the

Table 5
The content of lignin, cellulose and hemicellulose in plant residuals, frass and residue after BSFL composting on a total solids (TS) basis in the studied treatments. Treatments with no additions (C), added enzymes (E), frass (F), half substrate volume (H) and cover (T). Different letters within the columns indicate significant difference ($p < 0.05$). Values are presented as mean ± SD (n = 3).

	Fiber concentration TS basis			
	Lignin [%]	Cellulose [%]	Hemicellulose [%]	Total [%]
Into treatment				
Plant residuals	7.3 ± 0.8	16.9 ± 2.4	9.1 ± 1.7	33.3 ± 3.9
Frass	10.7 ± 1.6	14.9 ± 2.2	22.1 ± 6.5	47.8 ± 8.5
For PR-C, -E	7.3 ± 0.8	16.9 ± 2.4	9.1 ± 1.7	33.3 ± 3.9
For PR-F, -EF, -H, -HT	8.3 ± 1.0	16.3 ± 2.3	13.3 ± 3.2	37.9 ± 5.1
In residue				
PR-C	4.5^{a}±0.5	23.8^{ab}±1.3	6.9^{a}±0.2	35.2 ± 2.0
PR-F	8.1^{abc}±1.4	32.6^{a}±4.4	17.4^{bc}±3.9	58.2 ± 7.7
PR-E	13.2^{d}±3.2	58.8 ± 6.3	20.6 ^{cd}±4.3	92.7 ± 10.9
PR-EF	5.1^{ac}±0.8	20.2^{bc}±4.0	11.8^{ab}±2.5	37.1 ± 7.3
PR-H	4.8^{ac}±0.4	21.7^{b}±0.1	10.9^{ab}±1.3	37.4 ± 2.2
PR-HT	10.4^{bd}±1.4	26.0^{ab}±4.7	13.9^{abc}±1.6	50.2 ± 6.2
F-C	8.6^{bc}±0.7	22.3^{b}±1.0	20.0^{c}±1.0	50.9 ± 1.2
F-E	7.3^{abc}±0.4	11.4^{c}±0.2	27.5^{d}±2.5	46.2 ± 3.1

Table 4
The process performance in terms of material reduction and biomass conversion efficiency on a wet weight (WW) and volatile solids (VS) basis, and larval yield on a total solids (TS) basis in the studied treatments. Treatments with no additions (C), added enzymes (E) of different concentrations (E/2, E2), frass (F), different larval densities (L2, L4), half substrate volume (H) and cover (T). Different letters within the columns indicate significant difference ($p < 0.05$). Values are presented as mean ± SD (n = 3).

	Larval weight [mg/larva]	Survival[i] [%]	$M.Red_{WW}$ [%]	$M.Red^{ii}_{VS}$ [%]	BCE^{ii}_{WW} [%]	BCE_{VS} [%]	Larval $yield_{TS}$ [mg TS/cm^2]
PR-C	81.7^{ad}±6.3	76.7^{a}±3.5	72.6^{ab}±1.4	34.3^{ac}±4.7	3.09^{a}±0.18	5.90^{a}±0.30	44.8^{a}±2.1
PR-F	96.5^{bd}±5.6	96.5^{abd}±3.8	70.1^{bc}±0.8	44.7^{bd}±1.5	6.29^{bcf}±0.28	7.85^{ab}±0.46	91.4^{bd}±5.8
PR-E	85.1^{ad}±7.1	97.5^{bd}±0.7	68.9^{c}±1.0	46.5^{bde}±4.2	4.10^{ad}±0.37	7.70^{ab}±0.71	58.6^{ac}±5.8
PR-EF	90.3^{ad}±5.9	105^{bc}±8	76.0^{d}±1.1	45.6^{bd} ±1.0	6.41^{bce}±0.05	8.11^{b}±0.34	94.9^{be}±4.8
PR-E/2	85.6^{ad}±4.8	103^{bc}±3	78.8^{e}±1.2	42.4^{ab}±4.4	5.98^{bd}±0.48	7.17^{ab}±0.80	87.0^{bd}±9.8
PR-E2	119^{c}±7	74.0^{a}±5.2	72.9^{abf}±0.3	52.4^{df}±1.8	5.94^{bd}±0.14	7.66^{ab}±0.21	85.5^{bd}±2.6
PR-L2	48.5^{ef}±3.5	111^{bc}±6	79.2^{e}±0.9	54.0^{ef}±1.8	7.23^{ceg}±0.26	7.28^{ab}±0.30	85.2^{bd}±2.4
PR-L4	44.1 ^{f}±7.5	78.0^{ad}±16.9	83.9 ^{g}±0.3	29.6^{c}±9.4	4.75^{adf}±1.67	6.84^{ab}±1.30	75.4^{cde}±15.1
PR-H	77.4^{ad}±7.4	101^{b}±5	86.9 ± 0.3	42.4^{ab}±1.5	5.28^{abd}±0.46	10.3^{c}±0.7	53.2^{a}±3.5
PR-HT	127^{c}±9	102^{b}±6	83.2 ^{g}±1.6	54.5 ^{f}±1.8	8.72^{eg}±0.48	13.6 ± 0.7	70.3^{acd}±4.7
F-C	69.4^{ae}±12.4	105^{bc}±4	75.5^{df}±0.7	44.3^{abd}±1.7	7.99^{cg}±1.16	8.41^{bc}±0.41	104^{b}±6
F-E	115^{bc}±6	118^{c}±5	74.2^{adf}±0.7	41.2^{abc}±1.3	14.9 ^{g}±0.7	18.7 ± 0.9	215 ± 11

[i] Square transformation.
[ii] Normality was not achieved, and rank transformation was used.

Table 6
The total initial amount of nitrogen, lignin, cellulose, hemicellulose and lipids provided to the larvae [mg/larva], and the nitrogen and lipid content [%] in larvae in the studied treatments. Treatments with no additions (C), added enzymes (E), frass (F), half substrate density (H) and cover (T). Different letters within the columns indicate significant difference ($p < 0.05$). Values are presented as mean ± SD (n = 3), where LD (low deviation) means SD < 0.05, and VD (very low deviation) means SD < 0.005.

	Total initial amount [mg TS/larva]					$Larvae_{TS}$ [%]		
	Nitrogen	Lignin	Cellulose	Hemi-cellulose	Lipids	Nitrogen	Protein	Lipids
PR-C	9.42^{a} ±VD	20.1^{a}±LD	46.9^{a}±LD	25.2^{a}±LD	8.21^{a}±VD	7.87^{a}±0.20	37.5^{a}±1.0	8.19^{ab}±0.73
PR-F	9.85^{b} ±VD	23.1^{b}±LD	45.1^{b}±LD	36.7^{b} ±LD	7.76^{b} ±VD	7.83^{a}±0.25	37.1^{a}±1.2	7.32^{ab}±0.14
PR-E	9.42^{a} ±VD	20.1^{a}±LD	46.9^{a}±LD	25.2^{a}±LD	8.21^{a}±VD	7.67^{a}±0.25	36.5^{a}±1.2	8.27^{ab}±0.55
PR-EF	9.86^{b} ±0.01	23.1^{b}±LD	45.1^{b}±LD	36.8^{b} ±LD	7.76^{b} ±VD	7.74^{a}±0.38	36.8^{a}±1.8	5.81^{bc}±0.50
PR-H	9.18^{c} ±0.02	21.5^{c}±LD	41.9^{c}±0.1	34.5^{c}±0.1	7.21^{c}±0.01	7.80^{a}±0.13	37.1^{a}±0.6	4.93^{b}±0.65
PR-HT	9.19^{c} ±0.01	21.6^{c}±LD	42.0^{c}±LD	34.5^{c}±LD	7.22^{c}±VD	7.25^{a}±0.06	34.5^{ab}±0.3	8.83^{ac}±1.99
F-C	11.3^{d} ±VD	30.8^{d}±LD	43.3^{d}±LD	64.2^{d} ±LD	7.13^{d} ±VD	7.21^{a}±0.21	34.3^{ab}±1.0	10.5^{ad}±0.8
F-E	11.3^{d} ±VD	30.8^{d}±LD	43.3^{d}±LD	64.2^{d} ±LD	7.13^{d} ±VD	7.25^{a}±0.18	32.6^{b}±1.4	13.8^{d}±2.5

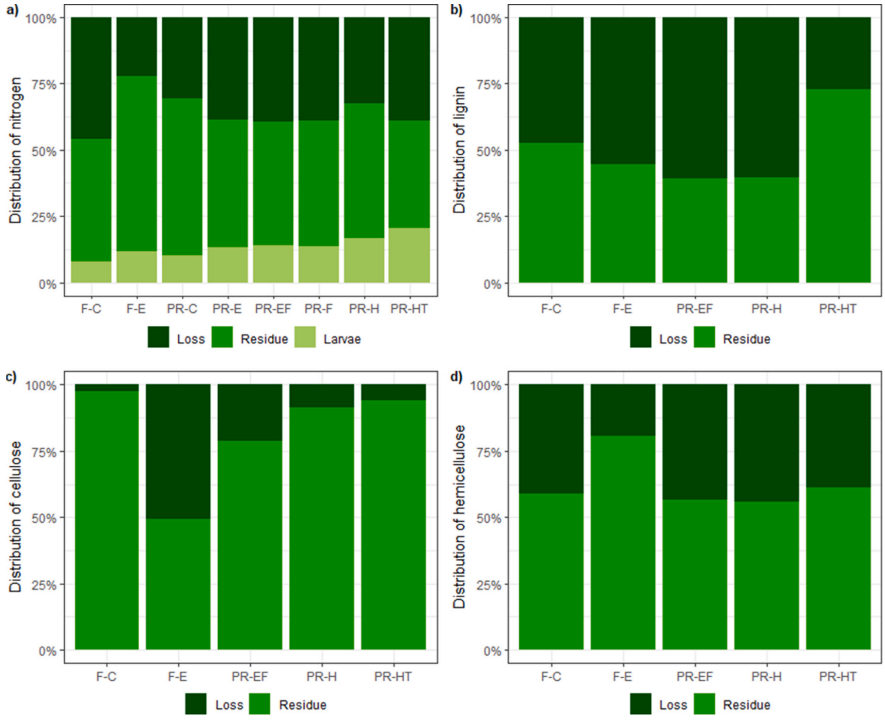

Fig. 3. Mass balance of TS in the black soldier fly larvae (BSFL) composting of plant residuals (PR) with the addition of enzymes (E) and frass (F), and half volumes of substrate (H) with a cover (HT), and only frass as a substrate with (F-E) or without the addition of enzymes (F-C): a) The distribution of nitrogen across three fractions, larval biomass (light grey), loss of nitrogen (grey) and treatment residue (black); b) The distribution of lignin, c) cellulose and d) hemicellulose across two fractions, loss (grey) and treatment residue (black).

residue, while less than 50 % remained in the residue in the other treatments (Fig. 3b). In the frass control with enzymes (F-E), a high degradation of cellulose was demonstrated (Fig. 3c), while the degradation of hemicellulose was lower (Fig. 3d) compared to the other treatments.

The lignin degradation was significantly lower ($p < 0.05$) in the treatment with a cover (PR-HT) (Table 6). The cellulose degradation was significantly higher in the frass control with enzymes.

4. Discussion

4.1. The effect of enzyme and frass additions on treatment efficiency

It was observed that almost all treatments had significantly increased BCE_{VS}, material reduction and larval yield compared to the control, which supports the initial hypothesis that the enzymes and frass improved BCE and material reduction (Table 4). The BCE and material reduction when rearing BSFL in plant residuals were in the same range as what has been reported for other fibrous substrates (*e.g.* grass clippings) [38]. When frass was added alone, it did not increase the dryness

of the residue to the expected extend. This is consistent with findings showing that additions of up to 10 % of rice husk, rice bran or coconut coir dust did not significantly increase the dryness of mixed vegetables during BSFL composting, with 25–50 % required to achieve a noticeably drier residue [21]. The addition of enzymes resulted in wetter residue (Table 2) [29]. However, the addition of frass in combination with enzymes resulted in significantly dried residue (Table 2). Combining frass and enzymes resulted in a treatment (PR-EF) with significantly higher BCE, material reduction and larval yield compared to the control. However, this treatment did not improve process performance significantly compared to the treatment with frass alone (PR-F), which raises questions about the contribution of the enzymes in increasing substrate availability. This finding is in accordance with studies that have reported no significant effect from the addition of 2.5 % yeast [51] or 1.0 % cellulase applied for 72 h [19] to fibrous substrates. The VS input per larva was 0.2 g in both treatments (Table 1), with frass accounting for 35 % of the total VS in the treatment with plant residuals. The process efficiency observed in the frass control (F-C), which was in the same range as reported by Lopes et al. [34] for frass derived from food waste (BCE 9–10 % and material reduction 30–45 %), was not higher than the process efficiency of the treatment with added frass (PR-F). This suggests that the nutrients required for larvae biomass accumulation originated from both the frass and plant residuals (Table 4). When adding enzymes to the frass control (F-E), the BCE_{VS} and larval yield were significantly higher than in any other treatment. This significant increase in process performance demonstrates the enzymes' ability to hydrolyse stable compounds in the frass, thereby enhancing its bioavailability to the larvae. Lopes et al. [34] investigated the impact of different inclusion levels of frass in BSFL composting and reported significantly lower process efficiencies compared to the control when adding frass derived from food waste as a dietary component in the BSFL bioconversion of food waste. This result can be explained by the fact that food is a more suitable substrate for the larvae than frass. In contrast, plant residuals were used in this study, which appear to be better suited to the larvae in combination with frass. The effect of frass addition may vary depending on its origin. Frass derived from plant residuals is unlikely to benefit the larvae, as it typically contains a high fibre content and even less nitrogen than before BSFL composting (Fig. 3a). The proportion of initial cellulose being lost in the frass control with enzymes (F-E) was significantly larger than in the other treatments (Fig. 3c), suggesting that the enzymes hydrolysed the cellulose into forms more readily available to the larvae. The fact that both BCE and larval yield were higher in the F-E treatment further supports the conclusion that enzymatic hydrolysis improved the bioavailability of cellulose (Table 4). Since the aim is to treat plant residuals, the proportion of the substrate replaced by frass, an additive originating from the BSFL bioconversion of pigfeed, should ideally be kept as low as possible to maintain process efficiency from a plant residual waste treatment perspective. However, none of the treatments with plant residuals had a high harvest efficiency, meaning that increasing the frass inclusion rate further could potentially have a positive effect. The treatment that had high harvest efficiency, the highest BCE_{VS} and material reduction of the plant residual treatments, and a trend of increased larval yield compared to the control was the treatment with half volumes of substrate, frass and enzymes and a cover (PR-HT). A lower substrate depth has been demonstrated to result in higher material reduction, BCE and larval survival depending on the substrate TS, which can be achieved with several feeding occasions when using a bulky substrate [33]. This was, however, not the case with the treatment without cover (PR-H), where the lower substrate depth only resulted in an increased BCE_{VS}, but no improvement in the material reduction. However, with a cover (PR-HT), there was a significant increase in material reduction as well as BCE.

4.2. Larval composition and size in the different treatments

The TS in the larval biomass varied greatly across treatments (19 % TS in treatment with double larval density, PR-L2, and 38 % TS in the frass control with added enzymes, F-E). This could be related to differences in larval weight, as these varied greatly, with 49 mg/larva in PR-L2 and 115 mg/larva in F-E (Tables 2 and 4). There was also a difference in the diets in terms of moisture content (10 %-unit difference) and ash content (3 %-unit difference), which could also have had an influence. The different TS content has, to this point, not been discussed greatly in literature, although it has been observed in other studies [8,13,28]. Lu et al. [36] state that differences in the nutritional value of BSFL may be the result of: (1) the developmental stage at which they are sampled, (2) the nutritional composition of the feed, (3) the processing method, or (4) various environmental factors such as temperature, humidity, moisture content and pH.

In this study, a 7 % *w/w* inclusion of frass was used. The addition of frass did not impact the larval weight, as evidenced by the fact that there was no significant difference between the treatments PR-C and PR-F (Table 4). A higher inclusion of frass could potentially result in a significant increase in larval weight, as observed in the frass control with enzymes. Lopes et al. [34] demonstrated that a higher frass inclusion increased the larval weight when BSFL composting food waste; however, this increase was not significant. Food waste alone generates heavier larvae than when larvae are reared on plant residuals (82 mg/larva in pure PR). Since all treatments had the same frass inclusion rate in this study, they must have been influenced by other factors. One potential explanation could be that the cover helped retain the moisture during the first nine days of treatment, thereby creating a more humid microenvironment compared to the uncovered treatments. Bekker et al. (2021) demonstrated that while BSFL were able to develop into prepupae across a moisture range of 45–75 %, the greatest larval biomass was achieved at moisture levels between 65 % and 75 %. However, microbial respiration was substantially higher at lower moisture contents, suggesting increased microbial competition for readily available nutrients in the drier treatments.

4.3. Impact of the nutritional composition of the substrate on process efficiency and larval composition

4.3.1. Nitrogen

The treatments with added frass had higher nitrogen content in the substrate compared to the treatments with no addition of frass. PR-F and PR-EF had a significantly higher initial nitrogen content per larva in the substrate than in the control, but the nitrogen content in the larvae did not differ significantly (Table 6). However, there were no significant differences between the treatments, indicating that the addition of enzymes with frass was not sufficient to increase the nitrogen content in the larval biomass or the BCE_{VS} compared to the treatments with the addition of frass only. Yildirim-Aksoy et al. [54] demonstrated that a 5 % inclusion of frass in the diet of channel catfish resulted in a significantly higher protein efficiency ratio (on a wet weight basis) compared to the control diet consisting of solely grain feed. This agrees with the findings of this study, where a higher protein efficiency ratio (nitrogen converted into protein by a factor of 4.76) was achieved in the treatments with controlled enzyme concentrations and a 7 % inclusion of frass (PR-F, PR-EF, PR-L2, PR-HT and F-E). This suggests that the larvae were able to convert the proteins in the substrate into their biomass more efficiently than in the other treatments and gain more larval biomass, yielding the larval biomass more attractive as a feed ingredient.

4.3.2. Lignocellulosic fraction on plant residuals

The degradation of lignin was significantly smaller in the treatment with a half volume of substrate, frass and enzymes and cover (PR-HT) compared to the other treatments, with the exception of PR with frass (PR-F, Fig. 3b). Xiang et al. [53] investigated lignocellulose-degrading bacteria and CAZymes genes in the substrate, residue and larvae intestines during BSFL composting, and demonstrated that the gene

coding for cellulase and hemicellulose was higher in the larval intestines and the genes for ligninase were higher in the substrate or residue. The reduced lignin degradation in the covered treatment could be the result of the cover limiting the oxygen availability to the microorganisms in the substrate, as most lignin degradation likely occurs in the residue and is carried out by bacterial species that are predominantly or strictly aerobic (*e.g.*, *Corynebacterium* and *Brevibacterium*) [53]. The degradation of lignin was higher in the treatments with added enzymes (Fig. 3b); however, this degradation was not significantly different compared to the control (PR-C). The mass balance of the lignocellulosic fraction in enzyme-only (-E), frass-only (-F) and the control (-C) treatments indicated apparent negative degradation. Since lignin formation during the treatment is highly unlikely, the most plausible explanation is contamination from chitin in the analytical process [9]. Neither cellulose nor hemicellulose degradation differed significantly across treatments, and the results were not sufficient to confirm the hypothesis that the addition of enzymes or frass enhanced lignocellulosic degradation (Fig. 3b-d).

Lignocellulosic degradation was higher for lignin (46.9 % vs 19.6 %) and hemicellulose (35.7 % vs 12.5 %), and lower for cellulose (12.0 % vs 41.5 %) in this study compared to what has been reported by Xiang et al. [53]. The total lignocellulose degradation found in this study (26.5 %) was comparable to that reported for the BSFL composting of domestic biowaste, such as food waste, kitchen waste, and fruit and vegetable waste (26.5 %), over a 14-day period [53]. Reducing substrate particle size has been shown to improve cellulose degradation [42], which could be a possible explanation for the varying cellulose degradation across the different treatments in this study. However, it does not explain why the degradation for lignin and hemicellulose would benefit from only roughly ground substrate and not substrate processed into a slurry [53]. Another possible explanation could be the difference of pH in the beginning of the BSFL treatment, which was lower than 5 for Xiang et al. [53], while it was 7.8 ± 0.4 in this study. In both studies, the pH in the residue was 9 at the end of the treatment. The high pH at the beginning of the treatment could theoretically enhance lignocellulosic degradation, since pH lower than 6 inhibits the lignocellulosic degradation of microorganisms [27].

A significantly higher degradation of cellulose was achieved in the frass control with enzymes (F-E, Fig. 3c), while the degradation of hemicellulose was significantly lower (Fig. 3d) compared to the other treatments.

4.4. Process efficiency from a large-scale treatment perspective

For BSFL composting to be relevant on an industrial scale, certain parameters must reach sufficient efficiency for the waste management to be economically viable [12]. Which parameters are considered most important varies depending on the intended perspective or objective of the treatment. From a waste management perspective, substrate reduction is crucial, as the waste producers are typically charged a fee per tonne of waste. From a protein producer perspective, high BCE_{vs} and short process times are key to maximising value. To fully utilise the value in the larvae, harvest efficiency is important in both scenarios, as larger larvae simplify the harvesting process. In this study, the residue resulting from the enzyme treatments (-E) had low TS, rendering dry separation of larvae and residue not feasible.

From a protein production perspective, the treatment with half volumes of substrate, frass and enzymes and cover (PR-HT) appears to be most suitable. It resulted in significantly higher BCE, material reduction and larval size, and the highest harvest efficiency compared to the control. Although the larval yield was slightly lower than in the treatments with full substrate volume, due to the inclusion of only half the number of larvae, the difference was marginal. From a waste management perspective, the treatment with double larval density (PR-L2) or double concentrations of enzymes (PR-E2) would be most suitable, as the material reduction was high. However, this is only relevant if the larvae are not intended for further use, such as in protein or feed production, which is unlikely even when the focus is on waste management. While the covered treatment (PR-HT) achieved a similar material reduction, it treated only half the amount of waste in the same space and time, potentially increasing costs due to the need for additional covers and labour. Nevertheless, forgoing the potential revenue from the larval biomass could ultimately be even more costly.

5. Conclusions

The hypothesis that enzyme addition would improve BCE was confirmed, as was the hypothesis that frass addition would enhance BCE and material reduction when BSFL composting of horticulturally produced cucumber plant residuals. A high harvest efficiency after BSFL composting was achieved with the treatment with half volumes of substrate, frass and enzymes, half larval density and a foam cover, which improved BCE_{VS} by 130 % and material reduction on a VS basis by 59 % compared to the control. However, the larval yield was not significantly different from the control treatment even though only half the number of larvae were added at the start.

Further studies are needed to assess whether these additions and adaptations are suitable for industrial-scale BSFL composting of plant residuals.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.118061.

Data availability

Data will be made available on request.

References

[1] N.E.A. Basri, N.A. Azman, I.K. Ahmad, F. Suja, N.A.A. Jalil, N.F. Amrul, Potential applications of frass derived from black soldier fly larvae treatment of food waste: a review, Foods 11 (17) (2022) 2664.
[2] S. Bortolini, L.I. Macavei, J.H. Saadoun, G. Foca, A. Ulrici, F. Bernini, D. Malferrari, L. Setti, D. Ronga, L. Maistrello, Hermetia illucens (L.) larvae as chicken manure management tool for circular economy, J. Clean. Prod. 262 (2020) 121289.
[3] M. Carlsson, M. Uldal, Substrathandbok för biogasproduktion, in: RAPPORT U2009, 14, Avfall Sverige, Malmö, 2009.
[4] J.Y.K. Cheng, S.L.H. Chiu, I.M.C. Lo, Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion, Waste Manag. 67 (2017) 315–323.
[5] S. Choi, N. Hassanzadeh, BSFL frass: a novel biofertilizer for improving plant health while minimizing environmental impact, Can. Sci. FAIR J. 2 (2) (2019).
[6] R. Core Team, 2019. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.
[7] K.M. Eggink, I.G. Donoso, J. Dalsgaard, Optimal dietary protein to carbohydrate ratio for black soldier fly (Hermetia illucens) larvae, J. Insects Food Feed 9 (6) (2023) 789–798.
[8] K.M. Eggink, I. Lund, P.B. Pedersen, B.W. Hansen, J. Dalsgaard, Biowaste and by-products as rearing substrates for black soldier fly (Hermetia illucens) larvae: effects on larval body composition and performance, Plos One 17 (9) (2022) e0275213.

[9] M. Finke, Estimate of chitin in raw whole Insects, Zoo. Biol. 26 (2007) 105–115.
[10] Y.S.M. Godliving, Recent advances in pretreatment of lignocellulosic wastes and production of value added products, Afr. J. Biotechnol. 8 (8) (2009) 1398–1415.
[11] M. Gold, J.K. Tomberlin, S. Diener, C. Zurbrugg, A. Mathys, Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review, Waste Manag. 82 (2018) 302–318.
[12] M. Grau, B.M.A. Dortmans, J. Egger, G. Virard, C. Zurbrügg, Modelling the financial viability of centralised and decentralised black soldier fly larvae waste processing units in Surabaya, Indonesia, J. Insects Food Feed 9 (2022) 1–14.
[13] J.B. Guillaume, S. Mezdour, F. Marion-Poll, C. Terrol, P. Schmidely, Asymptotic estimated digestibility, a new indicator of black soldier fly (Hermetia illucens) conversion efficiency in relation to larval density, J. Insects Food Feed 9 (7) (2023) 893–906.
[14] A. Hansson, K. Christensen, Biogas ger energi till ekologiskt lantbruk, Jordbruksverket, 2005.
[15] A. Isibika, B. Vinneras, O. Kibazohi, C. Zurbrugg, C. Lalander, Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae, Waste Manag. 100 (2019) 151–160.
[16] A. Isibika, B. Vinnerås, O. Kibazohi, C. Zurbrügg, C. Lalander, Co-composting of banana peel and orange peel waste with fish waste to improve conversion by black soldier fly (Hermetia illucens (L.), Diptera: Stratiomyidae) larvae, J. Clean. Prod. 318 (2021) 128570.
[17] S. Ites, S. Smetana, S. Toepfl, V. Heinz, Modularity of insect production and processing as a path to efficient and sustainable food waste treatment, J. Clean. Prod. 248 (2020) 119248.
[18] R.H. Janssen, J.-P. Vincken, L.A.M. van den Broek, V. Fogliano, C.M.M. Lakemond, Nitrogen-to-protein conversion factors for three edible insects: tenebrio molitor, alphitobius diaperinus, and hermetia illucens, J. Agric. Food Chem. 65 (11) (2017) 2275–2278.
[19] J. Jing Lim, C. Seng Liew, R. Raksasat, Z.M.A. Merican, K. Kiatkittipong, E. Alaaeldin Abdelfattah, M. Mohamad, M.J.K. Bashir, S. Karabo Obed Ntwampe, J. W. Lim, Cellulase pretreated palm decanter cake for feeding of black soldier fly larvae in triggering bioaccumulation of protein and lipid into biodiesel productions, Sustain. Energy Technol. Assess. 53 (2022) 102485.
[20] V. Joshi, M. Parmar, N. Rana, Purification, characterization of pectinase produced from apple pomace and its evaluation in the fruit juice extraction and clarification, J. BIOTECHNOL. J. BIOTECHNOL 136 (2008).
[21] P. Laksanawimol, P. Anukun, A. Thancharoen, Use of different dry materials to control the moisture in a black soldier fly rearing substrate, Peerj 12 (2024).
[22] C. Lalander, S. Diener, C. Zurbrugg, B. Vinneras, Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*), J. Clean. Prod. 208 (2019) 211–219.
[23] C. Lalander, E. Ermolaev, V. Wiklicky, B. Vinnerås, Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content, Sci. Total Environ. 729 (2020) 138968.
[24] C. Lalander, A. Nordberg, B. Vinneras, A comparison in product-value potential in four treatment strategies for food waste and faeces - assessing composting, fly larvae composting and anaerobic digestion, Gcb Bioenergy 10 (2) (2018) 84–91.
[25] C. Lalander, J. Senecal, M. Gros Calvo, L. Ahrens, S. Josefsson, K. Wiberg, B. Vinnerås, Fate of pharmaceuticals and pesticides in fly larvae composting, Sci. Total Environ. 565 (2016) 279–286.
[26] A. Lemme, P. Klüber, Rethinking amino acid nutrition of black soldier fly larvae (hermetia illucens) based on insights from an amino acid reduction trial, Insects 15 (11) (2024) 862.
[27] M.M. Li, R.R. White, L.L. Guan, L. Harthan, M.D. Hanigan, Metatranscriptomic analyses reveal ruminal pH regulates fiber degradation and fermentation by shifting the microbial community and gene expression of carbohydrate-active enzymes, Anim. Micro 3 (1) (2021) 32.
[28] L. Lindberg, E. Ermolaev, B. Vinnerås, C. Lalander, Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment, J. Clean. Prod. 338 (2022).
[29] L. Lindberg, B. Vinnerås, C. Lalander, Process efficiency in relation to enzyme pre-treatment duration in black soldier fly larvae composting, Waste Manag. 137 (2022) 121–127.
[30] Z. Liu, M. Minor, P.C.H. Morel, A.J. Najar-Rodriguez, Bioconversion of Three Organic Wastes by Black Soldier Fly (Diptera: Stratiomyidae) Larvae, Environ. Entomol. 47 (6) (2018) 1609–1617.
[31] Löfkvist, K., Hansson, T., Svensson, S.A., 2009. Förluster av växtskyddsmedel till omgivande mark och vatten vid användning i svenska växthus - en genomgång av möjliga riskmoment, Rapport 2009:6. Faculty of Landscape Architecture, Horticulture and Crop Production Sciences.
[32] I.G. Lopes, C. Lalander, R.M. Vidotti, B. Vinneras, Using Hermetia illucens larvae to process biowaste from aquaculture production, J. Clean. Prod. 251 (2020).
[33] I.G. Lopes, V. Wiklicky, E. Ermolaev, C. Lalander, Dynamics of black soldier fly larvae composting – Impact of substrate properties and rearing conditions on process efficiency, Waste Manag. 172 (2023) 25–32.
[34] I.G. Lopes, V. Wiklicky, B. Vinnerås, J.W.H. Yong, C. Lalander, Recirculating frass from food waste bioconversion using black soldier fly larvae: Impacts on process efficiency and product quality, J. Environ. Manag. 366 (2024) 121869.
[35] I.G. Lopes, J.W.H. Yong, C. Lalander, Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives, Waste Manag. 142 (2022) 65–76.
[36] S. Lu, N. Taethaisong, W. Meethip, J. Surakhunthod, B. Sinpru, T. Sroichak, P. Archa, S. Thongpea, S. Paengkoum, P. Paengkoum, Nutritional composition of black soldier fly larvae (hermetia illucens l.) and its potential uses as alternative protein sources in animal diets: a review, Insects 13 (9) (2022).
[37] N. Meijer, T. de Rijk, J.J.A. van Loon, L. Zoet, H.J. van der Fels-Klerx, Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (Hermetia illucens) larvae, Plos One 16 (4) (2021) e0249362.
[38] D.A. Peguero, M. Gold, A. Endara, M. Niu, C. Zurbrügg, A. Mathys, Evaluation of ammonia pretreatment of four fibrous biowastes and its effect on black soldier fly larvae rearing performance, Waste Manag. 160 (2023) 123–134.
[39] R. Raksasat, J.W. Lim, W. Kiatkittipong, K. Kiatkittipong, Y.C. Ho, M.K. Lam, C. Font-Palma, H.F. Mohd Zaid, C.K. Cheng, A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources, Environ. Pollut. 267 (2020) 115488.
[40] P. Saroj, P. Manasa, N. Korrapati, Biochemical characterization of thermostable carboxymethyl cellulase and β-glucosidase from aspergillus fumigatus JCM 10253, Appl. Biochem. Biotechnol. 194 (2022) 1–25.
[41] D. Sarpong, S. Oduro-Kwarteng, S.F. Gyasi, R. Buamah, E. Donkor, E. Awuah, M. K. Baah, Biodegradation by composting of municipal organic solid waste into organic fertilizer using the black soldier fly (Hermetia illucens) (Diptera: Stratiomyidae) larvae, Int. J. Recycl. Org. Waste Agric. 8 (1) (2019) 45–54.
[42] S.K. Sharma, I.M. Mishra, M.P. Sharma, J.S. Saini, Effect of particle size on biogas generation from biomass residues, Biomass 17 (4) (1988) 251–263.
[43] S. Song, A.W.L. Ee, J.K.N. Tan, J.C. Cheong, Z. Chiam, S. Arora, W.N. Lam, H.T. W. Tan, Upcycling food waste using black soldier fly larvae: Effects of further composting on frass quality, fertilising effect and its global warming potential, J. Clean. Prod. 288 (2021) 125664.
[44] J. Suckling, A. Druckman, R. Small, F. Cecelja, M. Bussemaker, Supply chain optimization and analysis of Hermetia illucens (black soldier fly) bioconversion of surplus foodstuffs, J. Clean. Prod. 321 (2021) 128711.
[45] K.C. Surendra, J.K. Tomberlin, A. van Huis, J.A. Cammack, L.-H.L. Heckmann, S. K. Khanal, Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.)) (Diptera: Stratiomyidae) (BSF), Waste Manag. 117 (2020) 58–80.
[46] Y. Tadele, N. Amha, Use of different non protein nitrogen sources in ruminant nutrition: a review. International knowledge sharing plattform IISTE, Adv. Life Sci. Technol. 26 (2015).
[47] W. Theron, Faculty of Engineering. Stellenbosch University, Stellenbosch University, Steam. Pretreat. Wheat Straw Sugarcane Bagasse Prod. Feed Black Soldier Fly. Larvae (2022).
[48] W. Theron, G.M. Teke, D. Diedericks, E. Van Rensburg, J.F. Görgens, Steam pre-treatment of sugarcane bagasse and wheat straw as a cleaner feedstock for black soldier fly larvae rearing, J. Insects Food Feed 10 (4) (2023) 585–599.
[49] C. Vahlberg, E. Nordell, L. Wiberg, A. Schnürer, Method for correction of VFA loss in determination of dry matter in biomass, Sven. Gastek. Cent. AB Malmö Swed. (2013) 53.
[50] P.J. Van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (10) (1991) 3583–3597.
[51] C.Y. Wong, M.N. Mohd Aris, H. Daud, M.K. Lam, C.S. Yong, H. Abu Hasan, S. Chong, P.L. Show, O.D. Hajoeningtijas, Y.C. Ho, P.S. Goh, H. Kausarian, G.-T. Pan, J.W. Lim, In-situ yeast fermentation to enhance bioconversion of coconut endosperm waste into larval biomass of hermetia illucens: statistical augmentation of larval lipid content, Sustainability 12 (4) (2020) 1558.
[52] C.-Y. Wong, S.-S. Rosli, Y. Uemura, Y.C. Ho, A. Leejeerajumnean, W. Kiatkittipong, C.-K. Cheng, M.-K. Lam, J.-W. Lim, Potential protein and biodiesel sources from black soldier fly larvae: insights of larval harvesting instar and fermented feeding medium, Energies (2019).
[53] F. Xiang, Q. Zhang, X. Xu, Z. Zhang, Black soldier fly larvae recruit functional microbiota into the intestines and residues to promote lignocellulosic degradation in domestic biodegradable waste, Environ. Pollut. 340 (2024) 122676.
[54] M. Yildirim-Aksoy, R. Eljack, C. Schrimsher, B.H. Beck, Use of dietary frass from black soldier fly larvae, Hermetia illucens, in hybrid tilapia (Nile x Mozambique, Oreocromis niloticus x O. mozambique) diets improves growth and resistance to bacterial diseases, Aquac. Rep. 17 (2020) 100373.

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Black soldier fly larvae (BSFL) composting was evaluated for plant-based waste using various pre-treatments and co-substrate additions. Fungal and enzymatic treatments improved material reduction, though effects on biomass conversion efficiency (BCE) were substrate- and scale-dependent. Enzyme and frass additions enhanced BCE in small-scale systems. Methane and nitrous oxide emissions were low. Despite limited larval yields, BSFL composting reduced waste volume and degraded pesticides, suggesting its suitability for fibrous residues where environmental benefits may outweigh the larval biomass production.

Lovisa Lindberg received her graduate education at the Department of Energy and Technology, Swedish University of Agricultural Sciences (SLU), following the award of her undergraduate degree from Uppsala University.

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