Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2022:30
Faculty of Natural Resources and Agricultural Sciences

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DOCTORAL THESIS
Uppsala 2022

Acta Universitatis agriculturae Sueciae
2022:30

Cover: Black soldier fly larvae treatment of fruit waste
Photo: Alice and Irene Isibika

ISSN 1652-6880
ISBN (print version) 978-91-7760-935-3
ISBN (electronic version) 978-91-7760-936-0

Uppsala
Print: SLU Service/Repro, Uppsala 2022

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Abstract

Black soldier fly larvae (BSFL) composting is a biowaste treatment that converts biomass into valuable animal protein and fertiliser, but low protein content and complex molecules (*e.g.* fibre) in substrate reduce BSFL composting efficiency. This thesis evaluated the impact of using pre-treatments and blending substrates on BSFL process efficiency. The feasibility of using BSFL composting to treat available food industry waste streams in Tanzania and physical-chemical characteristics of these wastes were also assessed.

The pre-treatments tested were biological, chemical, heat-based, biochemical and combinations of these, while blending involved mixing banana and orange peels with fish waste. All pre-treatments except heating and all substrate blends improved BSFL conversion efficiency in composting. The conversion efficiency was reduced by high concentrations of tannins, phenols, carbohydrates, fibre and fat, but increased by high protein and nitrogen concentrations.

The available food industry waste from single companies in Tanzania, in quantities of ~100,000-1,000,000 kg y^{-1}, was not sufficiently nutritionally balanced as a standalone feedstock for BSFL composting. However, with pre-treatment and substrate blending, BSFL composting could be successfully implemented to valorise biowaste streams in cities in low and middle-income countries such as Tanzania and other similar settings globally.

Keywords: *Hermetia*, *Trichoderma*, *Rhizopus*, bacteria, heat, chemical, enzyme

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Förbehandling och substratblandning för ökad processeffektivitet vid fluglarvskompostering av livsmedeindustriavfall

Sammanfattning

Teknologin för omvandling av bioavfall till djurfoder och gödselmedel med den Amerikanska vapenflugan (*Hermetia illucens*) är en biologisk behandling, där bioavfall med lågt proteininnehåll och högt innehåll av komplexa, svårnedbrytbara, molekyler som fibrer sänker processens effektivitet. I denna studie utvärderades påverkan på processeffektiviteten av förbehandlingar och av olika substratblandningar. Vidare undersöktes tillgången av olika livsmedelsindustriavfall i Tanzania. Dessa jämfördes med avseende på avfallens potential att användas i fluglarvskomposteringen i relation till tillgänglighet samt näringssammansättning. De förbehandlingar som utvärderades var: biologisk, kemisk, värmebaserad, biokemisk samt kombinationer av behandlingarna. Vidare så utvärderades påverkan på fluglarvskomposteringseffektiviteten av att blanda bananskal, apelsinskal och fiskavfall. Alla förbehandlingar, utom värmebehandlingen, och alla substratblandningar ökade processeffektiviteten. Höjda koncentrationer av tanniner, fenoler kolhydrater, fibrer, och fett hade negativ påverkan på processen. Ökad koncentration av protein eller kväve påverkade däremot processen positivt. I Tanzania utvärderades flera industrier med en avfallsproduktion på mer än 100 ton livsmedelsindustriavfall per år. De tillgängliga avfallsströmmarnas näringssammansättning var obalanserade för att ensamma utgöra lämpliga substrat till fluglarverna. Förslag att förbättra processen genom att förbehandla avfallet eller blanda olika bioavfallsströmmarföreslås för att uppnå ett mer näringsmässigt balanserat substrat. Genom dessa metoder kan avfallsströmmar med lågt värde omvandlas till larvbiomassa och på så sätt ge bioavfallet ett värde, för effektivare hantering av dessa avfallsströmmar i städer i låg-, och medelinkomstländer med liknande förutsättningar som Tanzania.

Keywords: *Hermetia, Trichoderma, Rhizopus*, bacteria, heat, chemical, enzyme

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Dedication

To my kids, Adrian Mahenda, Lucy Qwihaya and Aidan Masuka

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. 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 Management* 100, 151-60.

II. Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C. & Lalander C. (2022). Pre-treatment of banana peels and orange peels to improve composting process efficiency by larvae of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae). (Manuscript)

III. 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.

IV. Isibika, A., Simha, P., Vinnerås, B., Zurbrügg, C., Kibazohi, O. & Lalander, C. (2022). Food industry waste, an opportunity for black soldier fly larvae protein production in Tanzania. (Manuscript).

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The contribution of Alice Isibika to the papers included in this thesis was as follows:

I. Planned and designed the experiments together with Lalander and Vinnerås. Performed the experiments. Carried out the formal data analysis with Lalander and inputs from Vinnerås. Wrote the paper, with revisions by the co-authors.

II. Planned and designed the experiments together with Lalander and Vinnerås. Performed the experiments. Carried out the formal data analysis with revisions and inputs from Lalander and Vinnerås. Wrote the paper, with revisions by the co-authors.

III. Planned and designed the experiments together with Lalander, Zurbrügg and Vinnerås. Performed the experiments. Carried out the formal data analysis, with revisions and inputs from Lalander and Vinnerås. Wrote the paper, with revisions by the co-authors.

IV. Planned and designed the experiments together with Lalander and Simha. Carried out data collection in the field. Carried out the formal data analysis and wrote the paper, with inputs from the co-authors.

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Abbreviations

BCE	Biomass conversion efficiency
BOP	Banana and orange peel mixture
BP	Banana peel
BSF	Black soldier fly
BSFL	Black soldier fly larvae
DM	Dry matter
OP	Orange peel
Red	Reduction efficiency
VS	Volatile solids
WW	Wet weight

1. Introduction

The global population is predicted to increase to 9.7 billion by 2050 (UN, 2019), and waste generation per capita is also expected to increase (Chen et al., 2020). These two factors in combination are a concern, since global municipal solid waste generation is predicted to increase from 2.0 billion tonnes to 3.4 billion tonnes per year, with the main increase occurring in low-income countries in sub-Saharan Africa and South Asia (Kaza et al., 2018). Increasing volumes of municipal solid waste in low-income countries are challenging, as these countries already struggle to treat their waste adequately and current solid waste management practices involve open dumping, uncontrolled burning and disposal in open dumps or landfills (Ferronato and Torretta, 2019). These practices lead to environmental pollution, such as greenhouse gas emissions and eutrophication through leaching of plant nutrients into surface water bodies, and a risk of disease transmission, resulting in risks to the environment and to human health (Ferronato and Torretta, 2019). Municipalities in most cities in low-income countries are responsible for solid waste management, but they commonly have insufficient capacity and resources to handle large amounts of waste (Agamuthu Pariatamby et al., 2019). The informal and private sector has thus become involved in solid waste management, aiming for profit making (Aryampa et al., 2019).

The biodegradable waste (biowaste) originates from households or from production, post-harvest handling, storage and industrial or domestic processing of food products such as fruit juice, fish and bread (Gustavsson et al., 2011). Municipal biowaste is usually mixed with other waste fractions (paper, metals, plastics *etc*.), making it difficult to extract the valuable resources, such as nutrients (*e.g*. proteins, carbohydrates, fibre, lipids), plant nutrients (nitrogen, phosphorus) and energy (Katinas et al., 2019, Torres-

León et al., 2018), contained in the waste (Hettiarachchi et al., 2018). One biowaste stream that often is not mixed with other waste fractions is food industry waste. It is therefore a good biowaste stream to start with for assessing technologies for resource recovery. However, in resource recovery technologies of biowaste, the cost of treatment often exceeds the value of the products generated (Lohri et al., 2014). There is therefore a need to establish adequate and sustainable management solutions that can increase the value of biowaste through generation of valuable products from the treatment step. Increasing the value of biowaste could act as an incentive to segregate biowaste at source, which would improve the current biowaste management system.

Composting performed by the larvae of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) (BSF) is an emerging technology that has promising potential to valorise biowaste (Čičková et al., 2015). This treatment reduces the volume of the biowaste, while the resources biowaste contains are used to produce valuable larval biomass of BSF larvae (BSFL) and treatment residues (frass) (Čičková et al., 2015, Lalander et al., 2018). The protein in BSFL biomass can be used in animal feed (Henry et al., 2015, Surendra et al., 2016), the lipids can be used in animal feed and/or biodiesel production (Heuel et al., 2021, Ishak and Kamari, 2019) and the BSFL frass can be used as organic fertiliser (Setti et al., 2019, Beesigamukama et al., 2020a, Chiam et al., 2021). The main revenue from BSFL composting comes from the BSFL biomass (Lalander et al., 2018), so maximising the amount of BSFL biomass generated is recommended to make the treatment more economically viable.

Black soldier fly larvae have a voracious appetite and can feed and grow on a wide range of waste substrates, including food waste (Lalander et al., 2019), dairy manure (Myers et al., 2008), rice straw (Liu et al., 2021), maize straw (Gao et al., 2019), faecal sludge (Nyakeri et al., 2019), aquaculture waste (Lopes et al., 2020) and abattoir waste (Silva et al., 2019). Even though BSFL can feed on many different waste substrates, the nutritional composition of the substrate influences the process efficiency (Ewald et al., 2020, Kawasaki et al., 2019, Palma et al., 2019). Food industry biowaste streams are often single stream biowaste of *e.g.* peels fruits or vegetables, which are low in protein and high in fibre (Lalander et al., 2019, Nguyen et al., 2013), a nutritional composition that does not match the nutritional

requirements of BSFL (Lalander et al., 2019, Gold et al., 2020, Nguyen et al., 2013).

Methods to improve the nutrient availability and digestibility of waste substrates with imbalanced ratios of protein, carbohydrates, fibre and fat prior to BSFL composting have been investigated. These include application of a pre-treatment (Yu et al., 2011, Wong et al., 2020b, Franks et al., 2021) and blending of substrates (Nyakeri et al., 2019, Lopes et al., 2020, Lalander et al., 2019).

The effectiveness of different pre-treatments and of substrate blending using different types of biowaste in fly larvae composting has not been widely explored. Thus, more research is needed to evaluate and identify effective pre-treatments and substrate blends in terms of overall efficiency for composting, pre-treatment concentrations, pre-treatment time and substrate blend ratios based on different types of waste substrates. According to reports on available biowaste streams in cities in sub-Saharan African countries (Kaza et al., 2018), including countries in East Africa (Aryampa et al., 2019, Mbuligwe and Kaseva, 2006), large volumes of sorted agricultural waste and food industry waste are available for waste entrepreneurs to utilise. However, little is known about the actual feasibility of implementing BSFL treatment technology in these cities, especially treatment based on locally available waste streams.

2. Objective and thesis structure

2.1 Overall aim

The overall aim in this thesis was to develop, assess and compare pre-treatments and substrate blending to improve BSFL composting efficiency of food industry waste, and to assess the availability and suitability of food industry wastes for BSFL composting in low- and middle-income country settings.

Specific objectives were to:

- Evaluate and compare microbial, heat, chemical and enzyme pre-treatment methods to improve the nutritional composition of fruit industry waste (banana and orange peels) for BSFL composting (Papers I and II).
- Evaluate the impact of blending fruit wastes (banana and orange peels) and of fish waste addition as a protein source in fruit industry waste blends (banana and orange peels) on nutritional composition and BSFL composting efficiency (Paper III).
- Assess the availability and suitability of selected food industry waste in Tanzania for potential implementation of BSFL composting (Paper IV).

2.2 Structure of the thesis

This thesis is based on the studies described in Papers I-IV presented in schematic diagram in Figure 1. Papers I and II evaluated the effectiveness of using different types of pre-treatments with fungi, ammonia, heat and enzymes to improve the degradation of complex molecules such as fibre in single and mixed fractions of fruit waste to improve BSFL composting conversion efficiency. Paper III examined the possibility of blending different substrates to achieve better substrate composition in terms of carbohydrates, fibre and protein, and thereby improve BSFL composting conversion efficiency. Paper IV evaluated the feasibility of implementing BSFL treatment technology based on locally available food industry waste streams in Tanzania and their specific physical-chemical characteristics, using the process knowledge obtained in Papers I-III.

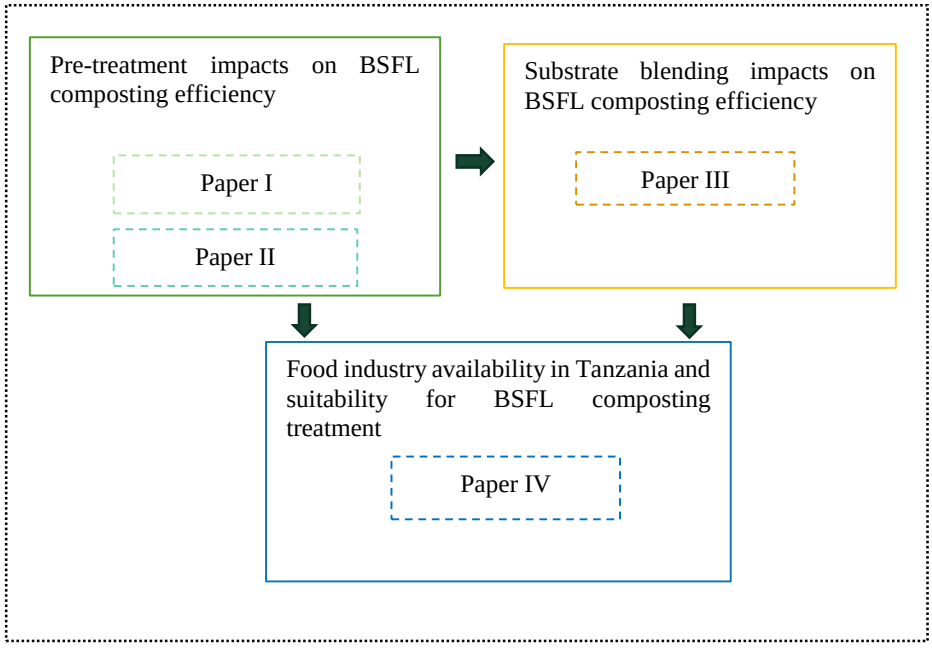

Figure 1. Structure of the research on black soldier fly larvae (BSFL) composting of biowaste performed in this thesis, involving: pre-treatments (Papers I and II), substrate blending (Paper III) and assessment of availability and suitability of food industry biowaste in Tanzania for BSFL composting treatment (Paper IV).

3. Background

3.1 Life cycle of black soldier fly *(Hermetia illucens* L.)

Black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae) is distributed mainly in tropical and subtropical regions of the world (Sheppard, 1994). It is originally native to North and South America (Callan, 1974), and is a common coloniser of organic wastes. Black soldier flies have five main stages in their life cycle, namely egg, larva, prepupa, pupa and adult. The adult fly lives for around 1-2 weeks, relying on body fat reserves acquired during the larval stage, which passes through six instars (da Silva and Hesselberg, 2020, Surendra et al., 2020). The adult flies do not feed, but only drink water, and BSF is therefore considered a non-pest and non-vector fly species (Tomberlin et al., 2002). In natural environments, the female flies lay 400-800 eggs close to decomposing organic materials, such as piles of manure, fruit and vegetable wastes and dead animals (Sheppard, 1994). After approximately four days at ~27 °C, the eggs hatch into hatchlings that begin exploring and eating food substrates nearby. In the final larval stage, the prepupal stage, the larvae stop feeding (Barros-Cordeiro et al., 2014, Myers et al., 2008). The prepupae migrate to dry areas, pupate and metamorphose into adult flies (Sheppard, 1994). The longest part of the BSF lifecycle is the larval stage, while the full life can stretch between around 40 and 150 days, depending on BSF rearing conditions (Popa and Green, 2012, Purkayastha and Sarkar, 2021). Optimal BSF rearing conditions vary depending on BSF development stage. Rearing involves conditioning and monitoring of optimal temperature (27 °C), relative humidity (~75%), light and nutrition requirements (Sheppard et al., 2002, Tomberlin and Sheppard, 2002,

Tomberlin et al., 2009, Tomberlin et al., 2002). For example, the fly environment needs to have sunlight, temperature around 28 °C and 75% relative humidity for successful mating. The pupae need a warm, dry and dark environment to turn into adult flies (Dortmans et al., 2017, Sheppard et al., 2002). Nutritional requirements are only considered during the larval stage, as the prepupae, pupae and flies do not feed (Čičková et al., 2015). Under optimal conditions in terms of substrate composition and rearing temperature, the larvae take around 14-16 days to develop into pre-pupae stage, in which the larvae empty their gut and crawl out of the substrate searching for a dry place (Dortmans et al., 2017, Purkayastha and Sarkar, 2021, Holmes et al., 2013).

3.2 Role of BSFL in waste management

Composting by BSFL is a treatment option for biowaste with potential to value and recycle nutrients that would otherwise not be reused, so it enables closing of the nutrient loop (Purkayastha and Sarkar, 2021, Lohri et al., 2017). Recycling of biowaste through BSFL composting also has potential to reduce the incidence of open dumping and landfilling of wastes, and is thus likely to alleviate some of the environmental problems, such as greenhouse gas emissions, eutrophication and public health risks, associated with inadequate waste management and disposal methods (Ferronato and Torretta, 2019). In addition, BSFL composting has the potential to convert biowaste into valuable marketable products, generating additional income that can cover the costs of waste management (Lohri et al., 2017).

3.2.1 BSFL treatment products

BSFL biomass

The larval biomass has a crude protein concentration of around 40%$_{DM}$, but the protein concentration varies somewhat (+/-5 percentage points) depending on the substrate and on larval size and age (Do et al., 2020, Lalander et al., 2019). The deviance in protein concentration based on age has been attributed to non-protein nitrogen in chitin compounds in the larvae giving a misleading protein value (Do et al., 2020, Lalander et al., 2019, Janssen et al., 2017). No conversion factor has identified for the protein

concentration in BSFL, but it has been suggested that a conversion factor of 4.76 should be applied, and not the commonly used value of 6.25 for generic protein (Janssen et al., 2017).

Varying fatty acid composition in the substrate has been demonstrated to affect the fatty concentration in the larvae, and thus BSFL biomass fat concentration varies greatly (+/-15 percentage points) with the substrate (Ewald *et al*., 2020). Moreover, the fat concentration in BSFL has been demonstrated to predominantly comprise short-chain saturated fatty acids (Meneguz et al., 2018, Spranghers et al., 2017, Ewald et al., 2020). BSFL biomass has been demonstrated to be a suitable animal feed alternative to the soybean meal and fish meal used in production of fish and in other animal diets due to their high protein and lipid concentration (Spranghers et al., 2017, Henry et al., 2015). Kawasaki et al. (2019) reported increased quality of products, such as increased eggshell thickness, egg yolk and egg albumin, in laying hens fed BSFL biomass. Other components of BSFL biomass, such as fats and chitin, have potential uses in industrial production of other valuable products, such as biodiesel and pharmaceuticals (Ishak and Kamari, 2019, Waśko et al., 2016).

Treatment residues

The BSFL composting process also results in another by-product, namely the treatment residues. These consist of larvae manure (called frass), non-degraded fractions of the waste and larval skins (Müller et al., 2017). The treatment residues have the potential to serve as a nutritious organic fertiliser, due to their functional similarity to existing commercial fertilisers (Tan et al., 2021, Beesigamukama et al., 2020b). The treatment residues could alternatively be used as a raw material in other processes, such as anaerobic digestion to produce biogas (Lalander et al., 2018).

3.2.2 Large-scale BSFL waste treatment

Large-scale BSFL composting facilities are already in use and there are several facilities treating more than 200 tonnes of waste per day. These generate high outputs of BSFL biomass, which is mainly used for protein production (da Silva and Hesselberg, 2020). Some countries, *e.g*. Spain,

USA, China, Canada, Germany, The Netherlands, France, Tanzania and Ireland, are already operating BSFL composting in large-scale production (Fowles and Nansen, 2020). The established companies, *e.g*. Protix, Ynsect, Hexafly, Enterra Feed Corporation, EnviroFlight, mostly aim for production of BSFL biomass for commercial purposes (Fowles and Nansen, 2020). Establishment of smaller companies, treating more than five tonnes of waste per day, is progressing in *e.g*. Tanzania and Kenya (Biobuu) (Recyclers, 2021). Unfortunately, these companies have not been publicly open about their operational procedure and financial aspects, mainly for reasons associated with maintaining competitive advantages of the business (Joly and Nikiema, 2019).

3.3 Potential environmental and health risks associated with BSFL composting

Implementing BSFL composting of biowaste as part of a circular economy to close the loop of nutrients and energy in the food system comes with the potential safety risk of accumulating toxic contaminants present in the food chain, such as residues of pharmaceuticals and pesticides, mycotoxins, bacterial toxins, heavy metals and pathogens (Lievens et al., 2021, Surendra et al., 2020). These toxic contaminants are a concern since they can cause harm through formation of stable toxic compounds that impair biochemical reactions and body functions of plant and animals when ingested (Yadav et al., 2020, Singh et al., 2017).

3.3.1 Metals

According to Purschke *et al.* (2017), feed conversion efficiency and growth of BSFL are reduced in the presence of metals. In that study, larvae fed corn standard substrate spiked with heavy metals (As, Cd, Pb, Hg, Cr, Ni) were found to be smaller than control larvae (45 and 74.8 mg $larva^{-1}$, respectively) and BSFL composting efficiency was negatively influenced by heavy metals, with significant bioaccumulation of lead (Pb) and cadmium (Cd) (Purschke et al., 2017). High substrate concentrations of lead and cadmium and their bioaccumulation in BSFL have also been associated with longer development time of larvae compared with a control (Diener et al., 2015, Van der Fels-Klerx et al., 2016). In contrast, Wu *et al.* (2020) observed no inhibition of larval weight gain when BSFL were exposed to increasing

concentrations of cadmium (100, 200, 400 and 800 mg Cd kg_{DM}^{-1} wheat bran) and copper (Cu) (10, 20, 40 and 80 mg Cu kg_{DM}^{-1} wheat bran). However, they observed substantial alteration and reduction (32-99%) in dominant families in larval gut microbiota, especially on high exposure of the BSFL (80 mg Cu kg_{DM}^{-1} wheat or 800 mg Cd kg^{-1} kg_{DM}^{-1} wheat). Thus heavy metals contained in waste substrates have the ability to reduce the efficiency of BSFL composting in terms of BSFL final growth weight, development time and the diversity and function of the gut microbiota.

Accumulation of heavy metals such as such as lead and cadmium in BSFL products used for feed and food is also a concern, since these metals have adverse effects on animal and human health when consumed in excess (Proc et al., 2020, Bessa et al., 2020, Wang and Shelomi, 2017). Moreover, high exposure to metals such as copper has been reported to enhance the abundance of pathogens such as *Escherichia coli, Enterococcus* spp. and *Salmonella* spp. in BSFL gut microbiota (Wu et al., 2021). Therefore, it is important to monitor and protect waste substrates used in the production of BSFL from heavy metal contamination at source, in order avoid the risks associated with heavy metals in the food chain.

3.3.2 Pathogens

Composting by BSFL is reported to reduce pathogenic microbial contaminants such as *Escherichia coli and Salmonella* spp. (Lalander et al., 2015, Erickson et al., 2004). However, some studies have found presence of oocysts or eggs of parasites such as *Eimeria tenella, E. nieschulzi* and *Ascaris suum* and pathogens such as *Salmonella* spp., *Enterococcus* microbes and *Bacillus cereus* in the larvae and treatment residues (Lalander et al., 2013, Wynants et al., 2019, Muller et al., 2019, Swinscoe et al., 2020). The presence of microbial contaminants in BSFL products intended for use in production of feed and food poses a risk of disease transmission to humans and animals. Several studies have thus recommended further processing of the feedstock, BSFL biomass and treatment residues, using treatments such as ammonia sanitisation (Lalander et al., 2013, Muller et al., 2019), drying (Lalander et al., 2013, Erickson et al., 2004), heating, acidifying, ultraviolet (UV) irradiation, microwaving (Wang and Shelomi, 2017, Muller et al., 2019) or blanching (Bessa et al., 2020) to eliminate microbial contaminants from the food production chain. For example, blanching at 100 °C for 60 s of BSFL biomass obtained from

seaweed substrate has been found to result in a 3-log reduction in *E. coli* and complete elimination of *Salmonella* spp. and *Listeria* spp. (Bessa et al., 2020).

3.3.3 Mycotoxins and chemical contaminants

Mycotoxins (*e.g*. aflatoxins and deoxynivalenol) (Gulsunoglu et al., 2019, Meijer et al., 2019) and chemical contaminants from pesticides (*e.g*. chlorpyrifos, chlorpyrifos-methyl, pirimiphos-methyl, azoxystrobin, propoxur, spinosad and propiconazole) (Lalander et al., 2016, Purschke et al., 2017, Meijer et al., 2021), pharmaceuticals (*e.g*. carbamazepine, roxithromycin, trimethoprim) (Lalander et al., 2016) and polycyclic aromatic hydrocarbons (*e.g*. naphthalene, fluorene, phenanthrene, pyrene) known for their carcinogenic, mutagenic and toxic properties (Fan et al., 2020), do not seem to affect BSFL composting process efficiency or accumulate in the products. However, while the studies cited above have demonstrated the ability of BSFL to degrade and eliminate or reduce undesired compounds, the actual elimination pathways and mechanisms are still not fully understood (Lievens et al., 2021). It has been speculated that the degradation, reduction and elimination of chemical contaminants is performed by the BSFL digestive system and/or associated gut microorganisms, and/or by other microorganisms contained in the waste substrate (Lievens et al., 2021). More efforts in identifying the mechanisms for degradation and excretion of chemical compounds from substrate and BSFL have been recommended, to help maximise the additional benefit of BSFL composting in decontaminating waste substrates while producing safe products (Lievens et al., 2021, Fan et al., 2020).

3.3.4 Environmental impact assessments

It has been shown that the environmental impact of BSFL composting in terms of resource utilisation, direct greenhouse gas emissions and global warming potential is largely dependent on the type of diet, the rearing method and the processing methods used for products (Bosch et al., 2019, Smetana et al., 2019, Ermolaev et al., 2019, Mertenat et al., 2019). It has been demonstrated that BSFL composting with food and feed production usually has a lower environmental impact than other comparable protein production systems, such as use of fish meal. The impact is reported to be lower

especially if the feed substrate cannot be consumed by humans or cannot be directly utilised in animal feeds, except for insects used for human consumption (Smetana et al., 2019, Bosch et al., 2019). Concerns arise mainly regarding the type of substrates fed and on emissions and energy input requirements in the BSFL treatment facility, to maintain optimal environmental conditions for rearing. Product processing steps such as drying the BSFL biomass and post-processing of the frass have greater potential to increase the negative environmental impacts (Smetana et al., 2019, Feng et al., 2019, Song et al., 2021, Mertenat et al., 2019). Therefore, more research is required to identify available diets, efficient and cost-effective BSF rearing methods and BSFL product processing methods with predictable and reliable outputs that have lower impacts on the environment in terms of resource use, pollution and greenhouse gas emissions. This is especially important in large-scale implementation of BSFL composting.

3.4 BSFL composting treatment process

3.4.1 Substrate feeding process

Black solider fly larvae ingest feed substrates through their mouthparts, which are capable of grabbing food particles in a sweeping action and grinding them into smaller particles (Bruno et al., 2020, Kim et al., 2010). For BSFL to ingest and degrade feed substrate efficiently, the substrate depth should not exceed 5 cm and composting should take place in a well-ventilated environment (Dortmans et al., 2017). Thick layers of feed substrate in feeding containers reduce the ability of BSFL to obtain a sufficient supply of oxygen. This leaves unprocessed substrates and creation of anaerobic pockets in the substrate, with negative effects on BSFL growth and increased greenhouse gas emissions (Čičková et al., 2015). Grinding and mixing of the feed substrate is usually recommended, to create a homogeneous mixture with desired particle sizes that can efficiently be consumed and digested by the BSFL (Parra Paz et al., 2015). The total amount of feed substrate required can be provided at the beginning of BSFL composting (Banks et al., 2014). However, Banks et al. (2014) recommend feeding the BSFL in portions of equal amounts provided 2-4 days apart within the first week of BSFL composting, in order to reduce crust formation and the presence of unprocessed substrates at the bottom of the treatment boxes.

3.4.2 Substrate degradation process in BSFL composting

The degradation process begins with the BSFL secreting enzymes, such as amylase and maltase, from their salivary glands into the feed substrates. These enzymes start to break down complex molecules into simpler forms that can easily be absorbed by the BSFL. Feed degradation has been demonstrated to take place primarily in the midgut section of BSFL (De Smet et al., 2018, Bonelli et al., 2019). The midgut has three sub-sections with differing pH, ranging from 6.0 in the anterior midgut to 2.0 in the central midgut and 8.5 in the posterior midgut (Bonelli et al., 2019), that drive the functions of enzymes, microorganisms and hormones. Generally, the functional and biological metabolism of BSFL depends on the diversity and composition of the gut microbial community (De Smet et al., 2018, Ao et al., 2021). The BSFL gut microflora, comprising fungal (Boccazzi et al., 2017) and bacterial (Jeon et al., 2011) communities, has been observed to vary and adjust to the type and composition of the feed substrate provided. The gut microflora can respond rapidly to differences in substrates by secreting the types of digestive enzymes required for efficient breakdown and absorption of complex molecules by the BSFL (Franks et al., 2021). Moreover, the gut microorganisms have been suggested to play a role in degradation of harmful pharmaceutical and pesticide compounds (Lalander et al., 2016) and in preventing pathogenic microorganisms from colonising the gut of BSFL (De Smet et al., 2018). *Firmicutes, Proteobacteria and Bacteroidota* have been identified as the most abundant phyla in the BSFL gut, while *Lactobacillales, Klebsiella, Providencia and Morganella* are the dominant genera, and these play an important role in the ability of BSFL to convert organic waste into biomass (Wynants et al., 2019, Bruno et al., 2019, Klammsteiner et al., 2021, Galassi et al., 2021). For example, *Providencia* has been suggested to play a role in protein and lipid conversion in the BSFL gut (Ao et al., 2021, Callegari et al., 2020), while members of the phylum *Firmicutes* are generally reported to have the ability to secrete a variety of proteases and pectinases that can degrade indigestible carbohydrates in substrates such as cow manure and straw (Sun et al., 2015, Zhang et al., 2018). Thus, through the presence of these gut microorganisms and enzymes that hydrolyse macronutrients into smaller molecules, BSFL have the ability to degrade a wide variety of organic materials (Gold et al., 2018). Various digestive enzymes are secreted into the gut of BSFL: *e.g.* amylase and cellulases that hydrolyse carbohydrate and starch macromolecules; protease and trypsin that

hydrolyse macromolecules of proteins; lipases that hydrolyse lipids macromolecules; and lysozymes enzymes that can decompose mycotoxins and pathogenic microbes ingested with feed substrate (Bonelli et al., 2019, Lee et al., 2014). Hydrolysed monomers of carbohydrates (such as glucose), monomers of protein (such as amino acids and insulin-like hormones) and monomers of lipids (such as free fatty acids) are all essential for BSFL metabolism and structural development and composition of the larvae (Gold et al., 2018). At the end of the degradation process, BSFL excrete non-digestible and non-absorbed substances, microorganisms, enzymes and antimicrobial peptides through the hindgut and these remain in the frass upon completion of treatment (Gold et al., 2018).

3.4.3 Effect of BSFL density, feeding rate, temperature and moisture content

Composting efficiency is influenced by factors such as BSFL density, feeding rate, and temperature and moisture content of the substrate. High feeding rates, of 100-200 mg larva^{-1} d^{-1}, have been shown to result in shorter development time for BSFL to reach the prepupal stage and in higher final larval weight than in BSFL grown at lower feeding rates (Parra Paz et al., 2015, Manurung, 2016, Diener et al., 2009). At low feeding rates, final larval weight is reported to be reduced by competition for nutrients by the BSFL, although low feeding rates at high BSFL densities have been observed to increase waste substrate reduction efficiency (Parra Paz et al., 2015). It has been demonstrated that feeding rates in BSFL composting can be regulated based on the BSFL density, depending on the end aim of the BSFL treatment process (Parra Paz et al. (2015). Moreover, since the waste-to-biomass conversion efficiency is dependent on the nutritional composition of the biowaste (Lalander et al., 2019), the optimum combination of feeding rate and BSFL density that results in the highest BCE and greatest waste reduction may vary with different types of biowaste (Diener et al., 2009). Lower feeding rates (30-60 mg larva^{-1}d^{-1}) and high BSFL density (5-7 larvae cm^{-2}) have been suggested for BSFL composting treatments aiming for high waste substrate reduction efficiency, while high feeding rates (130-230 mg larva^{-1} d^{-1}) together with low BSFL density (1.2-3 larvae cm^{-2}) have been suggested for treatments aiming for biomass production (Parra Paz et al., 2015). Composting with high BSFL density (>5 larvae cm^{-2}) and high feeding rate (≥95 mg larva^{-1} d^{-1}) is not recommended, due to temperature

increase and reduction of pH below 6 in the treatment process, which hinders relative growth of the BSFL and waste reduction efficiency (Parra Paz et al., 2015). Substrates high in moisture (>90%) create an unstable treatment process, with anaerobic conditions that lower the BCE and waste reduction by the BSFL (Lalander et al., 2020). In addition, high moisture content hampers separation of BSFL from treatment residues (Cheng et al., 2017, Lalander et al., 2020), resulting in wet, sticky and smelly treatment residues (Diener et al., 2011). During BSFL composting, the moisture content of the substrate decreases, while the temperature increases and the pH changes from acidic or neutral conditions to alkaline (Čičková et al., 2015, Meneguz et al., 2018). A study by Ma *et al.* (2018) investigating the impact of different pH levels on BSFL development observed higher BSFL (≥0.2g) and prepupa (≥0.18 g) final weight at pH 6, 7, 8 and 10 than at pH 4 or 2 (final weight ≤0.16 g). At higher temperatures (>30 °C), the BSFL are reported to crawl away from the food in search of a cooler location, while their metabolism has been shown to slow down at lower temperatures (<24 °C), resulting in BSFL eating less and developing more slowly at lower temperatures (Tomberlin et al., 2009).

The reported changes in moisture content, pH and temperature during BSFL composting, and the resulting BCE and waste reductions, are influenced by the metabolism and movement of the BSFL (Čičková et al., 2015, Diener et al., 2009). In literature, feeding rates of around 95-163 mg $larva^{-1}$ d^{-1} have been suggested for achieving favourable BSFL treatment efficiency while maintaining optimal process temperature (≤ 30 °C) and stabilised final pH greater than 6 (Parra Paz et al., 2015).

3.5 Impact of substrate nutrient composition on BSFL composting efficiency

Although BSFL can survive and grow on many different substrates, the nutritional composition of the substrate affects parameters such as larval development time, BCE, larval survival, final larvae weight and the quality of the products generated in BSFL composting (Ewald et al., 2020, Kawasaki et al., 2019, Palma et al., 2019). One parameter that has been demonstrate to impact the BSFL composting process in terms of larvae development time,

material reduction and BCE is the protein to carbohydrate ratio (Prot:Carb), *i.e*. the amount of readily available protein per amount of carbohydrates. A Prot:Carb ratio of around 1:1 has been shown to shorten BSFL development time, support BSFL growth and result in high material reduction and BCE (Lalander et al., 2019, Cammack and Tomberlin, 2017, Gold et al., 2020). Fibre-rich waste substrates such as dairy and cow manure, with high concentrations of cellulose (~32%$_{DM}$) hemicellulose (~17%$_{DM}$) and lignin (~14%$_{DM}$), are reported to be challenging in terms of substrate degradation and nutrient utilisation by BSFL, and thus result in lower BCE (~4%$_{DM}$) (Gold et al., 2020, Rehman et al., 2017a). Cohn *et al*. (2022) observed that substrates with hemicellulose constituents (*e.g*. xylan and galactose) significantly reduced BCE and waste reduction compared with substrates with other types of carbohydrates (*e.g.* sucrose, fructose and glucose). High concentrations of crude fat (≥6%$_{DM}$) in substrates such as fish waste have also been associated with low BCE and low final larval weight (Nguyen et al., 2013, Lopes et al., 2020). In order to achieve greater material reductions and higher BCE, it is thus important to optimise the ratio of protein to carbohydrate in the substrate. The concentrations of other nutrients such as fibre and lipids, which vary with type of feed substrate, should also not be too high. Applying a pre-treatment (Wong et al., 2021, Somroo et al., 2019, Palma et al., 2019, Liu et al., 2021) and blending (Lopes et al., 2020, Nyakeri et al., 2019) waste substrates prior to BSFL composting treatment have been suggested as possible approaches to meet the nutritional needs of the BSFL and thus increase the BSFL composting efficiency.

3.6 Blending of substrates

Blending of substrates involves mixing together two or more types of substrate to obtain a more balanced composition in terms of the nutritional needs of the larvae, overcoming the disadvantages of a homogeneous waste stream in BSFL composting. Compared with BSFL treatment of faecal sludge only, Nyakeri *et al*. (2019) reported increased BCE and waste reduction of a blended mixture of 30% faecal sludge with 50% mixed waste containing banana peels, food waste and brewers' yeast. Further, Lalander *et al.* (2019) demonstrated that supplementing low-protein substrates such as fruit and vegetable waste with a protein-rich substrate, such as abattoir waste, improved the BCE from 4%$_{DM}$ (pure fruit and vegetable waste) to 14%$_{DM}$

(1:1 fruit and vegetable waste:abattoir waste). Similarly, Lopes *et al*. (2020) evaluated combinations of 21 different mixtures of high-protein aquaculture waste with high-carbohydrate bread (0% to 100%, in 5% increments) and found that addition of even only 10-15% of aquaculture waste improved larval quality in terms of protein concentration and BSFL process performance in terms of material reduction and BCE.

3.7 Pre-treatments

Use of a pre-treatment has been demonstrated to enhance hydrolysis of the crystalline fibrous structures in complex macromolecules such as cellulose, hemicellulose and lignin in the substrate into simple monomers (Mtui, 2009). Various biowaste treatment processes, *e.g.* anaerobic processes and fermentation, have applied different types of pre-treatment methods to increase their production efficiency (Atelge et al., 2020, Öhgren et al., 2007, Harmsen et al., 2010). Pre-treatment methods fall into different categories, *e.g.* physical, thermal, chemical, biological and combinations of these (Atelge et al., 2020, Harmsen et al., 2010). Physical pre-treatments, such as milling and grinding, are used to increase the accessible surface area and pore size of all substrate components, while decreasing their crystalline complex structures (Harmsen et al., 2010).

3.7.1 Heat pre-treatment

Thermal pre-treatments are often applied prior to anaerobic digestion, with the substrate heated using an autoclave, oven, steam explosion or microwave (Atelge et al., 2020). Thermal pre-treatments involve timely application of heat and pressure to break down complex molecular structures in substrates, hence exposing and increasing the accessible surface areas for microorganisms and enzymes to easily degrade and utilise the digestibility of nutrients (Sarip et al., 2016).

3.7.2 Biological pre-treatment

Biological pre-treatments apply microorganisms such as fungi and bacteria to degrade complex molecular structures, such as those in fibre-rich substrates, into simple monomers such as glucose (Sindhu et al., 2016). Fungal species, *e.g. Rhizopus oligosporus* from the family Mucoraceae and *Trichoderma reesei* from the family Hypocreaceae, are examples of

microorganisms used in biological pre-treatment of cellulosic biomass, selected for their ability to produce and secrete enzymes such as cellulase and hemicellulase (Ling-qi, 2012, Sivaramakrishnan et al., 2021, Mustafa et al., 2016). Pre-treatment using microbial inoculation prior to BSFL composting has been shown to increase substrate conversion efficiency and final larval weight when applied to substrates such as soybean curd residue, dairy manure, coconut endosperm waste and chicken manure (Yu et al., 2011, Somroo et al., 2019, Rehman et al., 2019, Xiao et al., 2018, Mohd-Noor et al., 2017, Wong et al., 2020b). Application of several microbial strains, *e.g. Rhizopus oligosporus* (Wong et al., 2021), *Rhodococcus rhodochrous* (Franks et al., 2021), *R. rhodochrous* 21198 and *Arthrobacter* AK19 (Kooienga et al., 2020), the single-celled yeast *Saccharomyces cerevisiae* (Wong et al., 2020b) and several *Bacillus* spp. (Xiao et al., 2018, Somroo et al., 2019, Yu et al., 2011, Wong et al., 2019, Wong et al., 2020a) prior to BSFL composting has been reported to increase BSFL composting efficiency of several types of biowaste substrates. Pre-treatment time in microbial pre-treatment has been highlighted as one important parameter that can affect both overall process productivity and economic gains in anaerobic digestion (Xu et al., 2021, Hernández-Beltrán et al., 2019). A study by Wong et al. (2019) assessing the impact of inoculating coconut endosperm waste with a mixture of *Bacillus* spp. for 0, 7, 14, 21, and 28 days found that maximum protein conversion and BCE by BSFL were achieved with a microbial pre-treatment time of 14 days. Wong et al. (2019) also observed high protein conversion and BCE of 9% in treatments with a 0.5% wet weight (WW) dose of a mixture of *Bacillus* spp., compared with BCE of 6% and 8.5% with a low dose ($\leq 0.1\%_{WW}$) and high dose ($2.5\%_{WW}$), respectively.

3.7.3 Chemical pre-treatment

Chemical pre-treatment, with addition of various chemicals such alkalis, acids and salts, has been demonstrated to break down cell walls and convert lignin, cellulose and hemicellulose into simple molecules (Harmsen et al., 2010, Mahmood et al., 2019). Chemical pre-treatment involving addition of non-protein nitrogen to ruminant feeds has been demonstrated to result in successful conversion of the non-protein nitrogen, through ammonia assimilation by the microbial community in the rumen, into amino acids and proteins that can be utilised by the ruminant animal (Andrade-Montemayor *et al*., 2009; Tadele & Negassie, 2015). Carreiro *et al*. (2000) found that

nitrogen supplementation has the ability to improve the growth of microorganisms and synthesis of enzymes necessary to break down lignocellulose through increased cellulose activity in decaying leaf litter of flowering dogwood, red maple and red oak.

3.7.4 Enzyme pre-treatment

Enzyme pre-treatment uses specific proteins that act as biological catalysts, *i.e.* enzymes, to hydrolyse complex macromolecules such as cellulose, hemicellulose and lignin into sugars that can be readily utilised in biological processes (Moglia, 2008). Hydrolytic, degrading and ligninolytic enzymes normally involved in enzymatic pre-treatment include all or some of the enzymes cellulases, hemicellulases, pectinases, amylases, proteases, laccases, peroxidases and oxidases (Hosseini Koupaie et al., 2019). The different types of enzymes bind on active sites of selected substrates and catalyse the reactions that convert these substrates into new products (Hosseini Koupaie et al., 2019). Enzymatic pre-treatment of lignocellulosic biomass involves the use of oxidative and hydrolytic enzymes such as lignin peroxidase, xylanase, laccase, cellulases, β-glucosidase, hemicellulases, peroxidases and oxidases, often extracted from bacteria or fungi (Mtui, 2009, Hosseini Koupaie et al., 2019). Enzymatic pre-treatment is currently being explored in other biowaste treatments, such as anaerobic digestion, to enhance production of biogas from lignocellulosic biomass (Liu et al., 2021, Hosseini Koupaie et al., 2019). Enzyme pre-treatments are often performed at an optimal temperature range of 40-60 °C and a pH range of 4-7 (F and Shastri, 2016, Abdulsattar et al., 2020, Neesa et al., 2020). Compared with microbial pre-treatment, enzyme pre-treatment takes a relatively short time to hydrolyse complex molecules into monomers. Moreover, presence of microbial metabolism does not hinder the function of enzyme pre-treatment and no substrate is used by the enzymes (Zheng et al., 2014, Wei, 2016). Lindberg et al. (2022) investigated the impact on BSFL composting process efficiency of pre-treating cabbage and lettuce at 28 °C with an enzyme cocktail (SAE0020 Sigma-Aldrich, $1\%_{WW}$), consisting of cellulases, ß-glucosidases and hemicellulases. They observed that direct addition of enzyme without any retention time resulted in 22% higher BCE ($24\%_{VS}$) and 14% higher material reduction ($89\%_{VS}$) compared with the control, while longer pre-treatment of 2 d and 4 d did not improve BCE (Lindberg et al., 2022).

4. Material and Methods

4.1 BSFL composting (Papers I-III)

4.1.1 Materials

BSFL

Black soldier fly larvae (5-7 d old), >0.2 cm long and with an average weight of 1 mg larva^{-1} were obtained from a BSF colony that has been in operation at the Swedish University of Agricultural Sciences, Uppsala, Sweden, since 2015. The BSFL were reared for around 5 d on chicken feed (Granngården Hönsfoder Start, metabolisable energy content 11.2MJ kg^{-1}, 80% moisture) at a feed rate of 0.83 g 100 larvae^{-1}.

Waste substrates

Banana peels used in experiments were of two types: i) peel from *Musa acuminata* (Cavendish banana), referred to hereafter as dessert peel (Papers I-III); and ii) peel from ripe *Musa acuminata* x *M. balbisiana* (Pisang Awak banana) used specifically for juice production (Kibazohi et al., 2017), referred to hereafter as juice peel (Paper I). The Pisang Awak bananas were bought in Mabibo market, Dar es Salaam, Tanzania, and transported to SLU, where peels were separated from edible flesh. Cavendish bananas and orange peels were obtained from the fruit and vegetable wholesaler Sorunda Grönsakshall (Stockholm, Sweden). Different fish species of wild-caught fish comprising mainly perch and roach, but also some bleak, rudd, smelt, ruffe and herring, caught using multimesh gillnets in the Sea of Åland, were collected and supplied by the Department of Aquatic Resources (Kustlaboratoriet), Öregrund, Sweden.

Microorganisms

Isolation and growth of a consortium of bacteria from BSFL, as described in Paper I, was performed by Cecilia Lalander in the Environmental Engineering group at SLU. The mixture of bacteria isolated from the BSFL gut that was used in Paper I was identical to that used by Lundgren (2019) and contained 10 species: *Bacillus subtilis*, *Corynebacterium* spp., *Bacillus lichniformis*, *Lycinibacillus fusiformis* and six other bacteria species not identified by Lundgren (2019).

Pure strains of *Trichoderma reesei* and *Rhizopus oligosporus* fungi for the microbial pre-treatments in Paper I were obtained from Volkmar Passot at the Department of Molecular Sciences, SLU. These fungi strains were separately pre-cultured on malt extract agar (MEA) at 28°C for 7 d and harvested using sterile 0.9% NaCl.

4.1.2 Experimental set-up

Pre-treatments

The pre-treatment methods evaluated (Paper I) were microbial, chemical (non-protein nitrogen addition), heat-based, enzyme-based and combinations of these. The first pre-treatment study comprised six treatments. The first of these involved feeding the larvae with non-pre-treated banana peel prior to BSFL composting, while the remainder involved feeding the larvae with banana peels that had undergone one of five pre-treatments for different periods: i) microbial pre-treatment with BSFL gut bacteria mixture, *Trichoderma reesei* or *Rhizopus oligosporus* (7 days (d), 14 d, 21 d); ii) chemical pre-treatment with 0.8% non-protein nitrogen (N) and 1% N (7 d, 14 d); iii) heating pre-treatment at 120 °C under 2 bar (1 h); iv) combined chemical (1% N, 7 d) and microbial (7 d) pre-treatment; and v) heating combined with microbial (7d) pre-treatment (Paper I). The heating and microbial pre-treatments were applied based on the hypothesis that they would facilitate degradation of complex molecules in the substrate, making nutrients more available to the BSFL (Razaghi et al., 2016, Haddadin et al., 2009). The chemical pre-treatment with addition of non-protein nitrogen aimed at providing the BSFL with the nitrogen required for amino acid synthesis and potentially improving substrate nutrient digestibility and utilisation by the BSFL through breakdown of cell walls in biodegradable molecules (Carreiro et al., 2000, Palma et al., 2019).

In Paper II, enzyme treatments were performed based on the hypothesis that an enzyme cocktail containing mixtures of enzymes (cellulases, ß-glucosidases and hemicellulases) would convert hemicellulose and cellulose to readily available carbohydrates for utilisation by the BSFL. The ability of two different enzyme-based treatments to improve the BSFL composting efficiency in terms of BSFL survival, BCE, final larval weight and material reduction, compared with a control with no pre-treatment, was evaluated. The treatments were: i) non pre-treated substrate (control); ii) enzyme-treatment at 28 °C; and iii) enzyme-treatment at 50 °C for 24 h (Paper II). The hypothesis was that the increase in temperature from 28 °C to 50 °C for 24 h would result in more efficient enzymatic hydrolysis of fibres, and thus an improvement in BSFL composting efficiency. An enzyme concentration of 0.02 mL g^{-1} banana peel, 0.015 mL g^{-1} orange peels and 0.017 mL g^{-1} mixed banana-orange peel was used in the treatments, based on the fibre concentration in the substrates. All treatments were performed in triplicate. The enzyme-treatment at 50 °C for 24 h was performed in buckets placed in a specially designed insulated box (Figure 2).

Figure 2. Images of the insulated mixing box used for enzyme treatment at elevated temperatures.

Biological enzyme pre-treatments using a mixture of the fungi *Trichoderma reesei* and *Rhizopus oligosporus* were also performed in Paper II. The hypothesis was that using a mixture of two types of fungi would provide more types of enzymes to hydrolyse complex compounds such as

hemicellulose, cellulose and pectin into forms more available to the BSFL. The four conditions tested were: i) non-treated substrate (control); ii) heat pre-treatment at 50 °C for 24 h (control); iii) fungi-mix pre-treatment for 14 d; and iv) ammonia pre-treatment at 50 °C for 24 h, followed by fungi-mix pre-treatment for 14 d (Paper II). During the 14 d fungi pre-treatment, banana peels were separately inoculated with 1% (w/w) of *Trichoderma reesei* and orange peels were inoculated with 1% (w/w) of *Rhizopus oligosporus* for one week. The two sets of fungi-inoculated substrates were left to stand separately at 28 °C for 7 d and then mixed together, in order to give both fungal species an opportunity to colonise the substrate, after which they were mixed together and left for another 7 d at 28 °C. For the fungi-mix pre-treatment preceded by chemical pre-treatment, ammonia solution (4% w/w, 1% N) was added to the substrates and the chemical pre-treatments were carried out for 24 h at 50 °C. The pre-treated substrates were then neutralised to pH 7 by addition of 95% concentrated sulphuric acid (<2 mL). The fungi-mix 14 d pre-treatment was performed on the chemical pre-treated substrates, following the procedure described above.

Blending of substrates

The impact of mixing a protein-rich low-quality waste stream (fish waste) with a fibre-rich, low-quality waste stream (peels of orange and banana) in BSFL composting was assessed in Paper III using blended mixtures of banana peel, orange peel and fish waste, in 50 different combinations. Fish waste incorporation into the mixture was fixed at 0%, 10%, 50% or 75%, while the rate of banana peel and orange peel incorporation varied from 100% to 0%, with either 5% or 10% replacement increments between each step, as described in Paper III.

Processing of the waste substrates

Once fish waste was received, the fins and all internal contents of the fish, including gills, liver, kidney, intestines, heart, stomach and swim bladder, were collected and mixed (Figure 3c). The fish waste fraction represented available fish waste in Tanzania. All three substrates were homogenised separately using a blender (Robot Coupe Blixer 4 V, France), as shown in Figure 3a and 3b, to mimic the pre-treatments used in BSFL treatment facilities (Dortmans et al., 2017). They were then divided into feeding portions and stored at -20 °C until use.

Figure 3. Images of the a) banana peel b) orange peel and c) fish waste substrates before and after preparation.

BSFL composting

All treatments were conducted in plastic containers (Smartstore Classic 2, with reported dimensions L21xW17xH11 (cm^3) and with real measured dimensions of L17xW14xH10 (cm^3) (Figure 4a). Each container was covered with a plastic lid with a rectangular fabric mesh-covered opening (L9xW5 cm^2) to allow air circulation. The treatment boxes were placed in larger boxes (36.5 cm x 56.2 cm x 11.5 cm) arranged on racks (Figure 4b). All BSFL composting treatments were performed under the same controlled conditions, with an average temperature of 28.8 ± 0.8 °C. The larger boxes were regularly rotated on the racks to reduce effects of the temperature gradient (1 °C difference from bottom to top) and differences in air flow in the environment (Figure 4b).

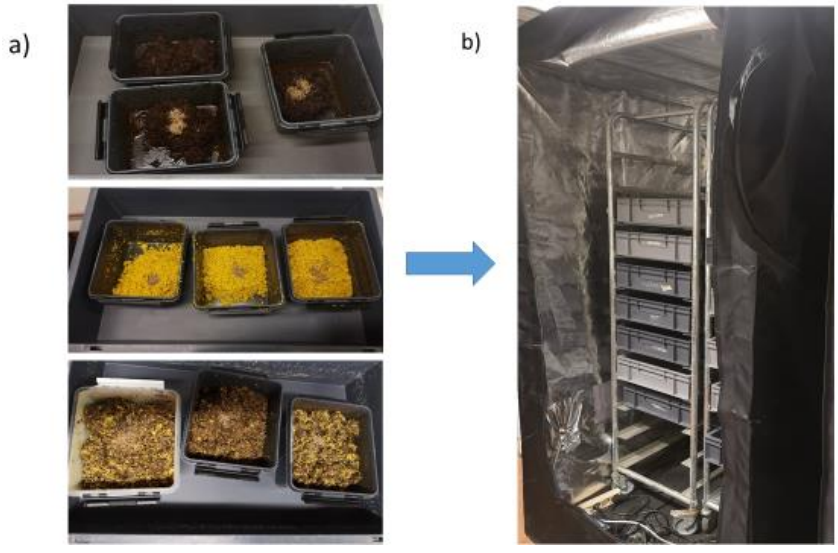

Figure 4. Black solider fly larvae (BSFL) composting set-up with a) treatment boxes, b) larger boxes holding treatment boxes on closable tents that maintain controlled constant environmental conditions.

The portioned and frozen banana peel, orange peel and fish waste were thawed at room temperature (28 °C) for 24 h, thoroughly mixed and administered to the BSFL in the treatment containers according to the set feed requirements.

In Paper I, treatments were applied in triplicate and each replicate contained 200 larvae (0.2 cm length, 1 mg) following an existing protocol (Lalander et al., 2019), resulting in a density of 0.8 larvae cm^{-2} and supplying a BSFL feeding rate of 0.04 g VS substrate $larva^{-1}$ d^{-1}. Feeding stopped on day 28, but composting continued up to day 30 (Paper I). The BSFL feeding was done every second or third day of the week, with waste substrates evenly distributed between the feeding events (Table 1).

In Paper II, treatment experiments were conducted in triplicate and each replicate received 700 larvae (average weight ~1 mg $larva^{-1}$), resulting in a larval density of ~2.9 larvae cm^{-2}, following the protocol described in Ermolaev et al. (2019). The depth of substrates did not exceed 6 cm and BSFL feeding rate was 0.2 g VS substrate $larva^{-1}$ over the entire treatment, evenly distributed between three feeding events on days 0, 4 and 7 (Table 1). After the last feeding event, the treatments were monitored until around 10%

of the BSFL had turned into pre-pupae, upon which the treatment was terminated. The BSFL treatment time varied between 3 and 4 weeks.

During the substrate blending experiments (Paper III), 50 treatment mixtures were conducted in singlets and each replicate received 700 larvae (average weight ~1 mg larva^{-1}), resulting in a larvae density of 2.9 larvae cm^{-2}, following the protocol described in Ermolaev et al. (2019). BSFL feeding was done on days 0, 4 and 7, supplying 0.25 g VS substrate larva^{-1} in total over the entire treatment, evenly distributed between the three feeding events (Table 1). After the last feeding event, the treatments were monitored until around 10% of the BSFL had turned into pre-pupae, upon which the treatment was terminated. The BSFL treatment time varied between 2 and 3 weeks.

Table 1. *Number of black soldier fly larvae (BSFL), larval density, larval volatile solids (VS) feeding dose, feeding methods and BSFL composting time applied in Papers I, II, II*

Paper	No. of larvae	Larval density (larvae cm^{-2})	Designed larval feeding dose (g VS larva^{-1})	Actual total larval feeding dose (g VS larva^{-1})	Feeding days (day)	BSFL composting time (days)
I	200	0.8	0.04 day^{-1}	1.46±0.05 - 0.75±0.05	Every 2nd or 3rd day	30
II	700	2.9	0.2	0.11±0.02 - 0.23±0.001	0, 4 and 7	18-32
III	700	2.9	0.25	0.27±0.004 - 0.33±0.04	0, 4 and 7	15-19

Sampling

In Paper I, samples of the treatment residues were collected once a week for determination of dry matter (DM) content, total volatile solids (VS) content and pH. Frozen portions of waste substrate were thawed at room temperature and added to the feeding containers after sampling (Paper I). No sampling during treatment was performed in Paper II, while sampling for DM, VS and pH determination was done only once a week in Paper III.

BSFL/pre-pupae were picked manually from the residues after termination of treatment. Samples of the harvested BSFL and of treatment residues were taken for DM and VS content determination and pH was measured in the treatment residues (Papers I-III).

To assess the impact of nutritional parameters (crude fibre, crude protein, carbohydrates, crude fat and total phenols) on BSFL composting efficiency, samples of untreated waste substrate (Papers I, II and III) and pre-treated waste substrate (Papers I and II) were taken for nutritional analysis. The nutritional analysis in Paper I was performed at the Department of Botany, University of Dar es Salaam, Tanzania. The nutritional analysis in Paper II and III was performed at Eurofins Food & Agro Testing Sweden AB (Swedac-accredited laboratory).

4.1.3 Physico-chemical and nutritional analysis

Dry matter and total volatile solids

Dry matter content of the substrates was determined by heating at 85 °C for 48 h. After drying, the materials were combusted in a furnace (LH30/12, Nabertherm GmbH, Germany) that was slowly heated to 200 °C for 2 h (to prevent sample losses due to rapid heating at high temperatures), before heating to 550 °C for 4 h (ISO 18122:2015) for determination of VS.

pH

The pH of the substrate mixtures and the treatment residues was determined using an InoLab Laboratory pH meter. For this, 10 g sample were placed in a 50-mL centrifuge tube, diluted to 50 mL with de-ionised water and left for 1 h at room temperature for acclimatisation prior to the pH readings.

C/N and Prot/Carb ratio

Carbon:nitrogen (C/N) ratio was calculated by dividing percentage of organic carbon (DM basis) (calculated as percentage VS divided by 1.8)

(Haug, 1980) by percentage of total nitrogen. Organic carbon and total nitrogen content were calculated based on the resulting total amounts of protein and the VS content established for the individual substrates in the substrate mixtures. The protein:carbohydrate (Prot/Carb) ratio was calculated by dividing the percentage protein concentration (DM basis) by the percentage carbohydrate concentration (DM basis).

4.2 Industrial field survey (Paper IV)

The food industry companies surveyed in Paper IV were selected from a list obtained from the Tanzanian Ministry of Industry, Trade and Investment database, which contains details of all food industry companies operating in the 25 regions of Tanzania. Food industry companies are categorised into 18 categories in the database, but only companies in five categories were selected for the survey, based on the likelihood of these companies to generate biowaste. The categories were: manufacture of malt liquors and wines, manufacture of other food products, manufacture of soft drinks and production of mineral waters and other bottled water, manufacture of vegetable and animal oils and fats, and processing and preserving of fish, crustaceans and molluscs (Paper IV). The survey involved a total of 42 food companies out of 91 companies listed under the five selected categories. The field survey was conducted in three regions of Tanzania that are urbanised, densely populated and have a high number of food industry companies. In these regions, Dar es Salaam, Mwanza and Dodoma, 17, 15 and 10 companies were evaluated, respectively.

Data were collected using a structured questionnaire comprising closed-ended questions administered in Swahili about the food companies' practices pertaining to generation, storage, collection, transportation, processing, final disposal and availability of organic process solid waste (Appendix 1 in Paper IV). Both qualitative and quantitative data were collected. Graphical representations of the data were produced using R statistical software (R Core Team, 2016).

The second part of the work in Paper IV involved assessing the suitability of biowaste from the participating food industry companies for use as feedstock in BSFL composting to produce animal protein. A multi-criteria analysis (MCA) procedure was applied to assess the suitability in terms of availability and physical-chemical properties of all available food industry waste from

the companies surveyed. The MCA methodology was taken from Lohri *et al*. (2015) and modified to analyse the selected criteria and their respective sub-criteria. Unlike in Lohri et al. (2015), the relative relevance of all sub-criteria was given a similar weighting, there was no total score summation or ranking procedure to compare the biowaste and there were no focus group discussions with solid waste experts (Paper IV). Biowaste suitability for production of animal protein using BSFL composting was graded using a three-colour traffic light method (Caddy, 2015), with a score of 1 (bad) represented by red, a score of 2 (medium) by yellow and a score of 3 (good) by green (Paper IV).

4.3 Calculations

Percentage dry matter (DM) was calculated as:

$$\mathrm{DM} = \left(\frac{\mathrm{m}_{dry.sample}}{\mathrm{m}_{wet.sample}}\right) \times 100 \qquad \text{Equation 1}$$

and total volatile solids on a dry matter basis (VS) as:

$$\mathrm{VS} = \left(\frac{(\mathrm{m}_{dry.sample} - \mathrm{m}_{ash.sample})}{\mathrm{m}_{dry.sample}}\right) \times 100, \qquad \text{Equation 2}$$

where $\mathrm{m}_{\mathrm{wet.sample}}$, $\mathrm{m}_{\mathrm{dry.sample}}$ and $\mathrm{m}_{\mathrm{ash.sample}}$ is sample weight before and after drying and after combustion (ashing), respectively.

Percentage material reduction on a VS basis (Red_{VS}) was calculated as:

$$Mat\ red_{VS} = \left(1 - \frac{m_{res}{}^{*}\ \mathrm{DM}_{res}{}^{*}\ \mathrm{VS}_{res}}{m_{mix}{}^{*}\ \mathrm{DM}_{mix}{}^{*}\ \mathrm{VS}_{mix}}\right) \times 100 \qquad \text{Equation 3}$$

where m_{res} and m_{mix} is the mass of residues and of substrate mixture, respectively, DM_{res} and DM_{mix} is the percentage dry matter content in treatment residues and substrate mixture, respectively, and VS_{res} and VS_{mix} is the total volatile solids in treatment residues and substrate mixture, respectively.

Percentage biomass conversion efficiency on a VS basis (BCE_{VS}) was calculated as:

$$\mathrm{BCE}_{VS} = \frac{m_{lv}{}^{*}\ \mathrm{DM}_{lv}{}^{*}\ \mathrm{VS}_{lv}}{m_{mix}{}^{*}\ \mathrm{DM}_{mix}{}^{*}\ \mathrm{VS}_{mix}} \times 100 \qquad \text{Equation 4}$$

where m_{lv} and m_{mix} is the mass of larvae and of substrate mixture, respectively, DM_{larvae} and DM_{mix} is the percentage dry matter content in the larvae and substrate mixture, respectively, and VS_{larvae} and VS_{mix} is the total volatile solids in the larvae and substrate mixture, respectively

Percentage survival rate (SR) of the larvae was calculated as:

$$\mathrm{SR} = \left(\frac{\mathrm{lv}_{end}}{\mathrm{lv}_{start}}\right) \times 100 \qquad \text{Equation 5}$$

where lv_{end} is the number of larvae that survived to the end of the treatment and lv_{start} is the initial number of larvae used in the treatment.

The VS loss in BSFL composting was calculated as:

$$\text{VS loss} \quad = \frac{m\mathrm{VS}_{mix} - m\mathrm{VS}_{lv} - m\mathrm{VS}_{res}}{m\mathrm{VS}_{mix}} \times 100 \qquad \text{Equation 6}$$

where $m\mathrm{VS}_{mix}$, $m\mathrm{VS}_{lv}$ and $m\mathrm{VS}_{res}$ is the mass of VS in the substrate mixture, larval biomass and treatment residues, respectively.

4.4 Statistical analysis

The Shapiro-Wilk test of normality was conducted to verify normality of data at 5% significance level for all statistical tests, with treatment groups with similar kind of substrates as a fixed factor (Papers I and II). The survival rate was found to be non-normal and was converted into $\log_{10}$ death rate (Paper I), which was found to be normally distributed (p>0.05). One-way analysis of variance (ANOVA) was used to determine whether there was any statistically significant difference between the treatment groups. Tukey's Honest Significant Difference (HSD) was used to verify, test and identify significant differences between treatment groups (Papers I and II). Kruskal-Wallis, a non-parametric test, was used to compare differences in treatment groups of variables that were not normally distributed in Paper II. Mann-Whitney U was then used to verify, test and identify significant differences between treatment groups in Paper II. Principal component analysis (PCA)

was performed to identify the variables that contributed most to the variation in the data, while multi-linear regression was used to verify correlations between selected variables (Papers I and III). Statistical Package for Social Sciences (SPSS) software (IBM version 23) (Paper II) and R statistical software (R Core Team, 2016) were used for statistical analyses and for graphical presentation of the data (Papers I and III).

5. Results

5.1 BSFL composting (Papers I-III)

5.1.1 Dry matter, total volatile solids and pH of substrates

The initial trials were performed with juice banana peels, which had a higher DM content (29.9%) than the dessert banana peels (12.5%$_{DM}$) used in the remainder of the research (Table 2). The pre-treatments resulted in either similar or decreased DM content, except in the case of 7 d bacteria pre-treatment combined with heat (Heat+Bac$_{7d}$), where the DM content almost doubled to 21%. A similar trend was seen for VS, with the juice peels having higher VS content (97%$_{VS}$) than the dessert peels (85%$_{VS}$). All pre-treatments, except heat only and heat combined with 7 d *rhizopus* (Heat+Rhiz$_{7d}$) decreased the VS content. The pre-treatments and BSFL composting increased the pH from weakly acidic to neutral and in some cases to weakly alkaline (Table 2).

The untreated banana peel (BP) substrate was wet (13%$_{DM}$), with 88% VS content and pH of 5.6 (Table 3). The percentage DM content varied somewhat in the enzyme-treatments, biological enzyme pre-treatments and different substrate mixtures of banana peel, orange peel and fish waste (Table 3). Enzyme plus heat pre-treatment (BP-Enz-24h$_{50°C}$) dried the substrate to 23%$_{DM}$, while the pH was lowered to acidic (4.8). Untreated orange peels (OP) were drier (25%$_{DM}$) than banana peels or the banana-orange peel (BOP) mixture (17%$_{DM}$) (Table 3). Enzyme plus heat-treatment of orange peels (OP-Enz-24h$_{50°C}$) made the substrate drier (39%$_{DM}$). All orange peel treatments had acidic pH, but the enzyme + heat pre-treatment lowered the pH from 4.8

to 3.9. Volatile solids content was not affected by enzyme pre-treatment of orange peels or banana-orange peel mixture. The fungi pre-treatments (BOP-$Fungi_{14d}$ and BOP-$NH3_{50°C}$-$Fungi_{14d}$) also dried the substrates and decreased the VS content, while increasing the pH (Table 3). With the exception of 75% fish waste substrate blend, which had similar VS content to the control, the substrate blends with and without fish waste had higher VS content compared with OP-control and BP-control. All the substrate blends had pH below 7.

The DM content of the residues increased strongly after enzyme-treatment of orange peels. It also increased in all the enzyme + heat treatments and all the fungi pre-treatments compared with the controls, and in all substrate blends with fish waste in comparison with the banana peel control (Table 3). The residue pH ranged between 3 and 10, with residues in enzyme-treatment OP-Enz-0d and enzyme + heat treatment OP-Enz-$24h_{50°C}$ having the lowest pH (<4.5) (Table 3).

5.1.2 Nutritional composition of substrates

The nitrogen concentration increased in all banana peel pre-treatments with addition of ammonia solution in Paper I with the exception of $NH3_{0.8\%N}$+$Trich_{7d}$, where nitrogen concentration was similar to that in the control (Table 4). The fat concentration was either similar or decreased in almost all the pre-treatments in comparison with untreated banana peels, with the exception of heat and combined heating-microbial pre-treatments. In comparison with untreated banana peel, the fibre concentration decreased in all pre-treatments except Heat+Bac_{7d}, where the fibre concentration was higher than in the control (Table 4). The carbohydrate concentration decreased in all pre-treatments except 21 d *rhizopus* ($Rhiz_{21d}$) and 7 days of bacteria combined with heat (Heat+Bac_{7d}), where the carbohydrate concentration was higher than in the control (Paper I). The phenol concentration was only elevated in the heat and combined heating-microbial pre-treatments. The C/N ratio was greatly reduced (<30) in the non-protein nitrogen pre-treatments $NH3_{0.8\%N}$+$Rhiz_{7d}$ and $NH3_{0.8\ \%N}$+Bac_{7d} of banana peel (Table 4).

Higher concentrations of nitrogen and crude protein compared with the respective control were observed in the combined heat and enzyme pre-treatments of banana peels and orange peels (BP-Enz-$24h_{50°C}$, OP-Enz-

$24h_{50°C}$) and direct enzyme pre-treatment of the banana-orange peel mixture (BOP-Enz-0d), the BOP-$NH3_{50°C}$-$Fungi_{14d}$ biological enzyme pre-treatment and all substrate blends with fish waste inclusion (Table 5). Crude fat concentration increased in the BOP-$Fungi_{14d}$ and BOP-$NH3_{50°C}$-$Fungi_{14d}$ pre-treatments compared with the control. Crude fat also increased in all substrate blends with fish waste compared with the orange peel control and in the 25%, 50% and 75% fish waste substrate blends compared with the banana peel control (Table 5). The carbohydrate concentration in BP-Enz-0d and OP-Enz-0d was similar to that in the control, but it was significantly increased in all remaining enzyme pre-treatments and biological enzyme pre-treatments (Paper II). The carbohydrate concentration increased in all substrate blends in Paper III compared that in the banana peel control, but decreased in all substrate blends compared with that in the orange peel control (Table 5). Phenol concentration increased in OP-Enz-$24h_{50°C}$, BOP-$Fungi_{14d}$ and BOP-$NH3_{50°C}$-$Fungi_{14d}$ compared with the controls, and in all substrate blends with fish waste compared with the banana peel control. The C/N ratio was significantly decreased in the BOP-Enz-0d and BOP-$NH3_{50°C}$-$Fungi_{14d}$ pre-treatments and significantly increased in BOP-$Fungi_{14d.}$ The C/N ratio decreased, while Prot/Carb increased, with fish waste inclusion in the substrate blends (Table 5).

Table 2. *Physical-chemical characteristics of different banana peel substrates and of pre-treated dessert banana peels and associated treatment residues. When triplicate analyses were conducted, values shown are mean (n = 3) ± standard deviation. In other cases, the value for the singlet sample is presented. Different letters within columns indicate significant differences (p<0.05)*

Treatment	Substrate			Residues	
	DM ($\%_{WW}$)	VS ($\%_{DM}$)	pH	DM ($\%_{WW}$)	pH
Untreated banana peels					
Juice banana peels	29.9 ± 0.40	96.9±0.22	4.0 ± 0.1	8.6±0.3	4.4±0.1
Dessert banana peels (control)	12.5 ± 0.34^{a,d}	85.2±0.08^{a,b,f}	5.7	6.6±1.1	5.9±0.3
Microbial pre-treatments					
$Trich_{7d}$	9.8 ± 0.12^{b,c}	81.6±0.93^{b,e,d}	5.0±0.9	6.8±0.3	7.6±0.4
$Trich_{14d}$	8.3	76.4	8.31	7.28	8.84
$Trich_{21d}$	7.6	73.5	8.1	7.4	9.28
$Rhiz_{7d}$	10.2 ± 0.06^{b,d,c}	80.8±0.56^{e}	6.9±0.4	6.6+0.05	7.0±0.7
$Rhiz_{14d}$	8.3	76.4	8.28	6.97	8.76
$Rhiz_{21d}$	8	77.1	6.76	7.2	8.48
Bac_{7d}	11.8 ± 0.23^{a,b,c}	78.6±2.3^{c,e}	8.0±0.5	6.3±0.2	7.7±0.2
Bac_{14d}	6.8	71.4	7.05	6.89	8.16
Bac_{21d}	7.9	87.8	7.3	6.95	7.99
Non-protein nitrogen pre-treatments					
$NH3_{0.8\ \%N}$	12.6 ± 0.65^{a,e}	84.4±0.32^{a,d,f}	7.3±0.2	7.5±0.7	7.6±0.2
$NH3_{1\ \%N}$	10.1 ± 2.4^{d,c}	83.1±2.2^{b,d,f}	5.8±1.2	7.3±0.4	7.6±0.1
$NH3_{0.8\ \%N\_14d}$	12.2	85.3	5.72	6.96	6.7
Heating pre-treatment					
Heat	9.4 ± 0.43^{b}	86.7±0.82^{a}	4.4	7.9±0.3	4.6±0.4
Non-protein nitrogen and microbial pre-treatments					
$NH3_{0.8\%N}$+$Trich_{7d}$	10.5	82.3	8.1	6.57	8.02
$NH3_{0.8\ \%N}$+$Rhiz_{7d}$	10.0 ± 0.21^{b,c}	78.2±0.53^{e}	8.6±0.2	7.1±2.0	8.5±0.2

$NH3_{0.8\ \%N}$+Bac_{7d}	8.5 ± 0.25[b]	80.3±0.34[e]	4.8±0.2	6.9±0.3	7.3±0.2
Heating and microbial pre-treatments					
Heat+$Trich_{7d}$	8.2	76.5	7.01	6.24	7.8
Heat+$Rhiz_{7d}$	12.2 ± 0.66[c,d,e]	86.0±0.70[a,f]	5.2±1.1	7.3±0.4	5.5±0.6
Heat+Bac_{7d}	21.8 ± 1.0	78.2±1.0[c,e]	7.4±0.3	6.4±0.5	7.2±0.4

Table 3. *Physical-chemical characteristics of the controls, enzyme treatments, biological enzyme pre-treatments, substrate blends of banana peels, orange peels and fish waste, and treatment residues, presented as average values (n=3) ± sd. Significant differences (*$p<0.05$*, Kruskal-Wallis test) are denoted *. Different letters within columns indicate significant differences (*$p<0.05$*) between treatments with banana peels (a-c), orange peels (d-f) and a mixture of banana and orange peels (g-k)*

Treatments	Substrate			Residues	
	DM ($\%_{WW}$)	VS ($\%_{DM}$)	pH	DM ($\%_{WW}$)	pH
Enzyme-treatments					
Banana peel, BP					
BP-control	13.1 ± 0.1^{a}	88.1 ± 2.1^{a}	5.6 ± 0.02^{a}	$6.7\pm2.0^{a*}$	7.9 ± 0.1^{a}
BP-Enz-0d	15.6 ± 2.5^{a}	90.0 ± 1.0^{a}	5.6 ± 0.02^{a}	$5.6\pm0.9^{a*}$	8.7 ± 0.2^{b}
BP-Enz-24$h_{50°C}$	23.2 ± 3.3^{b}	87.9 ± 0.3^{a}	4.8 ± 0.1^{b}	$27.4\pm9.8^{a*}$	9.5 ± 0.3^{c}
Orange peel, OP					
OP-control	$25.3\pm2.7^{d*}$	96.6 ± 0.1^{d}	4.8 ± 0.02^{d}	12.1 ± 1.0^{d}	7.2 ± 0.4^{d}
OP-Enz-0d	$24.2\pm0.2^{d*}$	96.0 ± 0.1^{d}	4.8 ± 0.01^{d}	20.7 ± 4.5^{e}	3.6 ± 0.1^{e}
OP-Enz-24$h_{50°C}$	$38.8\pm3.8^{e*}$	95.6 ± 0.2^{d}	3.9 ± 0.1^{e}	87.5 ± 2.9^{f}	4.4 ± 0.5^{e}
Banana-orange peel mix, BOP					
BOP-control	$17.1\pm0.2^{g*}$	$92.0\pm0.6^{g*}$	$5.3\pm0.1^{g*}$	$12.4\pm4.6g^{h}$	$7.9\pm0.1^{g*}$
BOP-Enz-0d	$17.9\pm1.3^{g*}$	$93.2\pm0.4^{g*}$	$5.1\pm0.02^{g*}$	8.5 ± 1.1^{g}	$6.1\pm0.3^{g*}$
Biological enzyme pre-treatments					
BOP-24$h_{50°C}$ - control	$19.6\pm2.0^{g*}$	$92.1\pm0.2g^{*}$	$4.6\pm0.2^{i*}$	18.0 ± 2.9^{gh}	$9.6\pm0.2^{g*}$
BOP-Fungi$_{14d}$	$38.6\pm6.2^{h*}$	$87.3\pm0.2i^{*}$	$9.2\pm0.6^{h*}$	24.8 ± 0.5^{h}	$9.9\pm0.2^{g*}$
BOP-$NH3_{50°C}$-Fungi$_{14d}$	$68.1\pm8.9^{i*}$	$90.2\pm1.7^{gi*}$	$6.0\pm0.04^{g*}$	34.6 ± 2.1^{i}	$9.4\pm0.2^{g*}$
Blending of substrates					
BP-control	11.3±0.01	86.3±0.1	5.75	10.19	8.85
OP-control	18.8±0.04	86.3±0.1	4.1	41.04	4.07
0% fish waste	18.6±3.7	93.0±2.3	4.9±0.5	19.2±10.0	8.0±2.0
10% fish waste	18.0±3.1	91.4±2.0	4.7±0.7	14.2±3.9	8.8±0.8

25% fish waste	22.0±3.4	89.5±1.9	5.9±0.6	16.8±9.3	8.6±0.5
50% fish waste	22.7±3.1	88.2±0.8	6.3±0.3	31.3±11.2	8.3±0.5
75% fish waste	24.6±1.6	86.0±2.1	6.6±0.1	28.1±5.5	8.3±0.3

Table 4. *Nutritional composition of untreated banana peels and pre-treated dessert banana peels. When samples from more than one replicate were analysed, values shown are mean ± standard deviation. In other cases, the value for the singlet sample is presented*

Substrate	Nitrogen	Crude fat	Crude fibre	Soluble carbohydrate	Total phenols	C/N	Crude protein	Prot/Carb
	($\%_{ww}$)	($\%_{ww}$)	($\%_{ww}$)	($\%_{ww}$)	(mg GAE g^{-1}_{00} extract)	ratio	($\%_{ww}$)	ratio
Untreated banana peels								
Juice banana peels	0.3	0.4	20.4	3.7	0.089	64.8	1.6	0.44
Dessert banana peels	0.11±0.03	0.2±0.001	8.5±0.1	1.7±0.2	0.98 ±0.05	66.0± 19.3	0.69±1.6	0.41±0.14
Microbial pre-treatments								
$Trich_{7d}$	0.06±0.01	0.1±0.002	3.2±0.3	1.4±0.4	0.14±0.12	96.3±20.3	-	-
$Trich_{14d}$	0.0	0.1	3.4	1.3	0.05	108.1	-	-
$Trich_{21d}$	-	-	-	-	-		-	-
$Rhiz_{7d}$	0.09±0.03	0.1±0.01	6.3±0.1	0.7±0.3	0.76±1.0	64.3 ±19.6	-	-
$Rhiz_{14d}$	-	-	-	-	-		-	-
$Rhiz_{21d}$	0.0	0.1	4.9	3.6	0.07	228.3	-	-
Bac_{7d}	0.07±0.02	0.2±0.01	8.0±1.9	0.7±0.2	1.0±0.10	104.1±30.1	-	-
Bac_{14d}	0.0	0.1	4.0	0.2	1.1	80.6	-	-
Bac_{21d}	-	-	-	-	-		-	-
Non-protein nitrogen pre-treatments								
$NH3_{0.8\ \%N}$	0.4	0.2	7.2	1.5	0.09	15.0	-	-
$NH3_{1\ \%N}$	0.36±0.1	0.1±0.1	7.3±2.1	1.1±0.6	0.47±0.30	15.8±0.4	-	-
$NH3_{0.8\ \%N\_14d}$	0.4	0.2	7.5	0.7	1.1	19.2	-	-
Heating pre-treatment							-	-
Heat	0.06±0.02	0.4±0.02	5.9±0.1	1.5±0.5	3.5±2.8	104.4±47.1	-	-
Non-protein nitrogen and microbial pre-treatments								
$NH3_{0.8\%N}$+$Trich_{7d}$	0.1	0.1	5.7	1.2	0.5	57.4	-	-
$NH3_{0.8\ \%N}$+$Rhiz_{7d}$	0.25±0.01	0.1±0.003	6.3±0.1	0.8±0.03	0.42±0.16	22.1±0.9	-	-
$NH3_{0.8\ \%N}$+Bac_{7d}	0.20±0.1	0.1±0.005	5.5±0.2	1.0±0.01	0.74±0.98	26.5±12.03	-	-

Heating and microbial pre-treatments								
Heat+Trich$_{7d}$	-	-	-	-	-		-	-
Heat+Rhiz$_{7d}$	0.1	0.4	7.2	1.4	0.2	54.7	-	-
Heat+Bac$_{7d}$	0.18±0.01	0.6±0.4	11.9±1.0	2.4±0.4	3.1±1.1	66.9±1.4	-	-

Table 5. *Nutritional composition of the controls, enzyme-treatments, biological enzyme pre-treatments and substrate blends of banana peels, orange peels and fish waste, presented as average values (n=3) ± standard deviation. Significant differences (*p*<0.05, Kruskal-Wallis test) are denoted *. Different letters within columns indicate significant differences*

Substrate	Nitrogen	Crude fat	Crude fibre	Carbohydrate	Total phenols	C/N	Crude protein	Prot/Carb
	(%ww)	(%ww)	(%ww)	(%ww)	(%ww)	ratio	(%ww)	ratio
Enzyme-treatments								
Banana peels								
BP-control	0.2±0.01^{a*}	1.2±0.3^{a*}	2.3 ±0.2^{a}	7.4 ±0.7^{a}	<0.2	42.3±3.5^{a}	1.0 ±0.1^{a*}	0.13±0.01^{a}
BP-Enz-0d	0.2±0.01^{a*}	0.9±0.1^{a*}	1.1±0.1^{b}	8.8±0.4^{a}	<0.2	38.4±8.5^{a}	1.3±0.1^{a*}	0.14±0.01^{a}
BP-Enz-24h$_{50°C}$	0.35±0.1^{a*}	2.1±1.0^{a*}	1.6±0.4^{b}	14.8±2.1^{b}	<0.2	34.0±8.7^{a}	2.2±0.5^{a*}	0.15±0.02^{a}
Orange peels								
OP-control	0.3±0.01^{d*}	0.7±0.04^{d}	2.8± 0.2^{d}	16.7±0.2^{d*}	0.3	47.0±6.0^{d}	1.8±0.1^{d*}	0.11±0.004^{d}
OP-Enz-0d	0.3±0.01^{d*}	0.8±0.3^{d}	2.0±0.2^{e}	17.6±0.4^{d*}	0.3	38.7±1.4^{d}	2.1±0.1^{d*}	0.12±0.004^{d}
OP-Enz-24h$_{50°C}$	0.6±0.1^{e*}	0.7±0.2^{d}	3.7±0.2^{f}	26.7±0.8^{e*}	0.5	34.9±7.1^{d}	3.8±0.6^{e*}	0.14±0.02^{e}
Banana-orange peel mix								
BOP-control	0.2^{g*}	1.0^{g*}	2.5^{g*}	10.6^{g*}	~0.2	43.8±0.2^{g}	1.2^{g*}	0.12^{g}
BOP-Enz-0d	0.28^{h*}	1.0^{g*}	1.5^{h*}	11.9^{h*}	<0.2	32.5±2.5^{hi}	1.8^{h*}	0.15^{h}
Biological enzyme pre-treatment								
BOP-24h$_{50°C}$ - control	0.45±0.03^{i}	0.9±0.2^{g*}	2.8±0.2^{i*}	10.3±0.4^{g*}	<0.2	22.2±3.6^{ij}	2.8±0.2^{i*}	0.28±0.01^{i}
BOP-Fungi$_{14d}$	0.74^{i*}	3.7^{h*}	11.2^{i*}	21.3^{i*}	0.4	25.2±4.0^{hij}	4.6^{i*}	0.22^{j}
BOP-NH3$_{50°C}$-Fungi$_{14d}$	0.25^{k*}	4.4^{i*}	14.3^{k*}	29.1^{k*}	0.4	13.5±1.9^{k}	15.8^{k*}	0.54^{k}
Blending of substrate								
Untreated banana peel	0.14	1.10	1.90	6.60	0.10	56.10	1.05	0.13
Untreated orange peel	0.17	0.27	2.60	14.10	0.37	78.9	0.86	0.07
0% fish waste	0.1±0.01	0.7±0.3	2.2±0.2	9.7±2.4	0.2±0.1	68.6±10.2	0.9±0.1	0.1±0.02
10% fish waste	0.3±0.04	1.0±0.2	2.1±0.2	9.2±2.2	0.2±0.1	35.0±2.2	23.0±1.6	0.2±0.02
25% fish waste	0.5±0.1	1.5±0.1	1.9±0.1	8.4±1.8	0.2±0.1	23.8±1.5	38.2±1.3	0.4±0.03
50% fish waste	1.0±0.1	2.5±0.1	1.5±0.1	7.0±1.4	0.2±0.1	12.8±0.6	63.7±1.0	0.9±0.1
75% fish waste	1.6±0.1	3.8±0.2	1.0±0.02	4.9±0.6	0.3±0.04	8.4±0.2	89.1±0.5	2.1±0.1

5.1.3 Process efficiency in BSFL composting treatments

The BSFL survival rate on the untreated banana peel substrate was above 90%, while the survival rate on the pre-treated banana peels varied from 51% to ≥90% (Table 6). Final larval weight was lower (<134 mg larva^{-1}) than in the control only in the 21 day *rhizopus* ($Rhiz_{21d}$), heat, and combined heat and 7 day *rhizopus* (Heat+$Rhiz_{7d}$) pre-treatments. High BCE, of around 15%, was achieved in 14 days of microbial pre-treatment with *rhizopus* and bacteria ($Rhiz_{14d}$, Bac_{14d}) and $NH3_{0.8\ \%N}$+$Rhiz_{7d}$ pre-treatment of banana peel, while material reduction of above 50% was obtained only in the 7 day bacteria (Bac_{7d}) and combined Heat+Bac_{7d} pre-treatments of banana peel. The highest VS loss, 64%, was observed in the combined Heat+Bac_{7d} pre-treatment, while heat pre-treatment had the lowest VS loss (5%) (Table 6).

Low survival rates (<70%) were observed in BP-Enz-$24h_{50°C}$, in all orange peel treatments, in BOP-$NH3_{50°C}$-$Fungi_{14d}$ and substrate blends with 10%, 50% and 75% fish waste (Table 7). Lower final larvae weights in comparison with the controls were seen only in OP-Enz-$24h_{50°C}$ enzyme pre-treatment, BOP-$Fungi_{14d}$ and the substrate blend with 0% fish waste (in comparison with the banana peel control). Highest BCE_{vs} compared with the control was seen in BP-Enz-0d (15%), OP-Enz-0d (8%), BOP-Enz-0d (16%), BOP-$NH3_{50°C}$-$Fungi_{14d}$ (9%) and the substrate blend with 75% fish waste (18%) (Table 7). Only the OP-Enz-0d enzyme-treatment and BOP-$Fungi_{14d}$ pre-treatment gave material reduction below 50%, while the VS loss was lowest (35%) in the OP-Enz-0d enzyme-treatment (Table 7).

Table 6. *Survival rate, final larvae weight, biomass conversion efficiency, material reduction and volatile solids (VS) loss in black soldier fly larvae (BSFL) composting of banana peels and pre-treated dessert banana peels. When triplicates were analysed, values are presented as mean (n = 3) ± standard deviation. In other cases, the value for the singlet sample is presented. Different letters within columns indicate significant differences (*$p<0.05$*, only triplicate samples)*

Substrate	Survival rate	Final larvae weight	Biomass conversion efficiency	Material reduction	VS loss
	(%)	(mg larva^{-1})	($\%_{VS}$)	($\%_{VS}$)	(%)
Untreated banana peels					
Juice banana peels	97.7±1.9	33±2^{a}	0.87±0.09^{a}	70.2±3.2^{a}	69.4±3.2
Banana desert peels	91.5±6.8	134±3^{b,c}	7.2±1.2^{b,c,e}	48.9±17.4^{a,c}	41.8±16.5
Microbial pre-treatments					
$Trich_{7d}$	90.8±4.5	169±60^{b,d,c}	8.2±1.4^{c,d}	36.3±2.9^{b,c}	28.1±4.3
$Trich_{14d}$	93.5	199	11.6	35.2	23.6
$Trich_{21d}$	60.5	226	9.5	35.1	25.6
$Rhiz_{7d}$	59.3±18.1	213±11^{d,e,f}	6.7±1.9^{b,c,d,e}	32.3±3.7^{b,c}	25.6±4.3
$Rhiz_{14d}$	99.5	220	15	38.3	23.3
$Rhiz_{21d}$	98	119	8	38.5	30.5
Bac_{7d}	89.2±7.7	194±15^{b,d}	8.1±1.0^{c,d,e}	51.0±1.2^{a,c}	43.0±0.6
Bac_{14d}	97	152	14.5	26.5	12
Bac_{21d}	94	151	9.9	36	26
Non-protein nitrogen pre-treatments					
$NH3_{0.8\ \%N}$	97.7±2.4	177±23^{b,d}	7.1±0.5^{b,c,e}	40.3±7.9^{c}	33.2±7.5
$NH3_{1\ \%N}$	89.3±9.4	176±44^{b,e}	9.6±3.9^{c,d,e}	25.6±17.5^{b,c}	16.0±21.5
$NH3_{0.8\ \%}$N_14d	97	140	6	49.5	43.5
Heating					
Heat	66.3±7.8	113±21^{b}	3.5±0.8^{a,b}	8.9±0.6^{b}	5.4±0.3
Non-protein nitrogen and microbial pre-treatments					

$NH30_{.8\%N}$+$Trich_{7d}$	51	231	6.1	44.8	38.8
$NH3_{0.8\ \%N}$+$Rhiz_{7d}$	94.0±2.3	229±22[d,e]	14.8±1.2[d]	47.8±4.3[a,c]	33.0±4.0
$NH3_{0.8\ \%N}$+Bac_{7d}	90.5±10.0	187±32[b]	11.0±1.7[d,e]	29.1±3.7[b,c]	18.1±4.8
Heating and microbial pre-treatments					
Heat+$Trich_{7d}$	94.5	136	7.4	24	16.6
Heat+$Rhiz_{7d}$	74.0±16.2	105±11[a,c,f]	2.6±0.10[a,b]	25.7±3.3[b,c]	23.1±3.3
Heat+Bac_{7d}	88.0±8.0	143±12[b,f]	3.3±0.43[b]	66±1.0[a]	64.7±1.9

Table 7. *Survival rate, final larvae weight, biomass conversion efficiency, material reduction and volatile solids (VS) loss in black soldier fly larvae (BSFL) composting of the controls, enzyme-treatments, biological enzyme pre-treatments and substrates blends of banana peel, orange peel and fish waste. Values shown are mean ± standard deviation (n = 3). Significant differences (*p<0.05,Kruskal-Wallis test) are denoted *. Different letters within columns indicate significant differences (p<0.05) between banana peel (a-c), orange peel (d-f) and a mixture of banana peel and orange (g-k) treatments*

Substrate	Survival rate	Final larvae weight	Biomass conversion efficiency	Material reduction	VS loss
	(%)	(mg larva^{-1})	(%$_{DM}$)	(%$_{DM}$)	(%)
Enzyme-treatments					
Banana peels					
BP-con	92.7±5.1^{a}	111.9±10.1^{a}	10.6±0.3^{a}	66.3±12.1^{a}	55.7±12.0^{a}
BP-Enz-0d	81.2±2.7^{b}	150.3±9.5^{a}	15.4±1.2^{b}	80.4±4.4^{a}	64.9±4.1^{a}
BP-Enz-24h$_{50°C}$	66.9±1.2^{c}	123.6±24.2^{a}	10.9±1.9^{a}	66.1±1.0^{a}	55.2±1.5^{a}
Orange peels					
OP-con	27.3±20.0^{d}	200.2±49.5^{d}	5.8±1.6^{d}	74.8±2.9^{d}	68.9±3.2^{d}
OP-Enz-0d	47.2±23.7^{d}	149.3±18.7^{d}	8.0±2.5^{d}	42.7±19.6^{d}	34.7±17.4^{d}
OP-Enz-24h$_{50°C}$	67.2±19.3^{d}	43.4±13.2^{e}	5.5±1.6^{d}	52.5±18.7^{d}	47.0±17.3^{d}
Banana-orange peel mix					
BOP-con	79.8±4.3^{g}	107±6.7^{gh}	6.7±0.5^{g*}	44.0±24.3^{g*}	37.3±23.9
BOP-Enz-0d	87.5±9.4^{g}	165.1±21.8^{i}	15.6±1.0^{h*}	65.3±5.1^{g*}	49.74±6.2
Biological enzyme pre-treatment					
BOP-24h$_{50°C}$ - control	91.2±8.0^{g}	88.2±3.6^{gh}	7.4±0.7^{g*}	59.7±1.5^{g*}	52.3±2.2

BOP-Fungi$_{14d}$	78.7±2.5^{g}	65.7±17.4^{h}	7.9±2.5^{g*}	47.8±25.4^{g*}	39.9±24.3
BOP-NH3$_{50°C}$-Fungi$_{14d}$	61.4±1.7^{h}	132.7±19.1^{gi}	9.4±0.6^{g*}	58.0±4.9^{g*}	48.6±4.5
Blending of substrates					
Untreated banana peels	100.00	83.00	5.80	49.50	44.20
Untreated orange peels	100.00	51.00	3.90	49.70	45.80
0% fish waste	96.4±11.3	70.3±0.2	4.5±1.3	61.8±8.5	57.3±9.3
10% fish waste	63.5±20.5	136.7±0.3	7.8±1.9	57.5±6.3	49.7±6.0
25% fish waste	83.0±10.7	167.9±0.2	12.3±2.1	69.5±4.2	57.41±4.1
50% fish waste	65.6±18.3	182.6±0.3	13.2±2.1	60.3±7.2	47.1±7.2
75% fish waste	66.0±27.6	219.6±0.3	18.0±5.8	56.4±8.9	38.4±4.4

5.2 Food industry waste survey (Paper IV)

5.2.1 Status of food industry waste generation and management

The survey on selected food companies in Tanzania showed that only 29 out of 42 participating companies produced biowaste in their production processes (Paper IV). The quantities of biowaste produced per year varied (≤500 to >1000 tons) between and within different company categories (Figure 5a). The quantity produced by most food companies (25 out of 29) also varied throughout the year, with type and nature of raw materials used (22 out of 25), demand and supply (5 out of 25) and technological reasons (2 out of 25) selected by the food companies in the questionnaire (Paper IV). Few food companies (4 out of 29) re-used ≥25% of their biowaste. The remaining companies either gave away or sold their biowaste for animal feeds or opted to dispose of it in landfill (Figure 5b). Having no value for usage (5 responses), high cost required for usage (9 responses) and waste contamination (10 responses) were reasons highlighted by the food companies for not re-using their biowaste (Paper IV). The biowaste management costs per year incurred by the food companies varied (<0 - ≥5000 USD) (Figure 5c). These costs arose during collection, storage, transportation and disposal of the solid waste (Figure 5d).

The food industry companies that generated biowaste perceived their current waste management practice to be good or very good (Figure 5e), but the majority (17 out of 29) still believed that there was room for improvement (Figure 5f). Almost all the food companies (28 out of 29) that produced biowaste agreed that their biowaste streams could have value if managed properly (Figure 5g). Almost all the biowaste streams (27 out of 29) were reported to be available for utilisation if other stakeholders outside the companies were interested in managing them (Figure 5h).

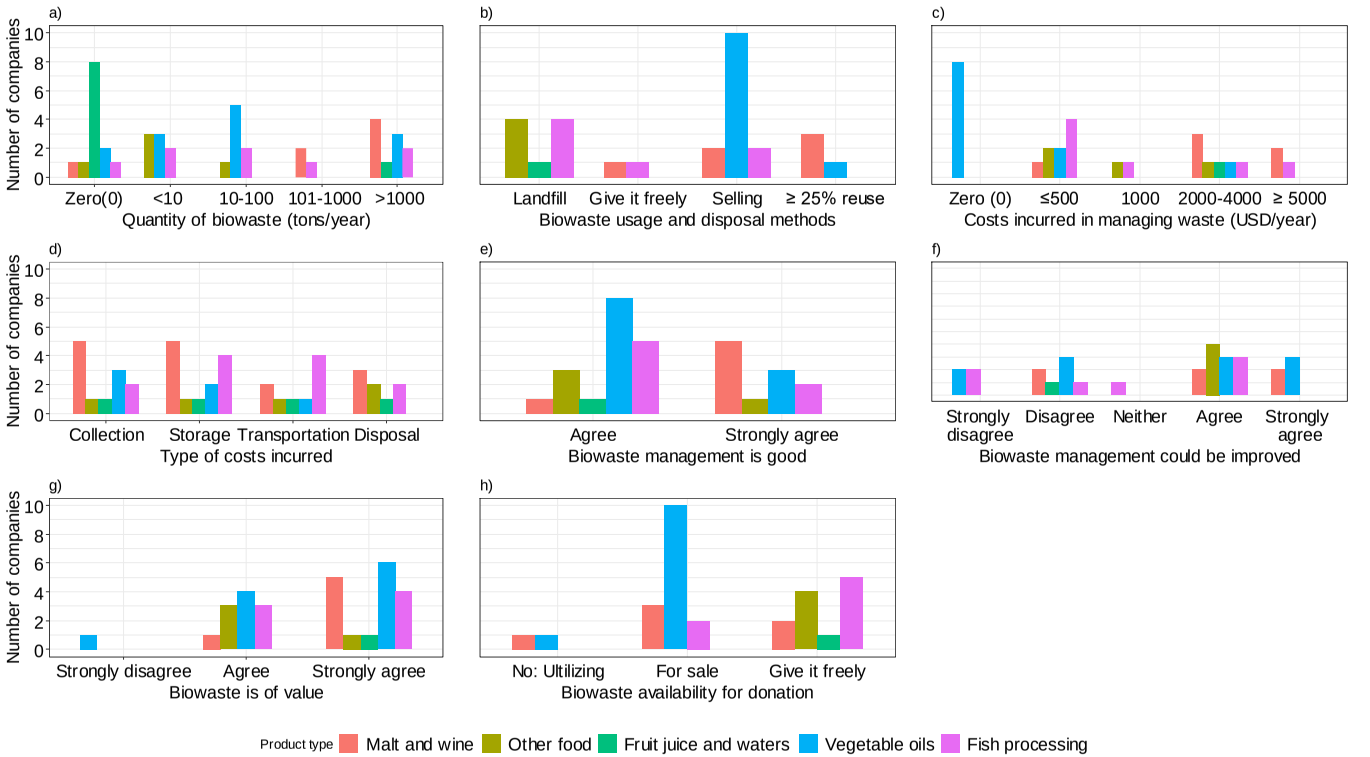

Figure 5. Graphical representation of the status of current production and management of biowaste at the Tanzanian food industry companies surveyed (n=29) and their perceptions on the food industry waste generated.

5.2.2 Assessment of food industry biowaste for production of animal protein using BSFL composting

Banana peels, mango seeds, sunflower press cake, brewery waste and coffee husks were the main types of biowaste produced by the food industry companies surveyed (Table 8 and 9). All the food industry waste was reported to be accessible and the amount of waste available ranged from 100,000 to 1,000,000 kg y^{-1} (Table 8). Brewery waste and banana peels were available in higher amounts that the other food industry wastes (Table 8). However, availability of brewery waste together with sunflower press cake waste for BSFL composting was limited by competition from existing users and high purchase cost (Table 8).

Fish waste was the only biowaste that had optimal moisture content (68%) while banana peel with the highest moisture content (87%) scored the least (Table 9). Moreover, banana peels together with mango seeds and coffee husks had the lowest protein concentration ($\leq 11\%_{DM}$). Mango seeds had too high carbohydrate concentration, while that in fish waste was too low, and thus both types of biowaste attained scores lower than 2 (Table 9). The VS content in all types of substrate was above the amount needed for adequate BSFL growth. Brewery waste, sunflower press cake and banana peels had an optimal Prot/Carb ratio (Table 9).

Table 8. *Average suitability scores of food industry waste types, based on four availability sub-criteria (accessibility, amount, purchase cost, competing use). Companies producing similar types of biowaste gave different responses to the same sub-criterion, so an average score was assigned for each sub-criterion based on fractions of a company's proportions of 'YES' and 'NO' responses in each food industry company category*

Bio-waste	Accessibility		Amount		Purchase cost		Competition	
	Response	Score	(Kg y^{-1})	Score	Response	Score	Response	Score
Brewery waste (n=4)	Yes	2.5±1.0	100,000-1,000,000	2.7±1.0	Yes	1.0±0.0	Yes	1.0±0.0
Sunflower press cake (n=12)	Yes	3.0±0.0	100,000-1,000,000	1.5±0.9	Yes	1.2±0.6	Yes	1.2±0.6
Banana peels (n=2)	Yes	3.0±0.0	100,000-1,000,000	2.5±0.7	Yes	2.5±0.7	Yes	2.0±0.0
Mango seeds (n=2)	Yes	3.0±0.0	100,000-1,000,000	2.0±1.4	No	3.0±0.0	No	3.0±0.0
Coffee husks(n=2)	Yes	3.0±0.0	< 10,000	1.0±0.0	No	3.0±0.0	No	3.0±0.0
Fish waste (n=7)	Yes	3.0±0.0	100,000-1,000,000	1.7±1.0	Yes	2.4±1.0	Yes	1.9±0.9

Table 9. *Calculated average scores attributed to physical-chemical properties of food industry waste, based on published data (n≥3 or 4).*

Biowaste	Moisture Content (%)	score	Protein (% DM)	score	Carbohydrates (% DM)	score	Volatile (%)	score	×Prot:Carb	Score
Brewery waste	5.5±2.1[a]	2.0±0.0	23.5±1.2[a]	3.0±0.0	51.5±7.9[a]	2.0±0.0	91.8±7.6[a]	2.3±0.6	1:02	3
Sunflower press cake	8.69±0.5[b]	2.0±0.0	30.5±6.5[b]	2.3±0.5	41.1±14.6[b]	2.3±0.5	91.6±3.5[b]	2.0±0.0	1:01	3
Banana peels	87.1±1.9[d]	1.0±0.0	6.7±3.4[c]	1.0±0.0	29.2±20.7[c]	2.3±1.0	87.0±2.8[c]	2.0±0.0	1:04	3
Mango seeds	47.1±4.0[e]	2.0±0.0	5.7±0.6[e]	1.0±0.0	35.0±25.2[e]	1.8±0.5	97.8±0.7[e]	2.0±0.0	1:06	2
Coffee husks	12.2±3.0[g]	2.0±0.0	10.8±4.1[f]	1.5±0.6	70.1±3.2[h]	2.0±0.0	91.0±9.3[f]	2.3±0.5	1:07	2
Fish waste	68.0±3.3[j]	3.0±0.0	47.2±17.3[i]	2.3±0.5	5.5±1.7[k]	1.0±0.0	93.5±6.2[j]	2.0±0.0	8:01	2

×Prot:Carb ratio, calculated from average literature values for carbohydrates and protein on a dry matter (DM) basis. Letters on each physical-chemical property indicate the source of the data listed immediately below:

[a](Farcas et al., 2021, Ajanaku et al., 2011, Naibaho and Korzeniowska, 2021).
[b](Rincón et al., 2011, Petraru et al., 2021, Chauhan, 2021).
[c] (Isibika et al., 2021, Isibika et al., 2019, Hassan et al., 2018, Ogunlade et al., 2021). Nmh
[d] (Isibika et al., 2019, Isibika et al., 2021, Khan and Perveen, 2010).
[e] (ELEGBEDE et al., 1995, Nzikou et al., 2010, Tesfaye, 2017).
[f](Setter et al., 2020, Oliveira and Franca, 2015, Murthy and Madhava Naidu, 2012, Elsawy et al., 2021).
[g] (Setter et al., 2020, Oliveira and Franca, 2015, Murthy and Madhava Naidu, 2012).
[h](Murthy and Madhava Naidu, 2012, Oliveira and Franca, 2015, Elsawy et al., 2021).
[i](Isibika et al., 2021, Lopes et al., 2020, Shanthi et al., 2021, Nguyen et al., 2015).
[j](Isibika et al., 2021, Lopes et al., 2020, Shanthi et al., 2021)
[k](Isibika et al., 2021, Shanthi et al., 2021, Nguyen et al., 2015)

6. Discussion

6.1 BSF larvae composting efficiency of pure fish waste, banana peel and orange peel

Rearing BSFL on pure banana peel and orange peel substrates resulted in low BCE_{vs} (<11%) (Tables 6 and 7), while no BSFL survival was observed on pure fish waste (Paper III). One of the reasons for the low BCE_{vs} observed for pure banana peels and orange peels could have been the low protein concentration (<2%$_{WW}$) in these substrates (Tables 4 and 5). Rehman *et al.* (2017b) and Lalander et al. (2019), among others, have emphasised the importance of sufficient protein feeding dose as one of the most limiting factors for BSFL growth. Moreover, the high fibre concentration (>2%$_{WW}$) in banana peels and orange peels (Papers I-III) could have impacted the BCE_{vs}. Waste substrates characterised by high fibre concentration (Rehman et al., 2017a) and high fat concentration (Nguyen et al., 2013, Lopes et al., 2020) have also been reported to be challenging in terms of nutrient availability and conversion into larval biomass. Orange peels, like other citrus fruits, have been demonstrated to contain phenolic acids and essential oils (mainly D-limonene) that have the potential to inhibit the growth of several fungal and bacterial strains (Bora et al., 2020). These compounds also exert insecticidal activity (Oboh et al., 2017), *e.g.* against larvae and pupae of housefly (Kumar et al., 2012) and Mediterranean fruitfly (Bachi and Ahmed, 2017). The high phenol concentration in banana and orange peels and the high fat concentration in orange peels could thus have contributed to the observed low BCE_{vs} (Tables 4-5; Paper III). The 100% mortality of BSFL on fish waste could have been due to high fat concentration together with lack of structure in the substrate, as observed by others (Nguyen et al., 2013, Lopes et al., 2020). The pure banana peel substrate resulted in low BCE_{VS}

(0.9-11%) in Papers I-III, on average lower than that (12%) reported by Nyakeri *et al*. (2017). This observed variation could have been caused by using different types of banana peel with varying nutritional composition in the different trials (Tables 4 and 5) and/or by modifications in the BSFL composting set-up. It has been demonstrated that factors such as larval density and larval feeding rates, which varied between the three experimental sets-up in this thesis (Table 1), can affect BSFL process efficiency (Parra Paz et al., 2015). Overall, the low BSFL process efficiency observed for the individual pure substrates emphasises the need to explore use of pre-treatments and blending of substrates to enhance bioconversion efficiency.

6.2 Impacts of applying pre-treatments and blending substrates prior to BSFL composting

6.2.1 Microbial pre-treatment with bacteria, *Trichoderma reesei* and *Rhizopus oligosporus*

Microbial pre-treatments of banana peel using one strain of microorganisms generally resulted in higher final larval weight and BCE_{vs} (Paper I). The exceptions were 7 d *R.* oligosporus ($Rhiz_{7d}$) pre-treatment of banana peel, which resulted in slightly lower BCE_{vs}, and 21 d *R. oligosporus* ($Rhiz_{21d}$) pre-treatment, which gave lower final larval weight compared with the control (Table 6). In Paper I, BCE_{vs} was found to correlate negatively with the concentrations of tannins and phenolic compounds, which might have contributed to the low BCE_{vs}. The reason for using microorganisms in pre-treatment was their known ability to produce and secrete enzymes, such as cellulase and hemicellulase (Ling-qi, 2012, Mustafa et al., 2016, Sivaramakrishnan et al., 2021). These enzymes are capable of degrading complex molecular structures, *e.g*. fibre (Sindhu et al., 2016), which were expected to increase the concentration of easily digestible simple monomers available for BSFL growth. The increase in BCE_{vs} and final larval weight in most treatments could have been influenced by the ability of the microorganisms to degrade fibre into more available nutrients, as lower fibre concentration ($<8.5\%_{WW}$) was observed in all microbial pre-treatments compared with the control (Table 6). Interestingly, with the exception of banana peels pre-treated for 7 d with *T. reesei* ($Trichd_{7d}$), the 14 d microbial pre-treatments of banana peels gave much lower fibre content ($<4\%_{WW}$) than

the 7 d and 21 d microbial pre-treatments and the control (Table 6). This suggests that the microorganisms needed a longer pre-treatment time (>7 d) to degrade fibre sufficiently to result in increased BCE_{vs} (>11%$_{DM}$ in the 14 d microbial pre-treatments) (Paper I). Similarly, a 14 d microbial pre-treatment time, using *Phanerochaete chrysosporium* and *Trichoderma reesei* fungi, has been found to be more effective in converting cotton stalks and banana peels into more easily available forms (Shi et al., 2008, Katongole et al., 2017). In accordance with the findings in this study, Wong et al. (2019) observed that a 14 d pre-treatment time using a mixture of *Bacillus* spp. on coconut endosperm waste resulted in higher BCE_{vs} than that obtained with 7 d and 21 d pre-treatments. These suggests that the 14 d microbial pre-treatment time resulted in increased availability of easily available carbon compared with 7 d microbial pre-treatment (Paper I). The low BCE_{vs} that resulted from the 21 d microbial pre-treatment time suggests that the microorganisms themselves consumed nutrients intended for larval growth. Microbial pre-treatment was thus found to be capable of increasing the amount of readily available nutrients in the fibre-rich banana peel and a 14 d pre-treatment time to yield the highest BCE_{vs} was recommended (Paper I).

6.2.2 Chemical pre-treatments with nitrogen supplementation

Chemical pre-treatment of banana peels with ammonia solution was intended to facilitate degradation of organic matter and provide an extra nitrogen source, which, with the help of ammonia-assimilating bacteria would be converted into forms available for BSFL growth (Paper I). With the exception of *T. reesei* and ammonia combined pre-treated banana peel ($NH3_{0.8\%N}$+$Trich_{7d}$), the nitrogen concentration increased in all chemical pre-treatments on addition of ammonia solution. The lack of nitrogen concentration increase in $NH3_{0.8\%N}$+$Trich_{7d}$ could have been due to higher nitrogen losses, perhaps facilitated by the consumption of the substrate by *T. reesei* during the pre-treatment process (Paper I). All chemically pre-treated substrates had lower concentrations of fibre and phenols compared to their controls. The combined chemical and microbial pre-treatments also had lower concentrations of fibre compared with the pre-treated substrates with ammonia addition only (Table 4). When the chemically pre-treated substrates were fed to the BSFL, the final larval weight of larvae reared on all these substrates was higher than that in their respective control (Table 6).

The increase in final larvae weight indicated that more nutrients were available to the BSFL in the banana peel substrate chemically pre-treated with ammonia solution than in untreated banana peel (Paper I).

However, the conversion efficiency, BCE_{vs}, was only increased (from 7% to 11%) for chemically pre-treated banana peels with ammonia solution ($NH3_{1\%N}$) and for the two banana peel types with combined ammonia and fungi pre-treatments ($NH3_{0.8\%N}$+$Rhiz_{7d}$ and $NH3_{0.8\%N}$+Bac_{7d}) (Table 6). The conversion efficiency of the pre-treated banana peels with lower concentrated ammonia solution ($NH3_{0.8\%N}$) or with ammonia solution for 14 d ($NH3_{0.8\ \%N\_14d}$) and the banana peel pre-treated by ammonia solution followed by *T. reesei* ($NH3_{0.8\%N}$+$Trich_{7d}$) was not improved (Paper I). Palma et al. (2019) observed increased substrate consumption and an increase in final larval weight when almond hull-based substrate was supplemented with urea as a model nitrogen source. Those authors concluded that the increase in BSFL composting efficiency obtained for the nitrogen-supplemented almond hulls was likely due to an observed decrease in substrate C/N ratio from 49 to 16 (Palma et al., 2019). In Paper I, the nitrogen concentration increased and the C/N ratio decreased in all chemical pre-treatments of banana peel substrates, except for $NH3_{0.8\%N}$+$Trich_{7d}$, where the nitrogen concentration was similar to that in the corresponding control (Table 4). Moreover, although the C/N ratio decreased in all chemical pre-treatments of banana peel substrates, that in $NH3_{0.8\ \%N\_14d}$ and $NH3_{0.8\%N}$+$Trich_{7d}$ was higher than that in the other pre-treatments involving non-protein nitrogen and a combination of non-protein nitrogen and microbial pre-treatment (Table 4). The low nitrogen concentration in $NH3_{0.8\%N}$+$Trich_{7d}$ and the higher C/N ratio in $NH3_{0.8\ \%N\_14d}$ and $NH3_{0.8\%N}$+$Trich_{7d}$ could have contributed to the observed lack of improvement in BCE_{vs} in these pre-treatments (Table 6). Higher VS loss (>33%) was observed in the $NH3_{0.8\%N,}$ $NH3_{0.8\ \%N\_14d}$ and $NH3_{0.8\%N}$+$Trich_{7d}$ pre-treatments than in the other chemical pre-treatments (VS loss <33%) (Table 6). The low BCE_{vs} in the $NH3_{0.8\%N}$, $NH3_{0.8\ \%N\_14d}$ and $NH3_{0.8\%N}$+$Trich_{7d}$ and relatively high VS losses suggest that these pre-treatments favoured microbial respiration rather than BSFL growth (Table 6). The increased BCE_{vs} and low VS losses seen for the other chemically pre-treated substrates (Table 6) suggests that BSFL growth was favoured rather than microbial respiration and could have been caused by the observed increase in nitrogen concentration and the lower concentrations of fibre, phenol and C/N ratio in those substrates (Table 4).

Interestingly, a greater increase in BCE_{vs} was observed for the $NH3_{0.8\%N}$+$Rhiz_{7d}$ and $NH3_{0.8\%N}$+Bac_{7d} combined pre-treatments of banana peel (Table 5, Paper I). Carreiro et al. (2000) demonstrated ability of nitrogen supplementation to improve growth of microorganisms and synthesis of enzymes necessary to break down lignocellulose through increased cellulase activity in decaying leaf litter of flowering dogwood, red maple and red oak. Moreover, Almqvist (2021), found an increase in total amino acid content (124% of inflow) in larvae and residue after BSFL composting ammonia pre-treated banana peels, suggesting that the BSFL, likely with the help of microorganisms, were able to convert non-protein nitrogen into protein. The increase in BCE_{vs} in the combined microbial and nitrogen supplementation pre-treatments (Paper I) likely increased degradation of complex molecules into forms more available to the BSFL, while also increasing substrate nutrient availability (to larvae and/or microorganisms) and thus utilisation by BSFL.

6.2.3 Blending fruit waste with fish waste

Blending banana peel, orange peel and fish waste substrates were intended to provide a more balanced nutritional composition compared with that in the individual biowastes (Paper III). Blending banana peel and orange peel with 0% fish waste resulted in low BCE_{vs} (~5%) and low final larval weight (~70 mg $larva^{-1}$), lower than the corresponding values obtained for the banana peel control (Table 7, Paper III). The protein concentration (~0.9%$_{WW}$) in the 0% fish waste blend was as low as that in the banana peel (1%$_{ww}$) and orange peel (0.9%$_{WW}$) controls, and was likely too low to support growth of large BSFL larvae (Table 5). Decreasing orange peel inclusion in the fruit-fish waste mixtures generally increased BCE_{VS} (Paper III). The negative impacts of phenols and fat in orange peels (see section 6.1) likely contributed to the observed low process efficiency for mixtures with high inclusion of orange peel, so that an increasing banana peel inclusion in the mixtures diluted the toxicity of the orange peels and thus led to increased BCE_{VS} (Paper III). Blending the fruit waste mixtures with fish waste resulted in increased protein concentration (Table 5), which likely explained the observed increase in final larval weight and BCE_{vs} (Table 7). The mixture with 75% fish waste was best in terms of final larval weight (~220 mg $larva^{-1}$) and BCE_{vs} (~18%) (Table 7). However, there were large variations in final larval weight (182-269 mg $larva^{-1}$) and BCE_{vs} (12-25%) for the 75% fish

waste mixtures (Paper III). These large variations were possibly partly the result of including different types of fish waste (species composition), and in particular differences in fat concentration in the fish species as a result of season variation (Paper III). Variations in process efficiency when treating substrates with similar and different nutritional composition can affect the reliability and sustainability of a BSFL composting facility (Gold et al., 2018). BSFL composting efficiency increased greatly with 25% fish waste inclusion, but did not increase further with 50% inclusion. Moreover, with 25% fish inclusion and 0.4 Prot/Carb ratio, the percentage increase in BCE_{vs} was more than 100% on increasing the protein from 1% to 38% (ww basis) in the substrates. Therefore, in Paper III it was concluded that BSFL composting efficiency can be increased by blending fruit waste with fish waste, and that inclusion of 25% fish waste in the substrate results in a great increase in conversion efficiency without any substantial variations in process efficiency. These conclusions were in agreement with findings by Lopes et al. (2020), who recommended 10-15% inclusion of aquaculture waste with reclaimed bread to improve BCE_{vs} and protein concentration in the larvae. Thus blending fruit waste with protein-rich substrate like fish waste can be recommended for large-scale BSFL composting applications, to reduce substrate toxicity and improve the nutritional composition of the substrate, and thus increase process efficiency (Paper III).

6.2.4 Heat pre-treatment

The intention with using heat as a pre-treatment method prior to BSFL composting was to enhance degradation of complex molecular structures in the substrates, in order to increase the amount of readily available nutrients for BSFL growth (Paper I). None of the heat pre-treatments resulted in a substantial increase in overall BSFL composting process efficiency, although final larvae weight (143 mg $larva^{-1}$) and material reduction ($66\%_{WW}$) increased significantly for banana peels pre-treated with combined heat and 7 d of bacteria pre-treatment ($Heat+Bac_{7d}$) (Table 6). This suggests that this pre-treatment favoured microbial respiration rather than BSFL growth. The concentrations of tannins (Paper I) and phenolic compounds (Table 4) in the heat-treated substrates were found to be negatively correlated with BCE_{vs} (Paper I). These substances are known to inhibit microbial and BSFL growth (Bora et al., 2020, Kumar et al., 2012). Pre-treating the banana peels with *T. reesei* alone for 7 d ($Trich_{7d}$) resulted in a greater decrease in

fibre concentration, similar to the concentration decrease resulting from 14 d microbial pre-treatments (Table 4). The Heat+Trich$_{7d}$ pre-treated banana peels did not have the same low BCE_{vs}, possibly due to the ability of *T. reesei* to break down compounds present in the peels following heat pre-treatment into more readily available nutrients that aided conversion into BSFL biomass (Paper I). Complex compounds in the fibre-rich substrates were degraded by heat pre-treatment, as shown by observed changes in the nutritional composition of the heat pre-treated substrates (Table 4). However, the pre-treatment process was found to increase the concentrations of compounds such as phenols and tannins, which can have toxic effects in BSFL composting (Paper I).

6.2.5 Enzyme pre-treatment

In the enzyme-treatments, the enzymes were expected to hydrolyse complex macromolecules such as cellulose and hemicellulose into readily available carbohydrates (Paper II). Interestingly, direct addition of enzymes to banana peels (BP-Enz-0d) and banana-orange peel mix (BOP-Enz-0d) was found to result in significantly higher BCE_{vs} compared with their respective controls (Table 6). With the exception of BOP-Enz-0d, the protein and carbohydrate concentrations were not significantly increased in the Enz-0d enzyme treatments (Table 5). Thus, the increased BCE_{vs} in the treatments with direct enzyme addition could have been due to the observed reduction in fibre concentration ($\leq$1.5%$_{WW}$), as this was the only significant difference found for the nutritional parameters compared with their corresponding controls (Table 5, Paper II). However, in the direct addition of enzymes, samples for fibre analyses were taken prior to the addition of larvae. The hydrolysis of fibres into carbohydrates was thus not documented, thus the actual impact of hydrolysis was not known for that treatment. The high increase in BCE_{VS} found for this treatment suggest that the hydrolysis was efficient, potentially because larval activity somehow increased enzyme activity, either through their movement and/or due to an increased enzyme activity in the BSFL gut.

Biochemical enzyme-treatment at higher temperature (50 °C) was intended to increase enzyme activity and improve substrate degradation, to increased BCE_{vs} (Paper II). It was found that enzyme-treatment at 50 °C increased the carbohydrate concentration significantly compared with the control (Table 5). However, it did not result in improved survival rate, final

larval weight, BCE_{vs} or material reduction in either the banana peel (BP-Enz-$24h_{50°C}$) or orange peel (OP-Enz-$24h_{50°C}$) enzyme-treatments (Paper II).

It should be noted that carbohydrate type has been shown to influence BSFL survival rate, BCE and crude lipid concentration in the larvae produced (Cohn et al., 2022). Substrates with high concentrations of galactose, arabinose and xylan have been shown to result in low survival rate, BCE_{vs} and Red_{vs} while diets high in glucose, sucrose and fructose result in higher BCE_{vs} and Red_{vs} (Cohn et al., 2022). Furthermore, enzyme pre-treatment of orange peels using an enzyme cocktail containing cellulase, ß-glucosidases and pectinase at 45°C for 24 h has been reported to result in nearly complete conversion of carbohydrates into easily available sugars such as glucose (Grohmann et al., 1995). In that study, lower hemi-cellulolytic activity resulted in high sugar production with low amounts of galactose and arabinose (Grohmann et al., 1995). In Paper II, a slightly greater increase in carbohydrate concentration, from 11% to 12%, in the BOP-Enz-0d pre-treatment at 28 °C more than doubled the BCE_{vs} from 7% to 16%, while the BP-Enz-$24h_{50°C}$ and OP-Enz-$24h_{50°C}$ treatments, with a more significant increase in carbohydrate concentration, did not result in improved BCE_{vs} (Table 5). In the direct addition of enzymes, samples for fibre analyses were taken prior to the addition of larvae. The hydrolysis of fibres into carbohydrates was thus not documented, thus hydrolysis rate is not known for that treatment. The high increase in BCE_{vs} found for this treatment suggest that the hydrolysis was efficient, potentially because larval activity somehow increased enzyme activity, either through their movement and/or due to an increased enzyme activity in the BSFL gut. The enzyme cocktail containing cellulases, ß-glucosidases and hemicellulases applied at 50 °C for 24 h, on the other hand, could have enhanced enzyme activity and resulted in degradation products with inhibitory effects (Cohn et al., 2022, Grohmann et al., 1995) on BSFL composting process efficiency in treatments BP-Enz-$24h_{50°C}$ and OP-Enz-$24h_{50°C}$.

Another factor that could have contributed negatively on BSFL composting efficiency in the BP-Enz-$24h_{50°C}$ and OP-Enz-$24h_{50°C}$ treatments was their lower pH of 4.8 and 3.9 in comparison with their controls (Table 3, Paper II). Lower pH in BSFL composting has been found to inhibit BSFL growth and microbial activity (Ma et al., 2018, Chen et al., 2019, Liu et al., 2019). Starting BSFL composting with substrate initial pH of 6-10 has been found to enhance BSFL development and microbial community growth,

contributing to high BSFL survival rate, final larvae weight and biomass yield (Ma et al., 2018).

Overall, the BP-Enz-0d and BOP-Enz-0d direct enzyme treatments with lower crude fibre concentration of $\leq 2\%_{WW}$ (Table 5) resulted in the highest BCE_{vs} and Red_{vs} compared with their respective controls (Table 7). This is in agreement with Lindberg et al. (2022), who evaluated the effect of enzyme pre-treatment time of lettuce-cabbage waste (0, 2 and 4 d) and observed the highest BCE_{vs} and Red_{vs} for 0 d pre-treatment. Direct enzyme treatment appears to be the most effective strategy in BSFL composting for achieving high composting efficiency (Paper II). This higher efficiency suggests that the presence of BSFL in some way increased enzyme activity, while not resulting in the negative impacts observed in the enzyme treatment at elevated temperature.

The intention with using a fungi mixture containing *T. reesei* and *R. oligosporus* on banana-orange peel mixture was that fungi would produce and spread several types of enzymes (Paper II). The enzymes produced were expected to result in more complete break-down of fibre in the two types of peel, thus producing easily degradable carbohydrates readily available to the BSFL and ultimately resulting in higher BCE_{vs} (Paper II). However, the BCE_{vs} observed for BOP-$Fungi_{14d}$ was similar to that in the control, while the survival rate, final larvae weight and material reductions were lower than in the control (Table 7). This low efficiency BCE_{vs} could have been caused by observed increases in the concentrations of fats, phenols and fibre in the substrate after the pre-treatment (Table 5, Paper II). High concentrations of these compounds in waste substrates have been shown to reduce the availability of easily absorbable nutrients for BSFL growth and BSFL survival (Lopes et al., 2020, Nguyen et al., 2013, Rehman et al., 2017a). One reason why the fungi pre-treatment worked for banana peels only (Paper I), and not for the mixture of orange and banana peels (Paper II), could be the large material reduction in pre-treatment of the banana-orange peel mixture (Paper II). This could have concentrated toxic compounds such as phenolic compounds and essential oils present in the orange peels. The concentration of essential oil was not analysed, but the phenol concentration in orange peels was higher than that in banana peels (Table 5), and was also quite high in the fungi pre-treated banana-orange peel mixture (Paper II). The carbohydrate concentration in the fungi pre-treated orange-banana peel mixture increased greatly compared with the control (2-3-fold increase), but the increase in

fibre concentration was even higher (4-5-fold increase). The low BCE_{vs} despite the increase in carbohydrate concentration suggests that nutrients in the substrates were not available to the larvae. Thus it is likely that the increase in concentrations of crude fibre and potentially also undesirable carbohydrates in BOP-$Fungi_{14d}$ and BOP-$NH3_{50°C}$-$Fungi_{14d}$ contributed to the lack of improvement BCE_{vs} and material reduction (Paper II).

The biochemical direct enzyme-treatments of banana peel and banana-orange peel mixture degraded complex molecules into easily available nutrients (that could easily be utilised by the BSFL) in a shorter time than fungal pre-treatment (Paper II). Thus, biochemical direct enzyme-treatment was the best treatment choice for production of BSFL biomass. It should be noted that, even though BCE_{vs}, and material reduction BCE_{vs} were not improved in the biological fungi enzyme pre-treatments, the total material reduction resulting from the pre-treatment process and BSFL treatment was more than 80% (Paper II). Fungi and bacterial respiration plus consumption of a large proportion of the VS by fungi during pre-treatment is likely the reasons for this high material reduction (Paper II). Moreover, the residues of all biological fungi pre-treatments were dry (Table 3), enabling easy separation of the larvae from the treatment residues. Thus biological fungi pre-treatment could be an interesting option in BSFL composting treatments of wet substrates (fruit wastes) or if there is a waste management fee received per tonne of treated waste.

6.3 Availability and management of food industry waste in Tanzania - a case study

The intention with the survey in Tanzania was to assess and quantify available amounts of food industry waste for implementation of BSFL composting in the country and to understand the current waste management strategies (Paper IV). It was found that large volumes (<100,000 to >1,000,000 kg y^{-1}) of food industry waste are generated in single food industry companies in the target cities (Figure 5a). Of these biowastes, only brewery waste (25%) and vegetable oil waste were partly re-used at the companies and had a steady market (Figure 5b). Most fish, coffee and fruit wastes were not used for anything, and this biowaste was either given away free to anybody who was interested or dumped in landfill (Figure 5b). Other studies have also reported low utilisation of biowaste from primary

production, even though large volumes could easily be accessed directly at source (Kaza et al., 2018, Aryampa et al., 2019, Ravi et al., 2020). A survey almost 15 years ago on industry solid waste management in Tanzania, conducted by Mbuligwe and Kaseva (2006), found that brewery and vegetable oil waste were used as animal feed, while other unused waste streams ended up in landfill, indicating that little has changed since then. However, the companies surveyed in Paper IV perceived their current waste management to be good or very good (Figure 5e). These findings suggest that the companies surveyed are not likely to change how they manage their biowaste unless provided with the motivation to do so (Paper IV). There is potential for change if good options are provided, considering that the food industry companies believed that their biowaste had value (Figure 5g) and expressed willingness to give away the biowaste to any interested stakeholders outside the company (Figure 5h).

Suitability assessment of available biowaste based on availability criteria demonstrated that all biowastes were accessible to interested stakeholders, but the amounts accessible from the companies varied (Table 8). Treatment of 1000 kg of biowaste per day has been suggested to be an optimal starting amount for a small-scale BSFL composting facility, based on what has been learned at an Indonesian start-up facility (Dortmans et al., 2017, Zurbrügg et al., 2018). In Paper IV, biowaste generation rate at individual companies, in addition to costs and competition from existing users of brewery waste and sunflower press cake for animal feeds, reduced the availability of the biowaste (Table 8). Collection of large amounts (at least 1000 kg of waste per day) from each company seemed unachievable, but could be obtained from more than one company. Suitability assessment based on biowaste availability criteria showed that banana peels, mango seeds and fish waste were the most available substrates in terms of accessibility, amounts, purchasing cost and competition (Table 8).

Several factors, in terms of physical-chemical properties, were found to affect the suitability of food industry waste for use as feedstock for BSFL (Table 9). Low protein concentration in banana peels, mango seeds and coffee husks, high moisture content in banana peels, and low carbohydrate concentration in mango seeds and fish waste were identified as factors that lowered these biowaste's suitability as potential feedstock for BSFL composting based on their physical-chemical criteria (Table 9). Brewery

waste and sunflower press cake were the only biowastes for which all nutritional parameters scored above the critical value of 1 (Table 9).

On assessing the food industry waste based on both availability and physical-chemical properties (Table 10), its use as feedstock in BSFL composting was hampered by one or several criteria (red colour in Table 10). Either they were not easily available or they were not nutritionally well balanced for BSFL as a standalone feedstock. However, based on the characteristics of the existing food industry waste available (Table 10), blending different waste streams could result in a balanced nutritional composition of the mixture (Paper IV). Blending different substrates in BSFL composting could thus be an incentive for waste management stakeholders to start using these streams for protein production. BSFL composting could thus contribute to improve food industry waste management.

Table 10. *Traffic light display showing biowaste feedstock suitability in black soldier fly larvae (BSFL) composting based on combined availability and physical-chemical criteria*

	Brewery waste	Sunflower press cake	Banana peels	Mango seeds	Coffee husks	Fish waste
Accessibility						
Amount						
Purchase cost						
Competing use						
Moisture content						
Protein						
Carbohydrates						
Volatile solids						
Prot:Carb ratio						

Red = critically low or high, yellow = more/less than needed (opposite of critical values) and green = optimal range.

6.4 Feasibility of implementing research results in BSFL composting facilities

The overall hypothesis tested in this thesis was that application of pre-treatments (Paper I, II) and substrate blending (Paper III) can improve BSFL composting efficiency of low-protein, fibre-rich food industry waste. An increase in BCE_{VS} was demonstrated for several pre-treatments and substrate blends (Figure 6). The improvements achieved in process efficiency suggest potential for implementation of these methods in BSFL composting facilities for treating food industry wastes with similar properties to those studied here (Papers I-III). Considering that the main revenue in BSFL composting comes from the BSFL biomass (Lalander et al., 2018), the higher BSFL biomass yield obtained in some treatments increases the chances to treat the biowaste at a feasible economic cost, especially in low-income countries (Lalander et al., 2018, Lohri et al., 2017).

The confirmed existence of large amounts of biowaste in Tanzania (Paper IV), representing East African countries in the sub-Saharan region, and the biowaste accessibility from food industry companies to interested stakeholders (Figure 5) indicate prospects for easy acquisition of large quantities of segregated biowaste for use in BSFL composting facilities. However, other required resources capable of impacting operational and investments costs need to be evaluated and weighed against increases in BSFL biomass productivity brought by application of pre-treatments (Paper I, II) and substrate blending (Paper III), especially for an industrial set-up. The extra resources required could include additional infrastructure, 14 d retention time, availability of microorganisms, enzymes, nitrogen sources, protein-rich biowaste, specific technical knowledge and high substrate moisture content, as highlighted in Papers I-IV. Successful BSFL composting of low-value food industry waste streams such as banana peel, coffee husks and mango waste that currently mostly end up in landfill (Figure 5, Paper IV) would enable reuse of the resources sustainably in compliance with the principles of a circular economy, through closing nutrient loops, especially in large cities, in Tanzania and other similar low- and middle income countries globally.

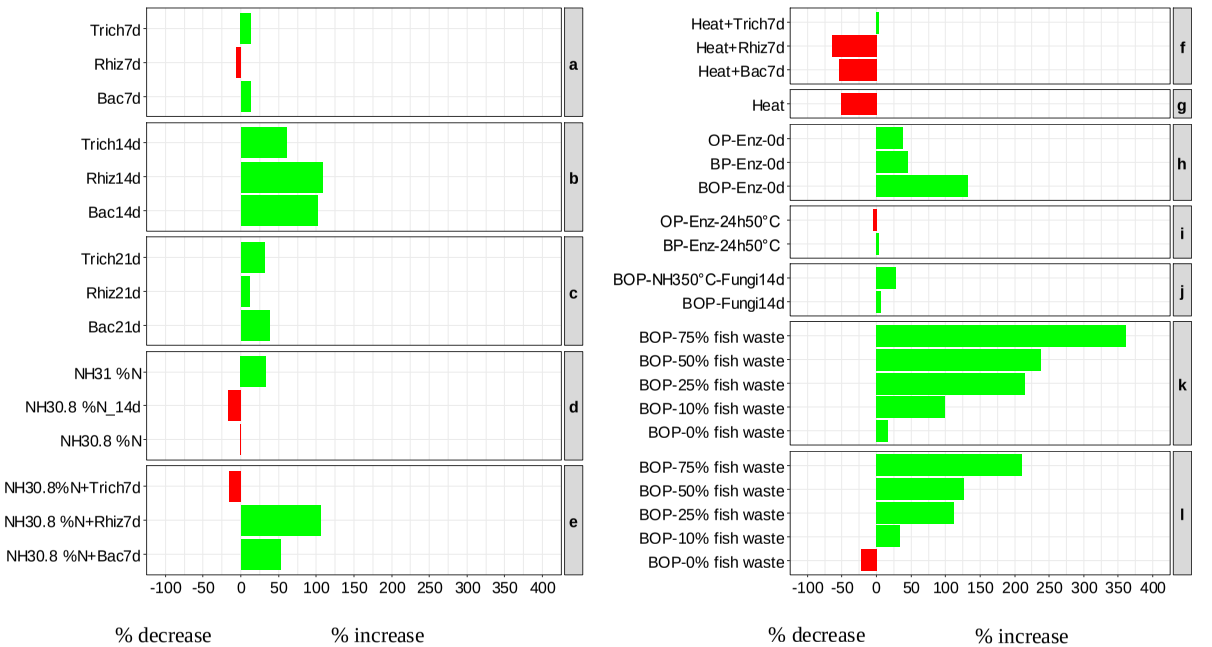

Figure 6. Percent increases (green colour) and percent decreases (red colour) in biomass conversion efficiency in black soldier fly larvae (BSFL) composting of pre-treatment a) 7 d microbial b) 14 d microbial c) 21 d microbial d) chemical e) chemical and microbial f) heat and microbial g) heat h) enzyme i) enzyme and heat j) fungi mixture, in comparison with their respective controls. The percent increases and percent decreases in biomass conversion efficiency of blended substrates k) were in comparison with orange peel control l) were in comparison with banana peel control.

7. Conclusions

- Almost all bacterial or fungi microbial pre-treatments applied to banana peels for 14 d resulted in more than a 50% increase in BCE_{VS}, likely due to lowering of substrate fibre concentration (to ≤4%ww) by the pre-treatments into easily available carbohydrates. A microbial pre-treatment time of 14 d can therefore be recommended for sufficient degradation of fibre in banana peels to achieve an acceptable increase in BCE_{vs}.
- Chemical pre-treatment of banana peels with ammonia solution resulted in increased substrate nitrogen concentration, which likely contributed to increased final larvae weight for the chemical pre-treated substrates. However, BCE_{vs} was increased in only some chemical and combined chemical-biological pre-treatments. High content of fat, fibre and phenols, low nitrogen content and high C/N ratio compared with controls for chemical pre-treatments were identified as parameters that probably reduced BCE_{vs}. Some of the chemical pre-treatments appeared to favour microbial respiration rather than BSFL growth. Chemical pre-treatment, alone or in combination with microbial pre-treatment, was capable of degrading banana peel fibre, providing extra nitrogen and balancing the C/N ratio, resulting in increased final larvae weight and BCE_{vs}. Predictions of what C/N combination that would render increased BCE_{vs} was not determined.
- Blending fruit peels with fish waste increased substrate protein concentration and resulted in a 112%, 127% and 210% increase in BCE_{vs} for substrate blends with 25%, 50% and 75% fish waste inclusion, respectively, in comparison with untreated banana peel. In comparison with the BCE_{vs} of untreated orange peel, BCE_{vs}

increased by 100%, 215%, 238% and 361% for fish waste inclusion of 10%, 25%, 50% and 75%, respectively. The highest BCE_{vs} (25%) was obtained for a substrate blend with 75% fish waste inclusion, but the BCE_{vs} in that treatment varied widely (18.0% ± 5.8), possibly due to varying fish waste composition and high lipid concentration. Blending fruit waste with fish waste balanced the carbohydrate and protein concentrations, which enhanced BSFL composting efficiency. Substrate blends with 25% fish waste inclusion are recommended, in order to increase BSF larvae composting efficiency without large variations.

- Neither heat alone nor combination of heat and microbial pre-treatment of banana peel gave a substantial increase in BCE_{vs}. In most cases, BCE_{vs} was lowered by heat pre-treatments increased concentrations of phenols and tannins, which negatively affected BCE_{VS}.
- Adding enzymes at the same time as the larvae in biochemical enzyme-treatment resulted in the highest BCE_{vs} for banana peel (45%) and banana-orange peel mixture (133%) in comparison with the respective control. The increase was likely due a reduction in the concentration of crude fibre into increased easily available carbohydrates. However, biochemical enzyme treatments at higher temperatures (50 °C instead of 28 °C) did not improve BCE. Likely because of increased concentrations of crude fibre, lower availability of absorbable carbohydrates for BSFL growth and lower pH in comparison with the controls.
- Biological fungi enzyme pre-treatment of banana-orange peel mixture did not improve BCE_{vs}, most likely due to increased fibre concentration and unavailable carbohydrates. However, the high total material reduction (80% of initial waste) suggests that fungi pre-treatment could be interesting for wet substrates (fruit wastes) or when high throughput of waste is an advantage.
- Food industry waste in Tanzania was found to be accessible, and available and suitable for use as feedstock in BSFL composting when blending biowaste streams.
- Low-value, fibre-rich biowaste streams with low protein content can be valorised with BSFL composting if first pre-treated or blended with a high-protein waste stream.

8. Recommendations for Future Studies

- Perform more detailed chemical analyses of substrates, BSFL and treatment residue, to get a better overview of changes occurring during pre-treatments and BSFL composting.
- Investigate whether improving the BSFL rearing process with additional pre-treatment methods such as adjustment of substrate pH and moisture content, together with other pre-treatments, affects BSFL composting process efficiency.
- Explore other types of enzyme cocktails, to determine whether hydrolysis of fibre-rich substrates such as banana peels and orange peels could be increased, resulting in higher BSFL composting process efficiency.
- Investigate whether the pre-treatments and substrate blending approaches applied in this thesis result in similar BSFL composting efficiency when applied to other waste streams.
- Investigate whether the small-scale results obtained in this thesis are transferrable to industry scale, in order to advance industrialisation of BSFL composting for treatment of biodegradable waste fractions.

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Popular science summary

Management of organic waste (biowaste) is a global problem, especially in low- and middle-income countries where large amounts of biowaste generated along the food production chain mostly end up in open dumps and landfills. Inappropriate handling of biowaste results in uncontrolled degradation of the waste, production of greenhouse gas emissions that can contribute to climate change, and disease transmission. It can also cause environmental problems such as eutrophication, since nutrients from the biowaste can leach to the nearby water bodies.

The energy and nutrients such as carbohydrates, fibre, proteins and lipids in biowaste can in fact be valuable resources, but utilisation is currently very low since food production is still operating as an open system. There is an urgent need to solve the challenges of biowaste management by closing nutrient loops in the global food production system.

Black soldier fly larvae (BSFL) composting is one promising option for biowaste treatment, since it converts nutrients from various types of low-value biowaste into high-value products in the form of larval biomass that can be used in animal feed and treatment residues that can be used as organic fertiliser. However, variations in the nutritional composition of biowaste affect the efficiency of conversion into high-value products.

This thesis focused on developing solutions to improve BSFL composting efficiency for nutritionally imbalanced biowaste. The solutions tested involved pre-treatments and substrate blending to add protein and nitrogen and improve degradation of complex molecules such as fibre into simpler forms that can be taken up by the larvae. The results showed that BSFL composting efficiency of banana and orange peels waste with low protein and high fibre content was improved by microbial, chemical and enzyme-treatments, alone and in combination. Heat pre-treatments did not result in

improved efficiency. Microbial pre-treatment of the substrates for 14 days was the best option, with more than 100% increase in conversion efficiency. Blending banana peels and orange peels with fish waste also improved BSFL conversion efficiency by more than 100%, with a maximum increase of 362%.

Treating food industry waste in Tanzania cities using substrate blending with BSFL composting was shown to be suitable in assessments considering biowaste suitability based on availability and physical-chemical characteristics. Use of pre-treatments and substrate blending combined with BSFL composting can be recommended as a treatment technology for biowaste streams in cities in low and middle-income countries such as Tanzania and other similar settings globally.

Populärvetenskaplig sammanfattning

Hantering av organiskt avfall är ett globalt problem, speciellt stort är problemet i låg-, och medelinkomstländer där en stor andel av avfallet från hela livsmedelskedjan hamnar på mer eller mindre kontrollerade soptippar. Felaktig hantering av det biologiska avfallet ger en okontrollerad biologisk nedbrytning som leder till utsläpp av växthusgaser som har stor klimatpåverkan. Det kan även bidra till spridning av sjukdomar samt övergödning om växtnäring läcker ut från avfallshanteringen till närliggande vattendrag. Matavfallets energi-, och innehåll av kolhydrater, fibrer, proteiner och fetter är värdefulla resurser som idag har en låg utnyttjandegrad. Därför finns det ett stort behov att lösa utmaningen med hanteringen av vårt bioavfall på ett sätt som gör att vi kan återvinna resurserna från dessa fraktioner.

Biologisk behandling med Amerikansk vapenfluga är en lovande teknisk innovation där näringsämnena i matavfallsfraktioner med lågvärdigt näringsinnehåll kan omvandlas till högvärdiga produkter. Processen ger två produkter, larver och kompost. Larverna kan användas som protein och fett i djurfoder. Komposten kan i sin tur användas som ett organiskt gödselmedel. Bioavfall med en för larverna obalanserad sammansättningen av dessa näringsämnen påverkar fluglarvskomposteringsprocessen negativt och resulterar i en läge effektivitet med avseende på omvandlingen till larvbiomassa. Den här avhandlingen fokuserar på utveckling av tekniker för att förbättra fluglarvskomposteringens effektivitet i behandlingen av substrat som har obalanserad sammansättning av olika näringsämnen. De metoder som utvärderas är olika förbehandlingar av enskilda substrat för att förbättra omvandlingen till fluglarvsbiomassa i fluglarvsprocessen, samt att blanda olika substrat för att därigenom få en bättre sammansättning och således en mer effektiv process. Resultaten visade att fluglarvskomposteringsprocessen

för banan-, och apelsinskal kunde förbättras om de förbehandlades med mikroorganismer, med en tillsats av ammoniak eller genom tillsats av fibernedbrytande enzymer. Förbehandling med värme var den enda behandlingen som inte ledde till någon processförbättring. Två veckors mikrobiell förbehandling var den behandling som hade den bästa effekten, då mängden larvbiomassa fördubblades. Genom att blanda skalen från bananer och apelsiner med fiskavfall gav liknande positiva effekter för processen, där en 362% ökning av utbytet i avfall till larvbiomassa uppnåddes som mest. Genom att blanda olika substrat som genereras i livsmedelsindustrin i Tanzania finns det möjlighet att skapa effektiva processer. Slutsatsen från detta projekt var att fluglarvskompostering är en lovande teknik för att behandla livsmedelsindustriavfall i låg-, och medelinkomstländer med liknade förutsättningar som Tanzania.

Acknowledgements

My academic achievements in this PhD journey were helped by great contributions from my supervisors, Björn Vinnerås, Cecilia Lalander, Oscar Kibazohi and Christian Zurbrügg, and support from the universities, SLU and UDSM.

My supervisors, thank you so much for the active support, guidance and encouragement that you provided continuously during my studies, I am immensely grateful. My main supervisor, Björn, thank you for the wonderful opportunity to join the Environmental Engineering group and for all the support, including the accommodation arrangements, making my stay in Sweden very pleasant. Cecilia, thank you so much for reading my work thoroughly several times and for your quick and kind responses to my spontaneous inquiries, also through phone calls and chats. Thank you for always turning my manuscripts inside out, with a tremendous number of valuable coloured comments! I have learnt a lot from you. I also enjoyed our detailed relaxing discussions on various topics about other aspects of life.

Thanks to Orjan Ostman at SLU Aqua for providing the fish waste and Volkmar Passot at SLU for providing *Trichoderma reesei* and *Rhizopus oligosporus*. This research was performed with Sida funding through the University of Dar es Salaam, Tanzania, and Swedish University of Agricultural Sciences, Sweden, within the sub-programme 'Sustainable Agricultural Productivity - Processing and Value Chain to Enhance Food Security in Tanzania'.

Present and past members of the Kretsloppsteknik group, many thanks to you for the team work and regular enjoyable work and social meetings and discussions, your friendship and support made my PhD journey a pleasant

experience. Thank you Jenna with your family for the support and warm welcome you gave me during my first visit to Sweden. Special thanks to Samual Blyth, Viktoria, Giulio and Emilien for the assistance you provided in my experimental work. Viki and Eliyan, thank you for the gifts and warm company. Thank you Prithvi for the great discussions and inputs to my studies, including the attractive BFSL life cycle illustration for my presentations. Thank you Cecilia with your family, Björn and Annika, for additional joy and comfort you provided during my family visits in Sweden.

Thanks to all at the Department of Energy and Technology, Sweden, and the former Department of Chemical and Mining Engineering, Tanzania, for being great colleagues. I want to send special thanks to Åke Nordberg for his valuable inputs and for always agreeing to be my opponent. Thank you Claudia von Brömssen for always providing statistical guidance in my studies. Thank you Sven Smårs for computer assistance and for construction of the heat insulated mixing box used for my experiments. Thanks to heads and administration offices at CME, CPE and ET departments for all the support you provided during my studies. Thanks to Victor for the assistance in following up the logistics regarding establishment of BSFL treatment facility at UDSM. Special thanks to Prof. Amelia Kivaisi and Mr. Charles Kweyunga at UDSM for their support in enabling the nutritional analysis to be performed despite the hardships encountered.

Thanks to Mary McAfee for fast and thorough language editing of all my work, Kigen from Recyclers Tanzania and Moritz Gold for their valuable inputs and interest in the study.

Thanks to all my friends for their friendship and their presence.

Very special thanks to my family; my husband Fabian, my babies Mahenda, Lucy and Masuka, my parents Robert and Lucy, my sisters Irene, Linda and Gloria, and all my in-laws. You all walked this journey with me, thank you all so much for the prayers, love and support, I feel so blessed and lucky to have you in my life. Asanteni sana.

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Waste Management 100 (2019) 151–160

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae

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ARTICLE INFO

Article history:
Received 22 July 2019
Revised 10 September 2019
Accepted 12 September 2019
Available online 17 September 2019

Post-print errata: We have in later research realised that we, with the way we handled the two fungi in this study, could not have guaranteed that there was no cross-contamination between the two.

Keywords:
Food industry waste
Fruit peel
Eco-technologies
Trichoderma reesei
Rhizopus oligosporus
Organic waste management

ABSTRACT

Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae (BSFL) is among the solutions being explored to shift the value chain in organic waste management by producing valuable products. Although BSFL consume a range of substrates, nutrient-imbalanced materials with high hemicellulose and lignin content, e.g. manure and banana peel, yield low conversion into larval biomass. This study explored pre-treatment methods to improve the nutrient composition and digestibility of banana peel to achieve higher substrate conversion into BSFL biomass. The pre-treatment methods evaluated were microbial, chemical (non-protein nitrogen), heat-based, and combinations of these. All pre-treatments tested except heating resulted in more efficient BSFL conversion in terms of final larvae weight. The low BSFL responses in pre-treatments were caused by the observed high amounts of tannins and phenolic compounds mainly from the heating pre-treatment. Waste to biomass conversion ratio correlated negatively with substrate volatile solids (VS) and positively with the decrease in VS in pre-treatment. Microbial – 14 days pre-treatments provided the optimum pre-treatment time for the microorganisms to achieve maximum degradation of the substrates, facilitating larval assimilation of the released nutrients. *Rhizopus oligosporus*-14 days and ammonia + *Rhizopus* resulted in the most efficient BSFL treatment, measured as protein produced per kg incoming material.

1. Introduction

Population growth and urbanisation is expected to increase municipal solid waste generation to 2.2 billion tonnes by 2025 and predicted to affect low- and middle-income countries (LMIC) most, due to unsustainable waste management practices (WorldBank, 2017). Solid waste management in most LMIC involves open dumping and burning (WorldBank, 2018). Municipal solid waste in those countries contains a mixture of plastics (<13%), metals (<4%), paper (<9%), glass (<4%) and more than 50% organic waste (Hoornweg and Perinaz, 2012). Solid waste dumped at landfill undergoes sorting by waste pickers, who remove a large proportion of the non-organic waste leaving high proportions of biodegradable organic waste (Komakech et al., 2014). Open burning is a source of dangerous carcinogenic gases and of black carbon, a short-lived climate pollutant (CCAC, 2015). Open dumping of waste allows biodegradable materials to decompose under unhygienic conditions leading to accumulation of greenhouse gases causing climate change (Cogut, 2016). The dumped waste attracts insects and rodent vectors that spread diseases such as cholera and malaria (Chowdhury et al., 2017). Untreated leachate from the decomposing dumped waste contaminate surface and groundwater supplies (Nagarajan et al., 2012).

Besides mixed municipal solid waste, homogeneous organic solid waste is generated by agricultural and industrial activities in LMIC during agricultural harvests and agro-industrial processes comprising stems, stalks, peel, seeds and pulp (Krishna and Chandrasekaran, 1996). For instance banana, belonging to the family Musaceae and genus *Musa*, is a crop grown in 120 countries throughout the world (Byarugaba-Bazirake, 2008). Tanzania contribute large quantities of bananas grown in Africa while generating around 60 tonnes ha^{-1} of banana-related organic solid waste per harvest (Tock et al., 2010; Emmanuel et al., 2014). Banana is a source of staple food and cash crop, and its juice is used commercially to produce alcoholic drinks (Carter et al., 2010). Banana waste from harvesting consists of stems and leaves, while banana

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https://doi.org/10.1016/j.wasman.2019.09.017
0956-053X/

waste from industrial production of e.g. banana crisps, banana juice and wine consists of peel and pulp, and both types are normally dumped in landfill, rivers or unregulated dumping grounds (Osma et al., 2007). There is also a growing industry of mass production of clear banana juice (Kibazohi et al., 2017). This creates an extra need for sustainable management systems to utilise the nutrients from the banana waste.

Use of insects for treatment of organic wastes is gaining increasing interest, as it uses organic solid wastes as a resource to produce valuable products (Čičková et al., 2015). Use of BSFL for organic waste treatment has the potential to add value to non-utilised organic wastes and also to act as an additional income generator for waste managers (Lohri et al., 2017). This technology converts organic waste efficiently and rapidly into protein-rich (40% dry matter (DM) and fat-rich (30% DM) larvae suitable for use in animal feed (Newton et al., 1977; St-Hilaire et al., 2007; Stamer, 2015) and biodiesel production (Li et al., 2011; Zheng et al., 2011), while the treatment residue is valuable fertiliser (Sheppard et al., 1994; Setti et al., 2019).

BSFL can be reared on different substrates, including animal manures (Sheppard et al., 1994; Myers et al., 2008; Zhou et al., 2013) pig liver, fish rendering waste and fruit waste (Nguyen et al., 2013), human excreta (Lalander et al., 2013; Banks et al., 2014) and food waste (Diener et al., 2011; Nguyen et al., 2015). Previous studies revealed that BSFL growth and waste conversion into biomass depend on the type, quantity and quality/nutrient composition of the substrate (Myers et al., 2008; Nguyen et al., 2013; Nyakeri et al., 2017; Gold et al., 2018; Meneguz et al., 2018). On substrates with imbalanced amounts of nutritional substances such as protein, fats and fibre, BSFL grow at a slower rate and achieve lower body weight (Nyakeri et al., 2017). Food substrates with a low protein to carbon ratio, high amounts of fat and fibre have also been shown to inhibit BSFL growth rate and biomass yield because of poor digestibility and low nutrient utilisation (Tomberlin et al., 2009; Tschirner and Simon, 2015; Lalander et al., 2019). Growth is particularly poor on homogeneous wastes with a high hemicellulose and lignin content, such as dairy manure and banana peel (Nyakeri et al., 2017; Rehman et al., 2017a; Kumar et al., 2018).

A few studies have been conducted on methods to improve the biodegradability/digestibility of substrate in order to increase conversion by BSFL (Yu et al., 2011; Li et al., 2015; Rehman et al., 2017b). One suggested method is to improve the nutrient balance by mixing waste with other substrates, e.g. rice straw with glucose and restaurant waste (Zheng et al., 2012; Li et al., 2015) or dairy manure with soybean curd residue (Rehman et al., 2017b). Another suggested method involves addition of microorganisms to the substrate, to improve the conversion efficiency and degradation of cellulose and hemicellulose, especially in homogeneous wastes such as poultry manure (Yu et al., 2011). A combination of these two methods can also be applied, e.g. microbe treatment together with mixing restaurant waste and rice straw (Zheng et al., 2012). However, due to the high lignin-cellulose content in rice straw, using this as a co-substrate has been found to produce small final BSFL weight compared with using other organic wastes rich in protein and lipids (Zheng et al., 2012; Li et al., 2015; Manurung et al., 2016). There is thus a need to identify inexpensive, simple and effective techniques to enable treatment of homogeneous and fibrous wastes with BSFL.

The main aim of this study was to explore the possibility of improving BSFL conversion of banana peel by using a pre-treatment method. Specific objectives were to:

a. Evaluate the efficiency of BSFL in degrading banana peel waste pre-treated with heat, ammonia or microorganisms.
b. Identify the nutritional parameters that affect BSFL treatment efficiency.

2. Materials and methods

2.1. Material and preparation

2.1.1. BSFL

The BSFL used in this study were obtained from a colony that has been in operation since 2015 at the Department of Energy and Technology, SLU. The BSFL were reared on chicken feed (Grangården Hönsfoder Start, metabolisable energy content of 11.2 MJ kg^{-1}, 80% moisture) for around 5 d. They were then transferred to the banana peel substrates and pre-treatments described below.

2.1.2. Banana peel

Banana peel used in this study were of two types: 1) Peel from *Musa acuminata*, Cavendish bananas, referred to in this paper as *dessert peel*; and 2) peel from ripe Pisang Awak bananas used specifically for juice production, referred to as *juice peel*. The dessert peel was collected every day in plastic bags at the Swedish University of Agricultural Sciences (SLU) campus in Uppsala, Sweden. The juice bananas were bought in Mabibo market, Dar es Salaam, Tanzania, and transported to SLU, where it was separated from edible flesh. The banana peel wastes collected were kept in the fridge and homogenised using a blender (Robot Coupe Blixer 4 V). They were then portioned and frozen at −20 °C until use.

2.1.3. Pre-treated banana peel

Only the dessert peel was used in the pre-treatments of banana peel as this was the only banana peel we found in Sweden where the study took place. The pre-treatment methods tested are shown in Table 1. In all experiments, after the defined pre-treatment period the pre-treated peel was mixed well, weighed, portioned into small daily feeding portions and frozen at −20 °C until use.

2.1.4. Chemicals

The chemical tested as pre-treatment was 24% technical ammonia (aq) (Nitor, Sweden) and analytical grade concentrated sulphuric acid (Fisher Chemicals, Uppsala, Sweden) was used for neutralising the pH after the pre-treatment period.

All chemicals and reagents used in the nutrient analyses were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany). These were: 80% ethanol, 1% methanol, 7.5% sodium hydrogen carbonate, chloroform, D-glucose, anthrone reagent, Folin-Denis reagent, tannic acid, diethyl ether, 1.25% sulphuric acid, 1.25% sodium hydroxide, Folin-Ciocalteau reagent, gallic acid, sodium potassium tartrate (Rochelle salt), Kjeldahl tablets, concentrated sulphuric acid (NH_3-free grade), sodium salicylate, sodium nitroprusside and 0.15% sodium hypochlorite solution.

2.1.5. Microorganisms

Isolation of BSFL gut bacteria: Instar 5 BSFL (reared on food waste) were collected and washed with deionised water, followed by rinsing with ethanol (70% EtOH). The disinfected larvae were manually chopped into pieces and immersed (4 larvae) in 10 mL unselective microbial nutrient broth (National Veterinary Institute, Uppsala, Sweden) at 70 °C for 20 min. Upon cooling to room temperature (RT), 1 mL broth was spread on taurocholate gelatine agar (TGA) (Miclev, Sweden) plates (∅ 14 cm) and incubated at 37 °C for 24 h. Ten visually different colonies were clean-spread on individual TGA plates and incubated at 37 °C for 24 h. Single individual colonies were then taken and heat-treated (70 °C, 20 min) once more in unselective nutrient broth (as described above). Upon reaching RT, 10 μL of broth were spread onto a TGA plate and incubated at 37 °C for 24 h. Once again, single colonies were collected from each plate, clean-spread on TGA plates and incubated at

Table 1
Summary of the banana peel wastes used and the pre-treatments tested.

Experiment	Banana peel type	Code	Pre-treatment 1		Pre-treatment 2	
			Treatment	Conditions	Treatment	Conditions
1. Non-pre-treated						
	Juice peel	Juice peel	–	–	–	–
	Dessert peel	Dessert peel	–	–	–	–
2. Microbial pre-treatment	Dessert peel	$Trich_{7d}$	*Trichoderma reesei*	7d	–	–
		$Trich_{14d}$		14d	–	–
		$Trich_{21d}$		21d	–	–
		$Rhiz_{7d}$	*Rhizopus*	7d	–	–
		$Rhiz_{14d}$	*oligosporus*	14d	–	–
		$Rhiz_{21d}$		21d	–	–
		Bac_{7d}	Bacteria	7d	–	–
		Bac_{14d}		14d	–	–
		Bac_{21d}		21d	–	–
3. Non-protein nitrogen pre-treatment	Dessert peel	$NH3_{0.8\ \%N}$	Ammonia	0.8%N, 7d	–	–
	Dessert peel	$NH3_{1\ \%N}$		1%N, 7d	–	–
	Dessert peel	$NH3_{0.8\%N\_14d}$	Ammonia	0.8%N, 14d		
4. Heating pre-treatment	Dessert peel	Heat	Heat, 120 °C	1hr	–	–
5. Non-protein nitrogen and microbial pre-treatment	Dessert peel	$NH3_{0.8\ \%N}$ + $Trich_{7d}$	Ammonia	0.8%N, 7d	*Trichoderma reesei*	7d
	Dessert peel	$NH3_{0.8\ \%N}$ + $Rhiz_{7d}$		0.8%N, 7d	*Rhizopus oligosporus*	7d
	Dessert peel	$NH3_{0.8\ \%N}$ + Bac_{7d}		0.8%N, 7d	Bacteria	7d
6. Heating and microbial pre-treatment	Dessert peel	Heat + $Trich_{7d}$	Heat, 120 °C	1hr	*Trichoderma reesei*	7d
	Dessert peel	Heat + $Rhiz_{7d}$		1hr	*Rhizopus oligosporus*	7d
	Dessert peel	Heat + Bac_{7d}		1hr	Bacteria	7d

37 °C for 24 h. The clean-spread colonies were kept at 4 °C until use. In total, 10 visually different bacterial colonies were observed. No bacterial typing was performed for the isolated bacteria. Before use, one colony from each clean-spread plate was added to 10 mL unselective bacterial nutrient broth and incubated at 37 °C for 24 h. A mixture of bacteria isolated from the BSFL gut was used in this study (Lundgren, 2019).

The fungi used in pre-treatment were *Trichoderma reesei* and *Rhizopus oligosporus,* obtained from the Department of Molecular Sciences, SLU. Both were pre-cultured on malt extract agar (MEA) at 28 °C for 7 d. The spores were harvested by pouring sterile 0.9% NaCl onto the MEA plates to wet the mycelium, inoculation loop removed mycelium from the agar surface. The spore suspension was transferred to a 50 mL centrifuge tube and diluted with 0.9% NaCl to 50 mL. The suspension was kept at 3–6 °C until use.

2.2. Experimental set-up

The study comprised six experiments (Table 1). The first experiment involved feeding the larvae with non-pre-treated dessert and juice peel, while the remaining five experiments involved feeding the larvae with dessert peel that had undergone one pre-treatment (Experiments 2–4) or two pre-treatments (Experiments 5–6) prior to BSFL composting.

2.2.1. Experiment 1 – No pre-treatment

The first experiment involved feeding the BSFL with dessert peel and juice peel to evaluate the effect of different banana peel types on BSFL growth.

2.2.2. Experiment 2 – Microbial pre-treatment

The second experiment aiming to make the nutrients in waste more available to the BSFL, evaluated the effect of microbial pre-treatment for different periods. Fungi, e.g. *Trichoderma* spp., have been demonstrated to facilitate composting, as they break down complex compounds like fibre into forms more available to other microorganisms (Haddadin et al., 2009). It was also demonstrated that solid-state fermentation with *Trichoderma reesei* increases the digestibility of banana peel to monogastric animals (Katongole et al., 2017). In this study, fungal species (*Trichoderma reesei* or *Rhizopus oligosporus*) or BSFL gut bacteria were seeded amounting to 1% (w/w) of dessert peel waste. The inoculated wastes were left for 7 d, 14 d and 21 d at 28 °C. The concentration of the each inoculation added was not measured but it approximated to 1.45×10^9 cells/mL.

2.2.3. Experiment 3 – Chemical pre-treatment

This experiment evaluated the effect of chemical treatment by addition of non-protein nitrogen to the peel. This was done to balance the carbon-nitrogen ratio, because BSFL gut microorganisms can probably utilise and convert non-protein nitrogen into proteins, as can other animals like ruminants (Amha, 2015). Ammonia solution with a concentration of 24.5% was added to dessert peel waste. Two different amounts of ammonia solution were tested, 0.8% and 1%, the former based on estimated amount of nitrogen and fibre in the dessert peel and the latter calculated as 4% (w/w) ammonia solution per wet weight of dessert peel. The peel pre-treated with ammonia solution was left to stand for 7 d at 28 °C. The pH of the substrate was then neutralised to pH 7 by addition of 95% concentrated sulphuric acid.

2.2.4. Experiment 4 – Heating

The fourth experiment evaluated the effect of heat pre-treatment of dessert peel prior to BSFL composting. The peel was heated at 120 °C under 2 bar for 1 h. The hypothesis was that heating would degrade macromolecules in the peel, such as fibre-bound polyphenols, to enhance availability of micronutrients for easy extraction and utilisation for growth by the BSFL.

2.2.5. Experiment 5 – Combined chemical and microbial pre-treatment

This set of experiments assessed the effect of addition of non-protein nitrogen followed by microbial pre-treatment prior to BSFL

composting. Ammonia (0.8%) solution was added to dessert peel as described in Experiment 3. The treated waste was left to stand at 28 °C for 7 d, and then the pH was neutralised using concentrated sulphuric acid as described for Experiment 3, but with further addition of 1% (w/w) of the selected inoculate and left at 28 °C for 7 d. Hence the combined pre-treatment lasted a total of 14 d.

2.2.6. Experiment 6 – Heating and microbial pre-treatment

The sixth experiment examined the effect of combined heating and microbial pre-treatment of dessert peel. The heated peel (Experiment 4) was seeded with 1% (w/w) of the selected inoculate (*Trichoderma reesei* / *Rhizopus oligosporus* / BSFL gut bacteria) and left at 28 °C for 7 d before BSFL composting.

2.3. BSFL composting

Composting in this study is defined as the degradation of organic material by BSFL under aerobic conditions for a specific period of time resulting in a larval biomass and compost, nutrient rich-product that can be used as fertilizer (Čičková et al., 2015; Lalander et al., 2015). All feeding experiments were conducted in triplicate plastic containers (Smartstore classic 2, 21x17x11 cm^3) with plastic lids with a hole covered with netting for air circulation, under the same controlled environment of 28 °C for 1 month. For feeding experiment, 200 larvae < 2 mm (around 10 d old) were added to each box, giving a density of 0.6 larvae cm^{-2}. The larvae were fed with the substrates every second or third day, at a feeding rate of 40 mg dry matter (DM) $larva^{-1}$ d^{-1}. The substrate portions were thawed and brought to room temperature before feeding. Growth of the larvae was monitored three days per week by weighing 10 randomly selected larvae. The larvae were then rinsed with water, dried on paper and placed back into the appropriate treatment container.

The BSFL composting units were run for one month to study the growth of BSFL on pre-treated and non-pre-treated banana peel. A sample of the treatment residues was collected once a week for total volatile solids (VS) and pH determination. After sampling, a fresh thawed ration of substrate was added to the containers. After 30 d of feeding, BSFL/Prepupae were manually picked from the residues. The DM and VS content of all harvested biomass of BSFL/prepupae and of treatment residues were determined. For the treatment residues, the pH was also measured.

2.4. Physico-chemical and nutrient analyses

2.4.1. Dry matter and total volatile solids

The DM content of weighed samples during the experiments, residues at the end of the experiments and pre-pupae was determined by drying the measured biomass at 85 °C for 48 h in a laboratory termaks oven. For VS measurements, the dried samples were heated to 200 °C for 2 h, to prevent sample losses due to heating directly at very high temperatures, and then heated to 550 °C for 4 h.

2.4.2. pH

The pH of the feed substrates was determined using an InoLab Laboratory pH meter. For this, 10 g sample were placed in a 50 mL centrifuge tube, diluted to 50 mL with deionised water and left to stand for 1 h at room temperature before the pH readings.

2.4.3. Nutrient analysis

Nutrient analysis was performed in order to evaluate the nutritional parameters influencing the efficiency of BSFL composting of banana peels. Total nitrogen content was determined using a semi-micro Kjeldahl method and indophenol-blue method (Allen et al., 1989). Total phenolic content was analysed according to the Folin-Ciocalteu method (Singleton et al., 1999), while soluble carbohydrates were determined using the anthrone method (Allen et al., 1989). Crude fibre content was analysed according to AOAC methods (AOAC, 1984), while total lipid content was determined using a chloroform-methanol mixture (Bligh and Dyer, 1959).

2.5. Calculations

To evaluate the efficiency of BSFL composting, material reduction and waste to biomass conversion ratio were calculated.

The percentage material reduction on a VS basis ($MR_{\%VS}$) during BSFL composting was calculated as:

$$\mathrm{MR}_{\%VS} = \left(1 - \frac{\mathrm{m}_{res} * \mathrm{TS}\%_{\mathrm{res}} * \mathrm{VS}\%_{res}}{\mathrm{m}_{inflow} * \mathrm{TS}\%_{\mathrm{inflow}} * \mathrm{VS}\%_{inflow}}\right) \times 100 \quad (1)$$

where m_{res} and m_{inflow} is the mass, $TS\%_{res}$and $TS\%_{inflow}$ is the total solids content, and $VS\%_{res}$ and $VS\%_{inflow}$ is the total volatile solids content in treatment residues and inflow substrate, respectively.

The percentage biomass conversion ratio on a VS basis ($BCR_{\%VS}$) was calculated as:

$$\mathrm{BCR}_{\%VS} = \frac{m_{pp} * \mathrm{TS}\%_{pp} * \mathrm{VS}\%_{pp}}{\mathrm{m}_{inflow} * \mathrm{TS}\%_{\mathrm{inflow}} * \mathrm{VS}\%_{inflow}} \times 100 \quad (2)$$

where m_{pp} and m_{inflow} is the mass, $TS\%_{pp}$and $TS\%_{inflow}$ is the total solids content, and $VS\%_{pp}$ and $VS\%_{inflow}$ is the total volatile solids content in pre-pupae and inflow substrate, respectively.

The percentage survival rate ($SR_{\%}$) of the BSFL was calculated as:

$$\mathrm{SR}_{\%} = \left(\frac{\mathrm{pp}_{out}}{\mathrm{BSFL}_{in}}\right) \times 100 \quad (3)$$

where pp_{out} is the number of larvae that survived to the end of the experiment and $BSFL_{in}$ is the initial number of larvae used in the experiment.

Volatile solids consumption ($VS_{con.}$) during pre-treatments was calculated as:

$$\mathrm{VS}_{con.} = \mathrm{VS}\%_{banana\ \ peel} - \mathrm{VS}\%_{pre-treated\ \ peel} \quad (4)$$

C/N ratio

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

2.6. Statistical analysis

Shapiro test of normality was conducted to verify normality of data. The survival rate was found to be non-normal and was converted into $\log_{10}$ death rate that was found to be normally distributed ($p > 0.05$). Analysis of variance (ANOVA) with 95% confidence interval was performed to identify statistically significant differences in treatment response variables between different treatments. When a significant difference was found, Tukey's HSD (honestly significant difference) *post-hoc* test with 95% confidence interval was performed. Principal component analysis (PCA) was performed to find the variables that mostly contributed to the data variance, while multi-linear regression was used to evaluate the variables selected in the PCA. R statistical software (R Core Team, 2016) was used for statistical analysis and for graphical representation of the data.

All experiments were conducted in triplicate with the exception of the microbial 14- and 21-day pre-treatments, which were planned and performed on single samples. The $NH3_{0.8\ \%N}$ + $Trich_{7d}$ and Heat + $Trich_{7d}$ were also performed on single samples, because

when performed on triplicates had very low survival rate and the experiment were repeated in singlets for verification of previous results. Singlet values were not included in ANOVA analysis, but were included in multi-linear regression analysis. Nutrition analysis were not conducted on all replicates due to lack of material. The nutritional analysis was conducted in order to find the substrate properties that contributed the most to the variance in treatment response values. The values of triplicates and doublets from the nutrition analysis were averaged prior to the multi-regression analysis. The failed $NH3_{0.8\ \%N}$ + $Trich_{7d}$ and Heat + $Trich_{7d}$ triplicate experiments were not included in the statistical analysis.

3. Results

3.1. Banana peels and properties of pre-treated peel

Dry matter content and VS content differed significantly between juice peel and dessert peel (Table 2). Juice peel had a 30% DM content and 97% VS content on DM basis, while dessert peel had a DM content of ~13% and a VS content of ~85% on a DM basis.

Pre-treatments of dessert peel altered DM content and total VS content. The pre-treatments that resulted in the highest DM content was Heat + Bac_{7d} (22%). The lowest DM content (~8%) was observed for Heat + $Trich_{7d}$ and all longer pre-treatments of 14 d and 21 d. Bac_{21d} resulted in the highest amount of VS (~88%). Pre-treatments resulting in lower VS content (73–78%) were Heat + Bac_{7d}, Heat + $Trich_{7d,}$ $NH3_{0.8\ \%N}$ + $Rhiz_{7d}$ and 14 d and 21 d microbial pre-treatments except for Bac_{21d} which had 88% VS, the highest content. The carbon to nitrogen ratio was different between the two banana peels, juice peel having a higher carbon to nitrogen ratio of 62.6 while dessert peel had 53.8. The carbon to nitrogen ration was altered by the pre-treatments and varied between pre-treatments with heat pre-treatments having 80 as the highest ratio while the non-protein nitrogen pre-treatments having the lowest ratio (13–16). The pH varied greatly among the different pre-treatments. Juice peel had the lowest pH values (around 4), while $Trich_{21d}$ pre-treatment of dessert peel gave the highest pH, ranging from 8 to 9.

3.2. Effect of pre-treatment on the nutrient content of banana peel

The heating pre-treatment and Heat + Bac_{7d} gave the highest amounts of total phenolic compounds (~3 mg gallic acid equivalents (GA) 100 g^{-1} extract), while the other pre-treatments gave ≤ 1 mg GA 100 g^{-1} extract (Table 3). Juice peel had different amounts of phenolic compounds and tannins (>0.1 mg GA 100 g^{-1} extract, 2% tannins) than dessert peel (~1 mg GA 100 g^{-1} extract, 0.5% tannins). $Trich_{14d}$ gave the lowest amount of total phenolic compounds (0.05 mg GA 100 g^{-1} extract). The heating, Heat + $Rhiz_{7d}$ and $NH3_{0.8\ \%N}$ pre-treatments gave the largest amounts of tannin, >2%.

All the pre-treatments with addition of ammonia solution gave total nitrogen content of >2%, with the exception of $NH3_{0.8\ \%N}$ + $Trich_{7d}$, which had 1% total nitrogen·$NH3_{0.8\ \%N}$ gave the highest amount (~4%), while $Rhiz_{21d}$ gave the lowest (~0.2%). Soluble carbohydrates, crude fibre and total lipids were not significantly different between the pre-treated and non-pre-treated dessert peel.

3.3. BSFL composting efficiency

Without any pre-treatment, $BCR_{\%VS}$ (7% VS) and the final larvae weight (134 mg $larva^{-1}$) were higher for BSFL composting of dessert peel than for BSFL composting of juice peel (0.9% VS and 33 mg $larva^{-1}$, respectively). The latter were the lowest values observed in all experiments (Table 4).

Table 2
Physico-chemical characteristics of peels before (experiment 1) and after pre-treatments (experiments 2–6). In cases when triplicates were conducted, values are presented as mean (n = 3) ± standard deviation, in other cases the value of the singlet sample is presented. Same letter column wise represent no significant differences ($p < 0.05$). Only sample conducted in triplicate are included in the statistical comparison. The juice peel were not included in the comparison as no pre-treatment was conducted on these peels.

	Dry matter (%)	Total volatile solids (% DM)	C/N ratio	pH Lowest - Highest
Experiment 1				
Juice peel	29.9 ± 0.40	96.9 ± 0.22	62.6	3.1 ± 0.03–4.4 ± 0.06
Dessert peel	12.5 ± 0.34^{a,d}	85.2 ± 0.08^{a,b,f}	53.8	5.3 ± 0.11–6.6 ± 0.37
Experiment 2				
$Trich_{7d}$	9.8 ± 0.12^{b,c}	81.6 ± 0.93^{b,e,d}	75.6	4.2 ± 0.08 –5.8 ± 0.13
$Trich_{14d}$	8.3	76.4	83.2	8.3–8.8
$Trich_{21d}$	7.6	73.5	–	8.1–9.3
$Rhiz_{7d}$	10.2 ± 0.06^{b,d,c}	80.8 ± 0.56^{e}	–	6.6 ± 0.19–7.4 ± 0.19
$Rhiz_{14d}$	8.3	76.4	49.3	8.2–8.8
$Rhiz_{21d}$	8.0	77.1	78.5	6.8–8.5
Bac_{7d}	11.8 ± 0.23^{a,b,c}	78.6 ± 2.3^{c,e}	76.6	7.0 ± 0.42–8.2 ± 0.15
Bac_{14d}	6.8	71.4	57.5	6.5–8.7
Bac_{21d}	7.9	87.8	–	7.3–8.8
Experiment 3				
$NH3_{0.8\ \%N}$	12.6 ± 0.65^{a,e}	84.4 ± 0.32^{a,d,f}	12.67	6.3 ± 0.45–7.7 ± 0.05
$NH3_{1\ \%N}$	10.1 ± 2.4^{d,c}	83.1 ± 2.2^{b,d,f}	13.19	5.4 ± 0.25–7.7 ± 0.09
$NH3_{0.8\%N\_14d}$	12.2	85.3	16.34	5.7–7.0
Experiment 4				
Heat	9.4 ± 0.43^{b}	86.7 ± 0.82^{a}	80.28	4.4 ± 0.04–5.0 ± 0.18
Experiment 5				
$NH3_{0.8\ \%N}$ + $Trich_{7d}$	10.5	82.3	45.72	8.0–8.6
$NH3_{0.8\ \%N}$ + $Rhiz_{7d}$	10.0 ± 0.21^{b,c}	78.2 ± 0.53^{e}	17.38	8.5 ± 0.11–8.9 ± 0.03
$NH3_{0.8\ \%N}$ + Bac_{7d}	8.5 ± 0.25^{b}	80.3 ± 0.34^{e}	19.40	4.8 ± 0.08–7.8 ± 0.09
Experiment 6				
Heat + $Trich_{7d}$	8.2	76.5	–	7–8.41
Heat + $Rhiz_{7d}$	12.2 ± 0.66^{c,d,e}	86.0 ± 0.70^{a,f}	47.78	4.8 ± 0.21–6 ± 0.30
Heat + Bac_{7d}	21.8 ± 1.0	78.2 ± 1.0^{c,e}	52.34	6.1 ± 0.21–7.5 ± 0.15

Different letters within columns indicate significant differences ($p < 0.05$). Only sample conducted in triplicate are included in the statistical comparison.

Table 3
Nutritional characteristics of juice peel and of dessert peel before (Experiment 1) and after pre-treatments (experiments 2–6). In cases when samples from more than one replicate were conducted, values are presented as mean ± standard deviation, in other cases the value of the singlet sample is presented.

	Soluble tannins (% DM)	Total nitrogen (% DM)	Soluble carbohydrates (% DM)	Crude fibre (% DM)	Total lipids (%)	Total phenolic compounds (mg GA 100 g^{-1} extract)	Volatile solids consumption (percentage points)
Experiment 1							
Juice peel	2.1	0.86	12.2	68	1.3	0.089	0
Dessert peel (n = 2)	0.53 ± 0.01	0.88 ± 0.26	13.6 ± 0.08	68.6 ± 2.8	1.4 ± 0.04	0.98 ± 0.05	0
Experiment 2							
$Trich_{7d}$ (n = 2)	0.60 ± 0.005	0.60 ± 0.12	13.7 ± 3.5	32.4 ± 2.9	1.4 ± 0.04	0.14 ± 0.12	4.0 ± 0.46
$Trich_{14d}$	1.1	0.51	15.4	40.8	1.4	0.05	8.8
$Rhiz_{7d}$ (n = 3)	0.79 ± 0.43	0.91 ± 0.25	7.1 ± 3.0	61.5 ± 1.4	1.4 ± 0.08	0.76 ± 1.0	4.4 ± 0.56
$Rhiz_{21d}$	1.2	0.24	44.9	61.9	1.3	0.07	8.1
Bac_{7d} (n = 3)	1.3 ± 0.33	0.57 ± 0.18	6.0 ± 1.9	67.9 ± 15.1	1.3 ± 0.07	1.0 ± 0.10	6.6 ± 2.3
Bac_{14d}	1.3	0.69	3.7	59.5	1.4	1.1	13.7
Experiment 3							
$NH3_{0.8\ \%N}$	2.6	3.7	12.5	60.9	1.4	0.09	1.2
$NH3_{1\ \%N}$(n = 2)	0.80 ± 0.29	3.5 ± 0.09	10.1 ± 2.4	72.1 ± 2.9	1.4 ± 0.07	0.47 ± 0.30	1.2 ± 2.2
$NH3_{0.8\%N\_14d}$	1.3	2.9	5.8	61.7	1.4	1.1	0
Experiment 4							
Heat(n = 3)	2.7 ± 0.59	0.60 ± 0.22	16.1 ± 5.4	63.0 ± 3.0	4.1 ± 0.27	3.5 ± 2.8	0
Experiment 5							
$NH3_{0.8\ \%N}$ + $Trich_{7d}$	1.6	1.0	11	54.3	1.3	0.50	2.9
$NH3_{0.8\ \%N}$ + $Rhiz_{7d}$ (n = 3)	0.85 ± 0.54	2.5 ± 0.09	7.9 ± 3.6	63.1 ± 1.5	1.4 ± 0.04	0.42 ± 0.16	7.0 ± 0.53
$NH3_{0.8\ \%N}$ + Bac_{7d}(n = 2)	1.1 ± 0.02	2.3 ± 1.1	12.0 ± 0.06	63.7 ± 1.5	1.4 ± 0.05	0.74 ± 0.98	5.1 ± 0.16
Experiment 6							
Heat + $Rhiz_{7d}$	3.1	1.0	12.6	62.4	3.6	0.20	0
Heat + Bac_{7d} (n = 2)	1.9 ± 0.48	0.83 ± 0.02	11.3 ± 1.1	55.6 ± 1.9	2.8 ± 1.9	3.1 ± 1.1	6.5 ± 1.0

Table 4
Process parameters in BSFL composting of peels before (experiment 1) and after pre-treatments (experiments 2–6). In cases when triplicates were conducted, values are presented as mean (n = 3) ± standard deviation, in other cases the value of the singlet sample is presented. Same letter column wise represent no significant differences ($p < 0.05$). Only sample conducted in triplicate are included in the statistical comparison.

	Final larval weight (mg $larva^{-1}$)	Survival rate (%)	Death rate (log_{10} %)[i]	Biomass conversion ratio (% VS)	Material reduction (% VS)
Experiment 1					
Juice peel	33 ± 2[a]	97.7 ± 1.9	0.65 ± 0.28[a]	0.87 ± 0.09[a]	70.2 ± 3.2[a]
Dessert peel	134 ± 3[b,c]	91.5 ± 6.8	0.80 ± 0.45[a,b]	7.2 ± 1.2[b,c,e]	48.9 ± 17.4[a,c]
Experiment 2					
$Trich_{7d}$	169 ± 60[b,d,c]	90.8 ± 4.5	0.91 + 0.27[a,b]	8.2 ± 1.4[c,d]	36.3 ± 2.9[b,c]
$Trich_{14d}$	199	93.5	0.81	11.6	35.2
$Trich_{21d}$	226	60.5	1.60	9.5	35.1
$Rhiz_{7d}$	213 ± 11[d,e,f]	59.3 ± 18.1	1.6 ± 0.20[b]	6.7 ± 1.9[b,c,d,e]	32.3 ± 3.7[b,c]
$Rhiz_{14d}$	220	99.5	−0.3	15.0	38.3
$Rhiz_{21d}$	119	98.0	0.3	8.0	38.5
Bac_{7d}	194 ± 15[b,d]	89.2 ± 7.7	0.89 ± 0.51[a,b]	8.1 ± 1.0[c,d,e]	51.0 ± 1.2[a,c]
Bac_{14d}	152	97.0	0.48	14.5	26.5
Bac_{21d}	151	94.0	0.79	9.9	36.0
Experiment 3					
$NH3_{0.8\ \%N}$	177 ± 23[b,d]	97.7 ± 2.4	0.19 ± 0.5[a]	7.1 ± 0.5[b,c,e]	40.3 ± 7.9[c]
$NH3_{1\ \%N}$	176 ± 44[b,e]	89.3 ± 9.4	0.69 ± 0.86[a,b]	9.6 ± 3.9[c,d,e]	25.6 ± 17.5[b,c]
$NH3_{0.8\%N\_14d}$	140	97.0	0.19 ± 0.50[a,b]	6.0	
Experiment 4					
Heat	113 ± 21[b]	66.3 ± 7.8	1.5 ± 0.10[c,b]	3.5 ± 0.8[a,b]	8.9 ± 0.6[b]
Experiment 5					
$NH3_{0.8\%N}$ + $Trich_{7d}$	231	51.0	1.7	6.1	44.8
$NH3_{0.8\ \%N}$ + $Rhiz_{7d}$	229 ± 22[d,e]	94.0 ± 2.3	0.76 ± 0.16[a,b]	14.8 ± 1.2[d]	47.8 ± 4.3[a,c]
$NH3_{0.8\ \%N}$ + Bac_{7d}	187 ± 32[b]	90.5 ± 10.0	0.81 ± 0.47[a,b]	11.0 ± 1.7[d,e]	29.1 ± 3.7[b,c]
Experiment 6					
Heat + $Trich_{7d}$	136	94.5	0.74	7.4	24.0
Heat + $Rhiz_{7d}$	105 ± 11[a,c,f]	74.0 ± 16.2	1.3 ± 0.36[a,b]	2.6 ± 0.10[a,b]	25.7 ± 3.3[b,c]
Heat + Bac_{7d}	143 + 12[b,f]	88.0 ± 8.0	1.0 ± 0.29[a,b]	3.3 + 0.43[b]	66 + 1.0[a]

Most of the pre-treatments improved $BCR_{\%VS}$ of dessert peel compared with the non-pre-treated peel, except for $Rhiz_{7d}$, $NH3_{0.8\%N\_14d}$, $NH3_{0.8\%N}$ + $Trich_{7d}$, heat, Heat + Bac_{7d} and Heat + $Rhiz_{7d}$. The Bac_{7d}, Heat + $Rhiz_{7d}$ and heat pre-treatments resulted in lower larval weights (<120 mg $larva^{-1}$) and $BCR_{\%VS}$ (2–4% VS), with exception of Bac_{7d} with 8% VS and addition of Heat + Bac_{7d} with 3% VS. BSFL reared on dessert peel subjected to a combination of microbial and ammonia pre-treatments developed rapidly and weighed more than those reared on non-pre-treated dessert peel, and had a final larval weight >190 mg $larva^{-1}$ and $BCR_{\%VS}$ranging from 6 to 14% VS. Higher $BCR_{\%VS}$ values were achieved with the 14 d long pre-treatments: 12% VS for $Trich_{14d}$, 15% VS for $Rhiz_{14d}$ and 15% VS for Bac_{14d}, while that for the 7 d and 21 d pre-treatments was less than 10% VS. None of the substrate properties measured was found to contribute to the material reduction. Similarly, the survival rate did not change considerably when rearing the larvae on differently pre-treated peel except for $NH3_{0.8\%N}$ + $Trich_{7d}$ where $SR_{\%}$ was considerably lower than in the other pre-treatments (Table 4).

i) The ANOVA analysis was conducted on the $\log_{10}$ death rate in order to comply with the test requirements of normality.

The PCA results demonstrated that $BCR_{\%VS}$was the only BSFL composting parameter affected by the pre-treatments and that it correlated with the nutritional parameters (Table 5, Fig. 1). Final larval weight, material reduction and survival rate were not affected significantly by the pre-treatments. A correlation between substrate VS and $BCR_{\%VS}$was observed in this study, with the larger the substrate VS, the lower the ratio (Table 5, Model 1.0). It was also observed that the higher the substrate VS consumption during pre-treatments, the higher the $BCR_{\%VS}$value achieved (model 2.0). The nitrogen-enriched treatments had higher $BCR_{\%VS}$, while the heated samples had lower values. On including both VS consumption during pre-treatments and the nitrogen content (Table 5, model 2.1), the adjusted R^2 value increased from around 0.3 to over 0.6. The adjusted R^2 value increased to almost 0.8 when the substrate content of tannins was also included (Table 5, model 2.2).

4. Discussion

4.1. BSFL composting efficiency with non-pre-treated banana peel

Although both juice peel and dessert peel were from ripe bananas, they differed in their composition (Tables 2 and 3). The differences might have been due to the different varieties/cultivars of bananas, geographical locations and soils at the growing sites, pesticides used, degree of ripeness and post-harvest handling. Larvae growth and biomass conversion ratio were much higher on the non-pre-treated dessert peel than on the non-pre-treated juice peel. Previous studies on BSFL composting of banana peel have reported a final larvae weight of 55 mg $larva^{-1}$ (Nyakeri et al., 2017). Final weight of the larvae in the present study was lower on juice peel (30 mg $larva^{-1}$) than on dessert peel (134 mg $larva^{-1}$). However, the $BCR_{\%VS}$values obtained for non-pre-treated juice and dessert peel in this study (0.9 and 7.2% VS) were lower than that reported in the previous study (11% VS) (Nyakeri et al., 2017). The reason for this could be the differences in the banana peel used and in the BSFL composting set-up, as it has been demonstrated that factors such as larval density and larval feeding rates affect process efficiency (Parra Paz et al., 2015).

Table 5
Model strength (adjusted R^2) and F-test significance value (p) for the evaluated models.

	Dependent variable	Predictors	Adjusted R^2
Model 1.0	Biomass conversion ratio	Substrate VS	0.438***
Model 2.0	Biomass conversion ratio	VS consumption	0.331**
Model 2.1	Biomass conversion ratio	VS consumption, nitrogen	0.558***
Model 2.2	Biomass conversion ratio	VS consumption, nitrogen, tannin	0.788***
Model 2.3	Biomass conversion ratio	VS consumption, nitrogen, tannin, phenolic compounds	0.795***

Significance level:
** $p < 0.01$.
*** $p < 0.001$.

4.2. Efficiency of BSFL composting with microbial pre-treatments

Microbial pre-treatment of dessert peel yielded larger larvae (>150 mg $larva^{-1}$) than produced on the non-pre-treated dessert peel (134 mg $larva^{-1}$), with the exception of larvae in the pre-treatment with $Rhiz_{21d}$ (190 mg $larva^{-1}$). *Trichoderma reesei* treatment also improved peel digestibility in monogastric animals (Katongole et al., 2017). Many types of yeast, bacteria and fungi, when applied to organic waste, produce enzymes that degrade the cellulose (the enzyme cellulase) and tannins (tannase) in the waste (Katongole et al., 2017). Furthermore, microbial pre-treatment is reported to increase the lactic acid bacteria content in banana peel, which when consumed by animals serves as a feed supplement and improves the intestinal microbial balance. According to those authors, the increase in final larvae weight due to microbial pre-treatment of banana peel might be explained by increased digestibility and utilisation of the nutrients in the peel caused by either improved availability or the presence of lactic acid bacteria. Similar microbial effects could explain the improved growth and development of BSFL fed on poultry manure inoculated with bacteria from the gut of BSFL (Yu et al., 2011). Comparing the 7 d, 14 d and 21 d microbial pre-treatments in the present study, the impact on biomass conversion ratio suggests that 14 d might be closest to the optimum pre-treatment time for the microorganisms to achieve maximum degradation of the substrates, facilitating larval assimilation of the released nutrients (Table 3, Fig. 1). Increasing the pre-treatment time to 21 d resulted in lower $BCR_{\%VS}$, possibly because the microorganisms themselves consumed the nutrient required by the BSFL.

4.3. Efficiency of BSFL composting with chemically pre-treated substrates

The BSFL grown on nitrogen-enriched dessert peel were larger and $BCR_{\%VS}$was higher than found for non-pre-treated dessert peel (Table 4). This could be due to the overall higher nitrogen level and lower carbon to nitrogen (C/N) ratio, which may improve BSFL conversion efficiency (Rehman et al., 2017b). However, it has been demonstrated that low C/N ratio is not enough for good conversion of substrate into larval biomass, and that the larval feeding dose of protein and VS is important (Lalander et al., 2019). The high final larvae weights and $BCR_{\%VS}$observed in the ammonia pre-treatments suggest that the BSFL process may be able to convert non-protein nitrogen to protein (Tables 2 and 3). Ruminant animals are also known to convert non-protein nitrogen into protein, since fermentation bacteria in the rumen can synthesis amino acids from non-protein nitrogen (Andrade-Montemayor et al., 2009; Tadele and Negassie, 2015).

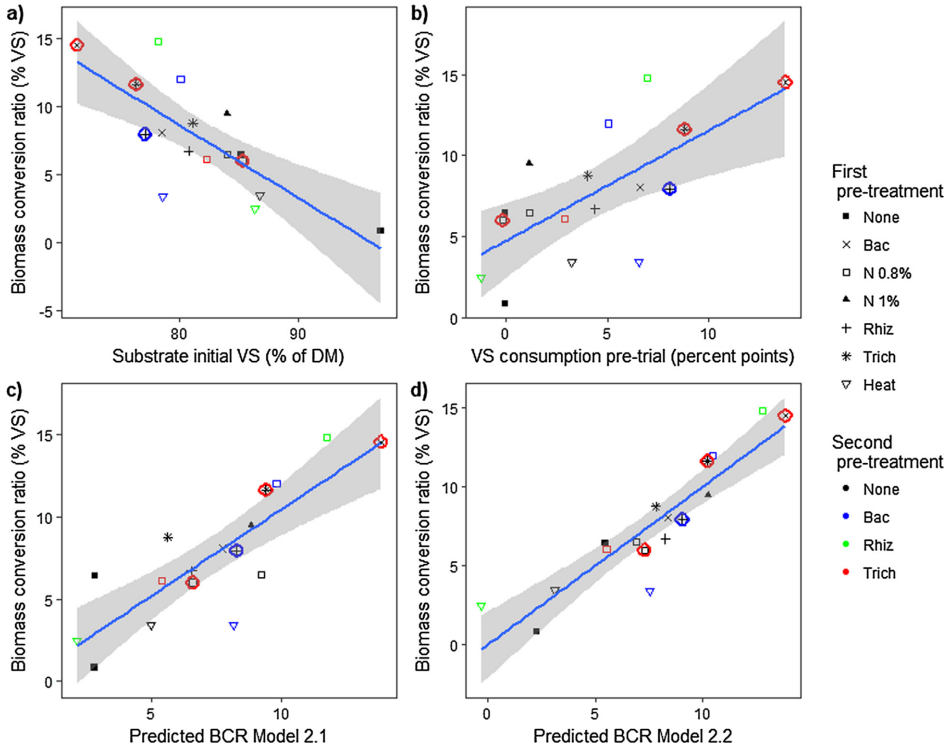

Fig. 1. Correlation between waste to biomass conversion ratio ($BCR_{\%VS}$) and (a) initial substrate VS (% of DM); (b) VS consumption during the pre-treatment (percent points); (c) predicted BCR values for Model 2.1, taking into account VS consumption during pre-treatment and substrate nitrogen content; and (d) predicted BCR values of Model 2.2, taking into account VS consumption during pre-treatment and the substrate content of nitrogen and soluble tannins. The points circled in red represent 14-day pre-treatments, while those circled in blue represent 21-day pre-treatments.

4.4. Efficiency of BSFL composting with heat pre-treatments

Heating the banana peel seemed to increase the amounts of tannins, which could explain the lower $BCR_{\%VS}$ ($\leq$4% VS) and larvae with low final weight (Fig. 1). Interestingly, similar findings in a herbivore study concluded that tannins, among other substances, are anti-nutritional parameters in food substrates that result in lower weight gain and lower efficiency of utilisation of dietary dry matter in herbivores (Butler, 1992).

4.5. Efficiency of BSFL composting with microbial pre-treatment and chemical pre-treatment combined

The BSFL grew larger (231, 229, 187 mg larva^{-1}) on dessert peel pre-treated with ammonia, followed by microbial pre-treatment. These combined pre-treatments also yielded a $BCR_{\%VS}$of 11% ($NH3_{0.8\ \%N}$ + Bac_{7d}) to 15% ($NH3_{0.8\ \%N}$ + $Rhiz_{7d}$), although $NH3_{0.8\%N}$ + $Trich_{7d}$ gave a value of 6.1%. The good BSFL responses to this combined method can be attributed to a more adequate protein dose and to the pre-treatments degrading complex carbohydrates into forms more available to the BSFL (demonstrated by the VS consumption during the pre-treatments).

4.6. Efficiency of BSFL composting with heating pre-treatment and chemical pre-treatment combined

The non-pre-treated dessert peel yielded BSFL with smaller final larval weight compared to the combined heat + chemical pre-treatments with exception of Heat + $Rhiz_{7d}$. All the combined heat + chemical pre-treatments gave lower waste to biomass conversion ratio, except for Heat + $Trich_{7d}$ (7.4% VS). The low BSFL responses in these treatments are likely to have been caused by the observed high amounts of tannins from the heating pre-treatment. The higher $BCR_{\%VS}$for Heat + $Trich_{7d}$ probably means that *Trichoderma reesei* was able to break the compounds present in the peel following heat pre-treatment into more readily available nutrients that aided its conversion into BSFL biomass. This suggests that effect of the substrate VS on BSFL efficiency depends on the composition of the VS under different pre-treatments, rather than the amount of VS in the substrate. Pre-treatments may then have the ability to transform the substrate VS into either

more digestible products or products that still do not enhance substrate nutrient utilisation by the BSFL.

4.7. Feasibility of using these pre-treatments on BSF treatment of banana peel waste

Pre-treatment increases waste to biomass conversion ratio, but also requires additional infrastructure and a longer retention time, of more than 7 d. The value of the increased productivity needs to be weighed against the additional operating and investment costs, especially for an industrial set-up. Operationally, to optimise pre-treatment solutions larger batches should be treated for cost efficiency, but BSFL treatment requires thin layers of substrate. Shorter pre-treatment may be the most economically viable option, even though it does not yield the highest output. Further process optimisation studies are required to find the best combination of pre-treatment and full process design.

5. Conclusions

Most pre-treatments of banana peel improved the waste to biomass conversion ratio compared with peel that was not pre-treated. The exception was heat pre-treatment and combined heat and microbial pre-treatment. Factors affecting BSFL composting were the efficiency of fungi/bacteria pre-treatment and the compounds present in the substrate after pre-treatment, such as total nitrogen, tannins and total phenolic compounds. Pre-treated substrates with high amounts of tannins and total phenolic compounds produced small, low- weight larvae and resulted in low biomass conversion ratio. Chemical pre-treatment with non-protein nitrogen produced largest final larvae weight and high biomass conversion ratios. Applying a pre-treatment such as those evaluated in this study could thus enable BSFL composting of currently unsuitable nutrient-imbalanced and fibrous wastes.

Acknowledgements

Trichoderma reesei and *Rhizopus oligosporus* were kindly provided by Volkmar Passot, Department of Molecular Sciences, SLU, Uppsala. This study was funded by the Swedish International Development Cooperation Agency (Sida - Styrelsen för Internationellt Utvecklingssamarbete) as part of the research Sustainable agricultural productivity, processing, and value chain for enhancing food security in Tanzania. The authors wish to thank Prof. Amelia Kivaisi of the University of Dar es Salaam and Moritz Gold of Eawag - Swiss Federal Institute of Aquatic Science and Technology, for their valuable comments and inputs to this article.

Declaration of Competing Interest

None.

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Journal of Cleaner Production 318 (2021) 128570

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

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

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ARTICLE INFO

Handling editor: Prof. Jiri Jaromir Klemeš

Keywords:
Protein-carbon balance
Organic waste
Fruit waste
Biodegradable waste
Fibrous waste

ABSTRACT

Black soldier fly (BSF) larvae composting is a promising waste treatment that can add value to available biodegradable waste. However, substrates that have low protein content and contain complex molecules (*e.g.* fruit peels) are not easily degraded by the larvae. This study evaluated the impact on the BSF larvae composting efficiency of co-composting different mixtures of banana and orange peels with incremental increase of fish waste. Mixtures (in total 50 distinct mixtures) of varying proportions of banana peels, orange peels and fish waste were evaluated. BFSL fed on orange peel and banana peel mixtures, containing no fish waste, resulted in a lower biomass conversion efficiency (4.5% ± 1.3) on a volatile solids (VS) basis (BCE_{vs}). Co-composting the fruit peels with fish waste increased the biomass conversion efficiency and the highest BCE_{vs} (25%) was attained when 75% fish waste was included. However, the BCE_{vs} varied greatly (18.0% ± 5.8), likely due to varying fish waste composition. A 25% fish waste inclusion resulted in more than twice as high BCE_{vs} (12.3% ± 2.1) compared to when no fish waste was included. As the conversion efficiency variance increased with increasing fish waste inclusion, it was recommended to keep the inclusions of the fish waste to around 25% of the total mixture, in order to increase the reliability of the BSF larvae composting efficiency.

1. Introduction

Globally, municipal solid waste generation was estimated to be 2 billion tons in 2016 and has been projected to increase to 3.4 billion tons by 2050 if the current waste generation rate continues (Kaza et al. 2018). This is a particular problem for low-, and middle-income countries with poor waste management, where waste generation is increasing faster than income changes, and is expected to more than double by 2050 (Kaza et al. 2018). Municipal authorities responsible for waste management logistics in these countries are not likely to have the capacity and resources to handle these increasing amounts of waste (Agamuthu Pariatamby et al. 2019). Currently, 93% of waste generated in most low-income countries (mostly containing > 50% biodegradable waste) end up being burned or dumped on roadsides or open land, or in waterways (Kaza et al. 2018). Other fractions, such as plastics and metals, are collected by the informal sector for recycling (Linzner and Lange, 2013). Inadequate biodegradable waste management can have detrimental impact on the environment, by emitting greenhouse gas emissions contributing to climate change, and leaching plant nutrients into water bodies which contribute to eutrophication (Ferronato and Torretta, 2019). The informal sector does not collect the biodegradable waste and there are a number of reasons that could explain why: for one thing, there is no source segregation, making collection more difficult (Hettiarachchi et al., 2018); furthermore, a reported problem in waste management is that treatment cost exceed the value of the generated products (Lohri et al., 2014). Composting, small-scale anaerobic digestion and use of insects and worms have been suggested as potential treatment technologies for converting the biodegradable waste into more valuable products (Lohri et al., 2017). One specific technology that has attracted great interest in the past decade is waste conversion using

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https://doi.org/10.1016/j.jclepro.2021.128570
Received 20 January 2021; Received in revised form 30 July 2021; Accepted 4 August 2021
Available online 11 August 2021

the black solider fly (BSF) larvae (Surendra et al., 2020).

The larvae of BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae) can process a wide variety of biodegradable wastes, even up to 80% on a wet weight basis for some substrates (Lalander et al. 2019). The larvae biomass have crude protein content of around 40% on a dry matter (DM) basis, but the content varies somewhat (by ±5 percentage points), depending on the substrate the larvae have been reared on, larval size and age (Wang and Shelomi, 2017; Do et al., 2020). The fat content of the larvae is around 30% but varies greatly (by ±15 percentage points) depending on the substrate (Ewald et al. 2020). The larvae can be used in animal feed or in production of biodiesel or other industrial products (Surendra et al., 2016). The treatment residues can be used as an organic fertiliser (Kawasaki et al., 2020) or as a raw material in other processes, such as feedstock in anaerobic digestion (Lalander et al. 2018).

Achieving high biomass conversion efficiency is important in BSF larvae composting, since the larvae biomass has higher economic value than the treatment residue (Lalander et al. 2018). Although the larvae can consume a wide variety of biodegradable substrates, low treatment efficiencies have been reported for substrates with imbalanced carbon: nitrogen ratio and/or high fibre content (Nyakeri et al., 2017) and substrates containing substances that could be toxic to the larvae, *e.g.* phenols (Isibika et al., 2019). Moreover, protein:carbohydrate ratios have been found to play a role and ratios between 1:2 (Lalander et al. 2019) and 1:1 (Cammack and Tomberlin, 2017; Gold et al., 2020) have been demonstrated to favour the conversion of waste into larval biomass. Homogenous substrates from a single source, such as food industry waste, are generally nutritionally imbalanced in some way, *e.g.* high content of fibre or carbohydrates. Different forms of pre-treatments have been demonstrated to improve the BSF larvae composting efficiency of these substrates. For example, different types of microbial inoculation was found to aid the breakdown of complex compounds into forms more easily available to the larvae and thereby increase the biomass conversion efficiency (Yu et al., 2011; Isibika et al. 2019; Somroo et al., 2019).

Co-composting fibre or carbohydrate-rich fractions with fractions that have *e.g.* higher protein content is another way of improving the BSF larvae composting efficiency of these substrates. Rehman et al. (2017) reported increased degradation of cellulose, hemicellulose and lignin, and increased final larval weight, reduction efficiency and biomass conversion efficiency when 40% dairy manure (fibre-rich) was co-composted with 60% chicken manure (nitrogen-rich). Lalander et al. (2019) demonstrated that supplementing low-protein substrates (fruit & vegetable waste) with a protein-rich substrate (abattoir waste) improved the biomass conversion efficiency from 4% DM (pure fruit & vegetable waste) to 14% DM (1:1 fruit & vegetable waste: abattoir waste). Nyakeri et al. (2019) demonstrated that co-composting 30% faecal sludge with other organic wastes, such as banana peels, increased the biomass conversion efficiency and waste reduction in BSF larvae composting compared with composting faecal sludge only. Lopes et al. (2020) found that a small inclusion (10–15%) of a protein-rich substrate (aquaculture waste) could improve the biomass conversion efficiency and protein content of generated larvae when BSF larvae composting a carbohydrate-rich waste (reclaimed bread).

Lopes et al. (2020) determined the impact of BSF larvae co-composting of two rather high-quality waste fractions, that is, whole fish carcasses of rainbow trout (*Oncorhynchus mykiss)* and reclaimed bread. However, the composition of many food industry waste fractions is not of such a high quality as the ones studied in Lopes et al. (2020). Therefore, the aim of this study was to assess the impact of co-composting a protein-rich low-quality waste stream (fish waste, comprising fins and all internal contents of the fish) with a fiber-rich low-quality waste stream (peels of orange and banana) on BSF larvae composting efficiency in term of biomass conversion, waste reduction and larvae survival. To understand the impact of inclusion of the protein-rich waste stream, incremental increase/decrease (from 0 to 100%) of orange peel, banana peel and fish waste were evaluated, totalling 50 distinct mixtures.

2. Materials and methods

2.1. Material and preparation

2.1.1. BSF larvae

BSF larvae (5–7 d old) were obtained from our BSF colony that has been in operation since 2015 (Swedish University of Agricultural Sciences, Uppsala, Sweden). The BSF larvae were reared for around 7 d on chicken feed (Granngården Hönsfoder Start, metabolisable energy content of 11.2 MJ kg^{-1}, 80% moisture) at a feed rate of 0.83 g 100 $larvae^{-1}$.

2.1.2. Waste substrates

2.1.2.1. Fish waste. Different fish species of wild-caught fish containing mainly perch and roach, but also some bleak, rudd, smelt, ruffe and herring were fished using multimesh gillnets in the Sea of Åland, collected and supplied by the Department of Aquatic Resources (Kustlaboratoriet, Öregrund, Sweden). Batches of approximately 100 kg fish were supplied on four different occasions and the batches differed in terms of both species and fish sizes, depending on availability at the source. Once received, the fins and all internal contents of the fish, including gills, river, kidney, intestines, heart, stomach and swim bladder, were collected and mixed. This fish waste fraction represented available fish waste in Tanzania.

2.1.2.2. Banana peels and orange peels. Banana and orange peels were provided in several different batches by the fruit and vegetable wholesaler Grönsakshallen Sorunda (Stockholm, Sweden). Only orange peels were provided, while the bananas were provided whole and peeled upon arrival.

2.1.2.3. Processing of the waste substrates. All three substrates were homogenised separately to mimic the pre-treatments used in BSF larvae treatment facilities (Dortmans et al., 2017), using a blender (Robot Coupe Blixer 4 V, France), divided into feeding portions and stored at −20 °C until use. The mixture substrates aimed at supplying 0.25 g VS $larva^{-1}$ in total over the entire treatment, however a range of 0.26–0.4 g VS $larva^{-1}$ was supplied (Supplementary information,Table S2).

2.2. Physico-chemical and nutritional analysis

2.2.1. Dry matter and total volatile solids

Dry matter content (DM) of the waste materials were determined by heating samples at 70 °C for 48 h. The drying was done at lower temperatures to prevent losses of volatile organic substances (Vahlberg C et al., 2013). After drying, the dried materials were heated in a furnace (LH30/12, Nabertherm GmbH, Germany) first at 200 °C for 2 h (to prevent sample losses due to rapid heating at high temperatures) then heated again to 550 °C for 4 h (ISO, 18122:2015) for determination of VS.

The percentage dry matter (DM) and total volatile solids on a dry matter basis (VS) were calculated as:

$$DM = \left(\left(m_{dry.sample} \right) / m_{wet.sample} \right) \times 100 \quad \text{Equation 1}$$

$$VS = \left(\left(m_{dry.sample} - m_{ash.sample} \right) / m_{dry.sample} \right) \times 100 \quad \text{Equation 2}$$

where, $m_{wet.sample}$, $m_{dry.sample}$ and $m_{ash.sample}$ are the sample weights before and after, the drying and after the combustion, respectively.

2.2.2. pH

The pH of the substrate mixtures and the treatment residues was determined using an InoLab Laboratory pH meter. For this, a 10 g sample was placed in a 50-mL centrifuge tube, diluted to 50 mL with deionised water and left to stand for 1h at room temperature before the pH readings.

2.2.3. Proximate analysis

Proximate analyses of protein, fat, fibre and total phenols (Table 1) were performed in triplicate on the first batch of the pure waste streams (fish waste, banana peel, orange peel) at Eurofins Food & Agro Testing Sweden AB (Swedac-accredited laboratory). Modified EU method 2009/152 was used to analyse protein and fat, while ISO 5498 modified method was used to analyse fibre. Total phenol content was analysed using the laboratory in-house Folin-Ciocalteu method, while carbohydrate content was estimated by subtracting the weight (g) of protein, fat, water and ash from the total weight (g) of the sample. The composition of these substances in the mixed substrates was calculated based on their concentration in each fraction (Supplementary information,Table S1) and the total amount of each fraction in respective mixture.

2.2.4. C/N ratio

Carbon:nitrogen (C/N) ratio was calculated by dividing percentage of organic carbon (calculated as percentage VS divided by 1.8; Haug 1980) by percentage of total nitrogen (DM basis). The organic carbon and total nitrogen were calculated based on the resulting total amounts of protein and volatile solids contents established from the individual substrates in the substrate mixtures.

2.3. Experimental set up

All treatments were performed at 28 °C in individual plastic containers (Smartstore classic 2, with dimensions L21xW17xH11 cm^3), each covered with a plastic lid with a rectangular fabric mesh-covered opening (L9xW5 cm^2) to allow air circulation. The portioned and frozen fish waste, banana peel and orange peel substrates were thawed at room temperature (28 °C) for 24 h and thoroughly mixed according to the required ratio in 50 different combinations (Table S1). Fish waste inclusion rate in the mixture was fixed at 0%, 10%, 25% 50%, 75% or 100%, while the banana peel and orange peel inclusion rates varied from 100% to 0%, at either 5% or 10% increments. Each treatment mixture received 700 larvae (>0.2 cm in size, 7 d old), resulting in a density of 2 larvae cm^{-2}. The larvae were fed on days 0, 4 and 7. After the last feeding event, the boxes were monitored until around 10% of the larvae had become pre-pupae, at which point the larvae were harvested and the treatment was terminated. The treatment time varied between 2 and 3 weeks.

One sample (~5 g) of each treatment mixture from any part of the treatment box was collected once a week for DM, VS and pH determination. After termination of treatment, larvae/pre-pupae were picked manually from the residues. The DM and VS content of all harvested biomass of larvae and of treatment residues were determined. For the treatment residues, the pH was also measured.

2.4. Calculations

Percentage material reduction on a VS basis (RED_{VS}) was calculated as (Diener et al., 2009):

$$RED_{VS} = \left(1 - \frac{m_{res} * DM_{res} * VS_{res}}{m_{mix} * DM_{mix} * VS_{mix}}\right) \times 100 \quad \text{Equation 3}$$

where, m_{res} and m_{mix} is the mass, DM_{res} and DM_{mix} is the percentage dry matter, and VS_{res} and VS_{mix} is the percentage total volatile solids in treatment residues and substrate mixture, respectively.

Percentage biomass conversion efficiency on a VS basis (BCE_{VS}) was calculated as (Lalander et al. 2019; Gold et al. 2020):

$$BCE_{VS} = \frac{m_{lv} * DM_{lv} * VS_{lv}}{m_{mix} * DM_{mix} * VS_{mix}} \times 100 \quad \text{Equation 4}$$

where, m_{lv} and m_{mix} is the mass, DM_{larvae}, $DM_{mixture}$ is the percentage dry matter, and VS_{larvae} and $VS_{mixture}$ is the percentage total volatile solids in the larvae and substrate mixture, respectively.

Percentage survival rate (SR) of the larvae was calculated as (Gold et al. 2020):

$$SR = \left(\frac{lv_{end}}{lv_{start}}\right) \times 100 \quad \text{Equation 5}$$

where, lv_{end} is the number of larvae that survived to the end of the treatment and lv_{start} was the initial number of larvae used in the treatment (n = 700).

The respired VS ($Resp_{VS}$) was calculated as (Lundgren, 2019):

$$Resp_{VS} = \frac{mVS_{mix} - mVS_{lv} - mVS_{res}}{mVS_{mix}} \times 100 \quad \text{Equation 6}$$

where, mVS_{mix} , mVS_{lv} and mVS_{res} are the mass of VS in the substrate mixture, the larval biomass and the treatment residues, respectively.

2.4.1. Statistical analysis

Principal component analysis (PCA) was performed to find the variables that contributed most to variation in the data, while multi-linear regression was used to verify correlations of selected variables. Normality with 95% confidence was verified in the model residuals with Shapiro-Wilk test. R statistical software (R Core Team, 2016) was used for statistical analysis and for graphical presentation of the data.

3. Results

3.1. Physico-chemical parameters

Dry matter content was below 28% in all substrate mixtures, while VS content ranged between 83 and 95% on a dry matter basis (Table S2). The pH of the substrate mixtures varied between 4 and 7. The protein content increased, while the carbohydrate content decreased, with increasing amount of fish waste in the mixture. The C/N ratio increased with orange peel inclusion and generally decreased with fish waste inclusion in the substrate mixture. The treatment residues of all mixtures had pH between 5 and 10, while the moisture content ranged between 52 and 90%.

3.2. BSF larvae composting efficiency

The larval survival in the substrate mixtures with 0% fish waste was

Table 1
Physico-chemical and nutritional properties of fish waste, banana peel and orange peel used for black soldier fly larvae composting.

	Dry matter	Total volatile solids	Protein	Fat	Fibre	Carbohydrate	Total phenols
	(%)	(%DM)	g $100g^{-1}$	g $100g^{-1}$	g $100g^{-1}$	g $100g^{-1}$	(%)
Fish waste	28.2 + 0.1	86.3 + 0.9	15.9 + 0.4	5.8 + 5.3	0.2 + 0.01	2.1 + 4.8	0.3 + 0.04
Banana peel	11.3 + 0.01	86.3 + 0.1	0.9 + 0.1	1.1 + 0.5	1.9 + 0.4	6.6 + 0.3	0.1 + 0.08
Orange peel	18.8 + 0.04	96.6 + 0.6	1.1 + 0.04	0.3 + 0.01	2.6 + 0.3	14.1 + 0.01	0.4 + 0.03

more than 97%, except for mixture 8 (F0%B70%O30%; 62% survival rate) (Table 2). All the substrate mixtures containing fish waste had a survival rate of more than 50%, with the exception of mixtures 46 and 48 (75% fish waste inclusion, survival rate 34% and 36%, respectively). The larvae from these two treatments had the highest final larval weight, 269 mg larva^{-1} for mixture 46 (F75%B10%O15%) and 255 mg larva^{-1} for mixture 48 (F75%B20%O5%). All 700 larvae died within two days when fed 100% fish waste.

Material reduction on a VS basis was generally greater for the substrate mixtures than for the homogenous peel substrates (mixtures 1 and 11, around 50%) (Table 2). Biomass conversion efficiency increased gradually with increasing inclusion of fish waste in the substrate mixtures. Substrate mixture 8, with 0% fish waste, had the lowest BCE_{VS} (2%), while substrate mixtures 45, 47 and 49, all with 75% fish waste, had the highest BCE_{VS}, 25%, 23% and 20%, respectively. VS respiration generally decreased with increasing inclusion of fish waste in the substrate mixtures.

3.3. Principal component analysis results and model strength

Principal component analysis was conducted to identify the most influential parameters contributing to BSF larvae composting efficiency. The first two principal components explained 73.3% of the variation in the dataset (PC1 47.3%, PC2 26.0%) (Fig. 1a), with phenol, protein, fat, fibre and carbohydrate content making the greatest contributions (Fig. 1b). Content of phenols correlated positively with orange peel inclusion and protein content correlated positively with fish waste inclusion, while no specific nutritional parameter appeared to correlate with banana peel inclusion. BCE_{VS} correlated positively with protein content, and negatively with carbohydrate content.

In the multilinear regression analysis, carbohydrate content was the only substrate property that contributed (66%) to the variation in final larval weight (Model 1.0, Table 3) and the found correlation was negative. Biomass conversion efficiency correlated positively with protein content in the substrate mixtures (Model 2.0, Table 3). The BCE_{VS} increased with increasing protein content in the substrate mixtures

Table 2
Process efficiency in BSF larvae composting. Larvae survival rate, final larval weight, biomass conversion efficiency, material reduction rate and respired volatile solids (VS) for substrate mixtures 1–49. All values are based on single samples.

Mix. no.	Substrate	Survival rate	Final larval weight	Biomass conversion efficiency	Material reduction	Respired VS
		(%)	(mglarva^{-1})	(%VS)	(%VS)	(%)
1	F0%B0%O100%	100.0	50.9	3.9	49.7	45.8
2	F0%B10%O90%	100.0	48.8	4.0	62.5	58.5
3	F0%B20%O80%	100.0	54.4	3.8	62.8	59.1
4	F0%B30%O70%	100.0	62.6	4.7	66.1	61.4
5	F0%B40%O60%	100.0	65.9	3.9	73.1	69.3
6	F0%B50%O50%	100.0	71.8	4.4	66.5	62.1
7	F0%B60%O40%	100.0	69.3	4.0	71.7	67.7
8	F0%B70%O30%	62.3	84.9	2.3	67.7	65.4
9	F0%B80%O20%	100.0	93.9	6.0	59.3	53.3
10	F0%B90%O10%	97.9	88.3	6.8	51.0	44.2
11	F0%B100%O0%	100.0	82.7	5.8	49.5	43.8
12	F10%B0%O90%	72.0	61.0	3.4	56.0	52.6
13	F10%B10%O80%	57.1	137.3	6.1	47.8	41.8
14	F10%B20%O70%	69.4	138.7	7.2	55.6	48.4
15	F10%B30%O60%	72.5	154.0	7.9	65.8	57.8
16	F10%B40%O50%	72.4	139.0	9.4	62.8	53.4
17	F10%B50%O40%	75.0	144.3	9.1	47.9	38.9
18	F10%B60%O30%	74.0	144.3	9.7	57.4	47.7
19	F10%B70%O20%	73.5	146.0	9.2	63.1	53.9
20	F10%B80%O10%	63.0	162.0	8.3	63.4	55.2
21	F10%B90%O0%	72.5	140.3	7.8	55.1	47.3
22	F25%B0%O75%	96.9	137.7	13.5	68.1	45.8
23	F25%B5%O70%	96.0	141.0	13.4	66.0	58.5
24	F25%B10%O65%	85.8	161.0	13.6	74.5	59.1
25	F25%B15%O60%	71.2	179.9	12.3	75.8	61.4
26	F25%B20%O55%	78.9	169.7	11.7	72.0	69.3
27	F25%B25%O50%	86.7	143.7	12.9	61.9	62.1
28	F25%B30%O45%	66.0	219.7	13.8	72.2	67.7
29	F25%B35%O40%	68.0	184.0	12.0	72.5	65.4
30	F25%B40%O35%	70.4	172.7	10.1	68.4	54.6
31	F25%B45%O30%	86.3	161.3	11.5	73.6	52.6
32	F25%B50%O25%	79.3	166.0	11.7	71.2	60.9
33	F25%B55%O20%	91.1	185.0	14.7	68.7	63.5
34	F25%B60%O15%	92.2	174.7	13.9	70.8	60.2
35	F25%B65%O10%	75.5	152.7	9.4	68.3	49.0
36	F25%B70%O5%	99.7	169.0	14.8	67.5	58.4
37	F25%B75%O0%	83.5	169.0	6.9	60.7	60.5
38	F50%B0%O50%	79.5	168.7	17.0	59.7	58.3
39	F50%B10%O40%	55.7	189.0	11.5	63.4	62.2
40	F50%B20%O30%	63.2	182.3	13.5	72.7	59.5
41	F50%B30%O20%	50.0	216.3	13.3	59.2	54.0
42	F50%B40%O10%	49.7	207.7	11.2	55.0	57.0
43	F50%B50%O0%	95.4	131.3	12.8	52.0	58.9
44	F75%B0%O25%	58.6	181.7	15.8	49.4	52.7
45	F75%B5%O20%	97.2	198.3	25.3	62.6	53.9
46	F75%B10%O15%	33.8	268.7	11.7	45.7	42.7
47	F75%B15%O10%	91.1	215.3	23.1	65.7	51.9
48	F75%B20%O5%	35.6	255.0	11.8	50.0	59.1
49	F75%B25%O0%	79.7	198.3	20.4	64.8	45.9

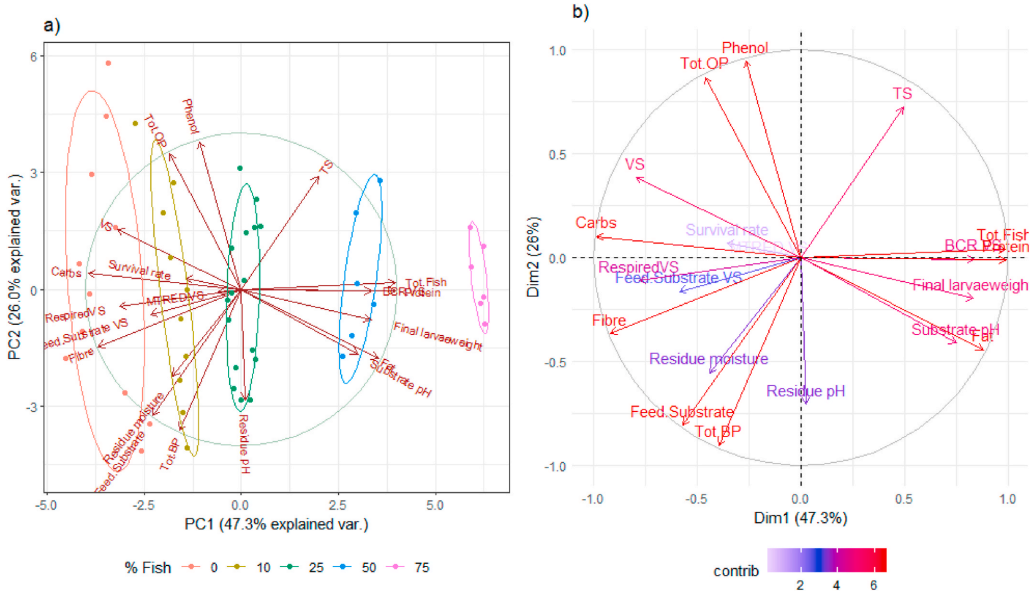

Fig. 1. Principal component (PC) plots obtained for fish waste, banana peel and orange peel substrate mixtures 1–49. Percentage of variation in the data explained by a) PC1 and PC2 and b) contribution of different variables (red indicates large contribution).

Table 3
Model strength (coefficient of determination adjusted R^2) and F-test significance value (p) of two models of dependent variables and predictors in the co-composting process. Negative correlations are denoted by the minus (−) sign. B represents coefficient slope values for the regression equation for predicting the dependent variable from the independent variable.

Model No.	Dependent variable	Model	Coefficients		Adjusted R^2	p- value
			B	Std. Error		
1.0	Final larval weight	Carbohydrates	−0.016	0.002	0.664	0.000
2.0	Biomass conversion efficiency	Protein	0.166	0.017	0.656	0.000

(Table 2, Fig. 1a).

4. Discussion

4.1. BSF larvae composting efficiency of pure fish waste, banana peel and orange peel

When the larvae were fed 100% fish waste, no observable larvae growth occurred, and all the larvae died within the first two days of treatment (Table 2, Fig. 1). This high mortality rate could have been caused by the high fat content in the fish waste. Nguyen et al. (2013) observed that high-fat restaurant and fish wastes decreased larvae growth and survival rates, with no survival being observed of larvae reared on fish waste, and attributed this to high amounts of fat and heavy metal contamination in the fish waste. Difficulties for the BSFL to metabolize and utilize the high fat contents (>6 g/100 g) in fish waste and bioaccumulation of heavy metals in the BSFL biomass were the factors that were associated with the observed inhibition in metabolism, health, and immunity of the BSF larvae that resulted in 100% larval mortality (Nguyen et al. 2013). Diener et al. (2015) observed no negative effects on larval survival when larvae were fed chicken feed spiked with heavy metals (cadmium, lead, zinc). The heavy metal content in the fish waste used in this study was not measured, but fish from the source area (Baltic sea north of Stockholm) are associated with high levels of heavy metals (Manzetti, 2020). Perch from the Baltic sea are known to have high levels of mercury (~209 μg kg^{-1} wet weight) of which depends on the mobility ability of perch along associated aquatic systems, that is, rivers, lakes and oceans (Suhareva et al., 2021). Accumulation of mercury by BSF larvae negatively impacted the size and development rate of the BSF larvae to pupae stage due to inhibition that also slowed the rate of food consumption when fed on food waste mixed with mercury (Attiogbe et al., 2019). Hence, presence of mercury could have similarly inhibited the BSF larvae growth when fed 100% fish waste in this study. Lopes et al. (2020) observed increasing larvae mortality with increasing aquaculture waste inclusion and it was speculated that the oily film formed on top of substrates with a high aquaculture waste inclusion prevented the larvae from breathing. The lack of structure and sticky nature of the fish waste substrate could also have led to suffocation of the larvae in this study, as the small larvae could not aerate, move and process this waste.

Waste substrates characterised by high fibre content (Rehman et al. 2017) and low nitrogen content (Lalander et al. 2019) have been reported to be challenging in terms of nutrient utilisation and conversion by larvae. The pure banana peel was low in protein and high in fibre (Table S2), which likely caused the observed low final larval weight and low biomass conversion efficiency (Table 2), and this is in accordance

with earlier findings for banana peel (Isibika et al. 2019).

Pure orange peel had the lowest protein content and highest amount of carbohydrates and phenols (Table S2). The final larval weight and BCEvs was also observed to increase with decreasing carbohydrate content in the mixtures (Fig. 1, Model 1.0 and Table 3). The high protein:carbohydrate ratio (1:14) in the orange peels could have contributed to the observed low material reduction, BCE_{VS} and final larval weight (Gold et al., 2020). Citrus peel is known to be difficult to treat biologically (Mizuki et al., 1990; Ruiz and Flotats, 2014). Calabrò et al. (2016) showed that anaerobic digestion of orange peel waste was inhibited by an increasing concentration of citrus essential oils (mainly D-limonene). In fact, citrus essential oils are used in insecticides as they have strong insecticidal activity, while they can also serve as antimicrobials, minimising the growth of several fungi and bacteria strains (Bora et al., 2020). Kumar et al. (2012) observed insecticidal activity against larvae and pupae of housefly (*Musa domestica*) from direct contact with essential oil from orange peel (*Citrus sinensis* L.). Presence of phenols in banana peel was also found to negatively impact the BCEvs (Isibika et al. 2019). Toxicity effects of essential oils (Kumar et al. 2012) and anti-nutritional effects (Isibika et al. 2019) from the high phenol content in orange peel could have also affected the conversion efficiencies of the 100% orange peels substrate in this study (Table 2).

4.2. BSF larvae composting efficiency with treatment mixtures of fish waste, banana peel and orange peel

Substrate mixtures of banana peel, orange peel and fish waste generally resulted in improved BFS larvae composting efficiency, compared with the individual substrates (Table 2). However, although all the peel-containing mixtures gave a very high larval survival rate, the BCE_{vs} (<7% VS) and final larval weight (<95 mg $larva^{-1}$) were still low. Pure fruits and vegetable wastes have previously been shown to be poor substrates for larval development, due to low protein and high carbohydrate content (Jucker et al., 2017). It was noticed that banana peel generated a slightly higher BCE_{VS} than orange peel: 5.8% compared to 3.9% for pure orange peel. Increasing the orange peel concentration generally reduced the BCE_{VS}, likely due to the orange peel toxicity discussed above. When diluting orange peel with other waste fractions, the toxicity of the orange impacted less on the overall efficiency, as the BCE_{VS} increased.

Adding fish waste as a protein source generally resulted in increased final larvae weight and biomass conversion efficiency (Table 2). The increase in BCE_{VS} and final larval weight was most likely due to the observed increase in protein content from the fish waste balancing the high carbohydrate and fibre content in the fruit peels.

The highest BCE_{VS} (25%) was achieved on substrate mixture 45, with 75% fish waste (F75%B5%O20%). However, large variations in BCE_{VS} (18 ± 6%) were observed for the six substrate mixtures containing 75% fish waste, indicating an unstable process (Fig. 2). In fact, supplementation with 25% fish waste more than doubled the BCE_{vs} compared with 0% fish waste, and thus could be considered sufficient.

Gold et al. (2020) found a 1:1 protein:carbohydrate ratio in substrate to be ideal for BSF larvae. However, the BCE_{VS} increased 2.7-fold (from 2% to 12%) on increasing the protein: carbohydrate ratio from 0.1 (0% fish) to 0.4 (25% fish) (Fig. 2b), while no significant increase in BCE_{VS} (13%) was found on doubling the protein: carbohydrate ratio to 0.9 (50% fish inclusion). Lopes et al. (2020) concluded that mixing bread with small quantities (<15%) of aquaculture waste was sufficient to maximise the positive impact of nitrogen supplementation on larval development. In the present study, a higher level of fish waste (inclusion of 25%) was needed to achieve larger positive responses in conversion efficiency. This was likely due to the fish waste in this study having a lower protein content (15.9% of DM) than the aquaculture waste (60.3% of DM) used by Lopes et al. (2020). The total protein addition in Lopes et al. (2020) for 15% addition of aquaculture waste was 10%, similar to the addition in this study for the 75% addition, which yielded a 12% protein addition. However, the bread had a somewhat higher protein content than the fruit peels in this study. The lower fat content of the fish waste, as well as the higher fibre content in banana and orange peel compared to bread, could explain why more fish waste could be added in this study without increasing the mortality of larvae (Table 2).

The observed variations in BCE_{VS} and final larval weight with higher fish waste inclusion could have been caused by differences in nutritional composition between the different batches of fish waste, which

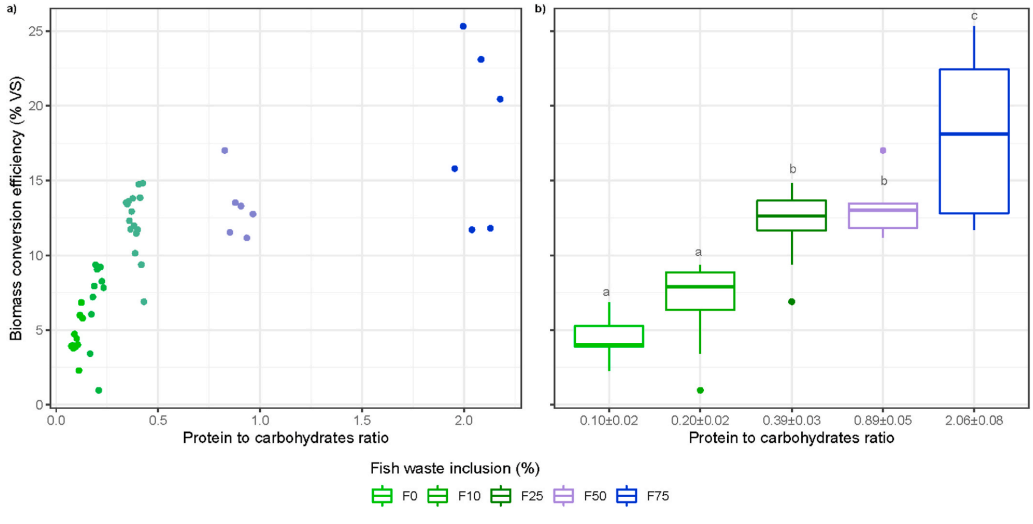

Fig. 2. a) Scatterplot and b) boxplot showing the impact of protein to carbohydrate ratio with increasing fish (F) inclusion level from 0% (green) to 75% (blue) on biomass conversion efficiency on a volatile solid basis (BCE_{VS}). Different letters on boxes in (b) indicate significant difference (95% confidence interval) in mean BCE_{VS}.

contained different types of fish species depending on availability at the source at the time of delivery. Furthermore, during processing of small and large fish species to obtain fish waste, it was found that the stomach contents differed between batches. In some batches great amounts of fat and/or eggs were found in the fish, while the fish in other batches had no or low amounts of fats and/or eggs in the stomach. Thus, the nutritional composition, including fat content and hormone composition of the fish waste, may have caused the large variations observed, particularly for the mixtures with 75% fish waste. Variations in process efficiency when treating substrates with similar and different nutritional composition have been reported to impair the reliability and sustainability of this treatment, especially in industrial-scale operations (Gold et al. 2018). Maintaining lower levels of fat (<40% of DM) and fibre (<50% of DM) in substrate mixtures has been suggested to increase fly larvae treatment performance and reduce variations (Gold et al. 2020). This may be difficult to achieve in a waste treatment facility, as all waste that arrives must be treated, however, keeping the inclusion of the more variable sources smaller (here around 25–50%) can minimise the variations.

Relatively high material reduction (50–75%) was achieved for most substrate mixtures in this study (Table 2). VS respiration correlated negatively with biomass conversion efficiency and positively with material reduction, indicating that the material reductions achieved probably resulted from both BSF larvae and microbial degradation. Microbial activity in the substrates could have partly contributed to the variable treatment conversion efficiencies (material reduction rates) seen for the different substrate mixtures.

Overall, this study demonstrated that it is possible for BSF larvae to degrade challenging low–quality substrates such as orange peel and banana peel, with almost doubled BCE_{vs}, with a relatively small inclusion of a low-quality protein-rich waste steam. The protein content of the fish waste was relatively low compared that reported for *e.g.* aquaculture waste by Lopes et al. (2020), who found that 15% inclusion was sufficient in co-composting with bread. This suggests that a smaller inclusion rate of the protein-rich fraction may be required with a higher-protein substrate. Many organic wastes are currently not fully utilised and end up polluting the environment. Composting by BSF larvae can add value to these wastes, by converting them into insect products potentially suitable for various applications, for example in animal feed.

5. Conclusions

The aim of this study was to see the potential of improving BSF larvae composting efficiency of low-quality food industry wastes by means of co-composting. A fibre-rich, hard to degrade waste stream such as fruit peels, was co-composted with a low-quality protein-rich waste stream (fish waste). BSF larvae did not survive in sticky, fat-rich fish waste and BSF larvae composting of pure and mixed banana and orange peel mixtures resulted in lower final larval weights and BCE_{vs}. Final larval weight and BCE_{vs} generally increased with increasing protein content in the substrate mixtures. Combining fish waste with fruit waste increased BCE_{vs}, to up to 25% with 75% fish waste (12% protein addition) in the substrate mixture. In other words, around 4-fold increase in BCE_{VS} was achieved for the 75% fish waste inclusion compared to what was achieved for pure banana and orange peels. However, large variation in BCE_{VS} (18.0% $\pm$ 5.8) and final larval weight (219 mg $larva^{-1}$ $\pm$35) was found when 75% fish waste was included. Lower inclusion rates of fish waste were thus suggested, as a 2.7-fold increase in BCE_{vs} was found when with 25% fish waste (4% protein addition) as compared to no fish waste inclusion, while the variance in efficiency was kept lower (BCE_{VS} 12.3% $\pm$ 2.1). Lower variations in process efficiency renders higher reliability of the treatment process. This study demonstrated the potential of using low-quality protein-rich waste to improve the BSFL composting of low-quality fibre-rich wastes. The present study provides a scientific basis for future studies that should investigate whether these small-scale results are transferrable to industry-scale in order to advance the industrialization of BSFL composting treatment of biodegradable waste fractions.

CRediT authorship contribution statement

A. Isibika: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **B. Vinnerås:** Conceptualization, Methodology, Supervision, Writing – review & editing. **O. Kibazohi:** Funding acquisition, Supervision, Writing – review & editing. **C. Zurbrügg:** Conceptualization, Supervision, Writing – review & editing. **C. Lalander:** Conceptualization, Methodology, Formal analysis, Supervision, 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.

Acknowledgements

We thank Örjan Östman at SLU Aqua for providing the fish waste, Viktoria Wiklicky for providing the BSF larvae and Giulio Zorzetto for assistance with experimental work at SLU, Uppsala, Sweden. This research was performed with Swedish International Development Cooperation Agency (Sida) funding through the University of Dar es Salaam, Tanzania, and Swedish University of Agricultural Sciences, Sweden, within the sub-programme 'Sustainable Agricultural Productivity - Processing and Value Chain to Enhance food Security in Tanzania'.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2021.128570.

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Supporting information

Table S1. Waste substrate amounts and composition in the 50 mixtures of banana peel, orange peel and fish waste tested

Mixture no.	Fish waste	Banana peel	Orange peel	Fish waste (F)	Banana peel (B)	Orange peel (O)	Total F	Total B	Total O	Feed treatment	Feed Substrate
	%	%	%	g VS	g VS	g VS	(g)	(g)	(g)	(substrate mixture)	(g)
1	0	0	100	0.0	0.0	175.0	0.0	0.0	962.6	F0%B0%O100%	962.6
2	0	10	90	0.0	17.5	157.5	0.0	180.3	866.4	F0%B10%O90%	1046.7
3	0	20	80	0.0	35.0	140.0	0.0	360.7	770.1	F0%B20%O80%	1130.8
4	0	30	70	0.0	52.5	122.5	0.0	541.0	673.8	F0%B30%O70%	1214.8
5	0	40	60	0.0	70.0	105.0	0.0	721.3	577.6	F0%B40%O60%	1298.9
6	0	50	50	0.0	87.5	87.5	0.0	901.7	481.3	F0%B50%O50%	1383.0
7	0	60	40	0.0	105.0	70.0	0.0	1082.0	385.0	F0%B60%O40%	1467.0
8	0	70	30	0.0	122.5	52.5	0.0	1262.3	288.8	F0%B70%O30%	1551.1
9	0	80	20	0.0	140.0	35.0	0.0	1442.7	192.5	F0%B80%O20%	1635.2
10	0	90	10	0.0	157.5	17.5	0.0	1623.0	96.3	F0%B90%O10%	1719.3
11	0	100	0	0.0	175.0	0.0	0.0	1803.3	0.0	F0%B100%O0%	1803.3
12	10	0	90	17.5	0.0	157.5	71.9	0.0	866.4	F10%B0%O90%	938.3
13	10	10	80	17.5	17.5	140.0	71.9	180.3	770.1	F10%B10%O80%	1022.4
14	10	20	70	17.5	35.0	122.5	71.9	360.7	673.8	F10%B20%O70%	1106.4
15	10	30	60	17.5	52.5	105.0	71.9	541.0	577.6	F10%B30%O60%	1190.5
16	10	40	50	17.5	70.0	87.5	71.9	721.3	481.3	F10%B40%O50%	1274.6
17	10	50	40	17.5	87.5	70.0	71.9	901.7	385.0	F10%B50%O40%	1358.6
18	10	60	30	17.5	105.0	52.5	71.9	1082.0	288.8	F10%B60%O30%	1442.7
19	10	70	20	17.5	122.5	35.0	71.9	1262.3	192.5	F10%B70%O20%	1526.8
20	10	80	10	17.5	140.0	17.5	71.9	1442.7	96.3	F10%B80%O10%	1610.8
21	10	90	0	17.5	157.5	0.0	71.9	1623.0	0.0	F10%B90%O0%	1694.9
22	25	0	75	43.8	0.0	131.3	179.8	0.0	722.0	F25%B0%O75%	901.8
23	25	5	70	43.8	8.8	122.5	179.8	90.2	673.8	F25%B5%O70%	943.8
24	25	10	65	43.8	17.5	113.8	179.8	180.3	625.7	F25%B10%O65%	985.8
25	25	15	60	43.8	26.3	105.0	179.8	270.5	577.6	F25%B15%O60%	1027.9

26	25	20	55	43.8	35.0	96.3	179.8	360.7	529.4	F25%B20%O55%	1069.9
27	25	25	50	43.8	43.8	87.5	179.8	450.8	481.3	F25%B25%O50%	1111.9
28	25	30	45	43.8	52.5	78.8	179.8	541.0	433.2	F25%B30%O45%	1154.0
29	25	35	40	43.8	61.3	70.0	179.8	631.2	385.0	F25%B35%O40%	1196.0
30	25	40	35	43.8	70.0	61.3	179.8	721.3	336.9	F25%B40%O35%	1238.1
31	25	45	30	43.8	78.8	52.5	179.8	811.5	288.8	F25%B45%O30%	1280.1
32	25	50	25	43.8	87.5	43.8	179.8	901.7	240.7	F25%B50%O25%	1322.1
33	25	55	20	43.8	96.3	35.0	179.8	991.8	192.5	F25%B55%O20%	1364.2
34	25	60	15	43.8	105.0	26.3	179.8	1082.0	144.4	F25%B60%O15%	1406.2
35	25	65	10	43.8	113.8	17.5	179.8	1172.2	96.3	F25%B65%O10%	1448.2
36	25	70	5	43.8	122.5	8.8	179.8	1262.3	48.1	F25%B70%O5%	1490.3
37	25	75	0	43.8	131.3	0.0	179.8	1352.5	0.0	F25%B75%O0%	1532.3
38	50	0	50	87.5	0.0	87.5	359.6	0.0	481.3	F50%B0%O50%	840.9
39	50	10	40	87.5	17.5	70.0	359.6	180.3	385.0	F50%B10%O40%	925.0
40	50	20	30	87.5	35.0	52.5	359.6	360.7	288.8	F50%B20%O30%	1009.1
41	50	30	20	87.5	52.5	35.0	359.6	541.0	192.5	F50%B30%O20%	1093.1
42	50	40	10	87.5	70.0	17.5	359.6	721.3	96.3	F50%B40%O10%	1177.2
43	50	50	0	87.5	87.5	0.0	359.6	901.7	0.0	F50%B50%O0%	1261.3
44	75	0	25	131.3	0.0	43.8	539.4	0.0	240.7	F75%B0%O25%	780.1
45	75	5	20	131.3	8.8	35.0	539.4	90.2	192.5	F75%B5%O20%	822.1
46	75	10	15	131.3	17.5	26.3	539.4	180.3	144.4	F75%B10%O15%	864.1
47	75	15	10	131.3	26.3	17.5	539.4	270.5	96.3	F75%B15%O10%	906.2
48	75	20	5	131.3	35.0	8.8	539.4	360.7	48.1	F75%B20%O5%	948.2
49	75	25	0	131.3	43.8	0.0	539.4	450.8	0.0	F75%B25%O0%	990.2
50	100	0	0	175.0	0.0	0.0	719.2	0.0	0.0	F100%B00%O0%	719.2

Table S2. Physico-chemical and nutritional properties of substrate mixtures 1-49. Dry matter (DM), volatile solids (VS), pH, protein, fat, fibre, carbohydrates, total phenols, carbon:nitrogen ratio (C/N) and moisture content in the substrates. All values are based on single samples

Mixture no.	Substrate	DM	VS	pH		Protein	Fat	Fibre	Carbohydrate	Total phenols	C/N	VS
		(%)	(%DM)	sub	res	(g)	(g)	(g)	(g)	(g)		(g)
1	F0%B0%O100%	23.9	95.2	4.1	5.1	10.1	2.6	24.7	135.7	3.6	34.2	219.3
2	F0%B10%O90%	21.0	95.3	4.5	4.9	10.7	4.3	25.7	134.1	3.4	32.5	209.7
3	F0%B20%O80%	21.6	94.1	4.5	6.3	11.2	5.9	26.6	132.5	3.2	31.0	230.0
4	F0%B30%O70%	18.6	93.3	4.6	8.9	11.7	7.6	27.6	130.9	3.0	29.6	210.4
5	F0%B40%O60%	22.2	92.9	4.8	9.2	12.2	9.2	28.5	129.3	2.8	28.3	267.9
6	F0%B50%O50%	18.4	94.1	4.8	8.8	12.8	10.9	29.5	127.7	2.6	27.1	239.2
7	F0%B60%O40%	19.2	92.6	5.1	9.3	13.3	12.5	30.4	126.1	2.4	26.1	261.3
8	F0%B70%O30%	19.1	95.2	5.0	9.5	13.8	14.2	31.4	124.5	2.2	25.1	281.5
9	F0%B80%O20%	14.5	91.8	5.4	9.3	14.4	15.9	32.4	122.8	2.0	24.1	217.7
10	F0%B90%O10%	12.5	90.5	5.7	9.4	14.9	17.5	33.3	121.2	1.8	23.3	193.8
11	F0%B100%O0%	13.8	87.7	5.8	8.9	15.4	19.2	34.3	119.6	1.6	22.5	218.9
12	F10%B0%O90%	24.0	93.9	3.5	7.0	20.6	6.5	22.4	123.7	3.4	16.9	211.9
13	F10%B10%O80%	21.0	93.6	4.2	8.1	21.2	8.2	23.3	122.1	3.2	16.4	201.2
14	F10%B20%O70%	20.5	93.1	3.9	8.4	21.6	9.8	24.3	120.4	3.0	16.0	211.4
15	F10%B30%O60%	19.4	92.0	4.4	8.9	22.1	11.5	25.3	118.8	2.8	15.7	212.8
16	F10%B40%O50%	16.4	91.2	4.6	8.9	22.7	13.1	26.2	117.2	2.6	15.3	191.0
17	F10%B50%O40%	17.4	91.5	4.8	9.5	23.2	14.8	27.2	115.6	2.4	14.9	215.8
18	F10%B60%O30%	14.9	91.2	4.9	9.3	23.8	16.4	28.1	114.0	2.3	14.6	195.9
19	F10%B70%O20%	15.4	90.6	4.9	9.3	24.3	18.1	29.1	112.4	2.1	14.3	213.1
20	F10%B80%O10%	15.3	89.9	5.2	9.3	24.8	19.8	30.0	110.8	1.9	14.0	221.8
21	F10%B90%O0%	16.1	87.3	6.0	9.6	25.4	21.4	31.0	109.2	1.7	13.7	237.9
22	F25%B0%O75%	26.8	91.2	5.2	7.9	36.3	12.3	18.9	105.6	3.2	9.6	220.1
23	F25%B5%O70%	25.7	91.3	7.0	7.8	36.5	13.2	19.4	104.8	3.1	9.5	221.2
24	F25%B10%O65%	24.6	89.8	6.5	8.6	36.8	14.0	19.8	104.0	3.0	9.4	217.8
25	F25%B15%O60%	27.3	86.7	5.0	8.5	37.0	14.8	20.3	103.2	2.9	9.4	243.4
26	F25%B20%O55%	24.6	90.4	5.3	8.9	37.3	15.7	20.8	102.4	2.8	9.3	238.2
27	F25%B25%O50%	23.4	88.8	5.0	8.7	37.6	16.5	21.3	101.5	2.7	9.2	230.9
28	F25%B30%O45%	22.4	91.7	5.3	8.9	37.8	17.3	21.8	100.7	2.6	9.2	237.3
29	F25%B35%O40%	22.1	91.5	5.7	8.6	38.1	18.1	22.2	99.9	2.5	9.1	242.2
30	F25%B40%O35%	22.8	90.8	5.7	9.2	38.4	19.0	22.7	99.1	2.4	9.0	256.6
31	F25%B45%O30%	21.6	89.5	5.8	8.8	38.6	19.8	23.2	98.3	2.3	9.0	248.1
32	F25%B50%O25%	21.4	85.3	6.1	8.9	38.9	20.6	23.7	97.5	2.2	8.9	241.7
33	F25%B55%O20%	19.4	88.0	6.1	8.9	39.2	21.4	24.1	96.7	2.1	8.9	234.0

34	F25%B60%O15%	18.8	90.9	6.2	8.5	39.4	22.3	24.6	95.9	2.0	8.8	240.4
35	F25%B65%O10%	17.4	89.9	6.2	9.1	39.7	23.1	25.1	95.1	1.9	8.7	227.3
36	F25%B70%O5%	18.3	87.0	6.1	8.6	40.0	23.9	25.6	94.3	1.8	8.7	237.3
37	F25%B75%O0%	15.1	89.8	6.5	7.2	40.2	24.8	26.1	93.5	1.7	8.6	209.1
38	F50%B0%O50%	26.8	87.9	6.0	7.7	62.4	22.1	13.0	75.4	2.8	5.6	198.8
39	F50%B10%O40%	25.4	89.4	5.8	7.8	62.9	23.7	14.0	73.8	2.6	5.5	210.8
40	F50%B20%O30%	23.2	87.3	6.2	8.8	63.4	25.4	15.0	72.2	2.4	5.5	204.4
41	F50%B30%O20%	19.8	89.0	6.4	8.7	64.0	27.0	15.9	70.6	2.3	5.4	193.1
42	F50%B40%O10%	21.0	88.0	6.5	8.0	64.5	28.7	16.9	69.0	2.1	5.4	218.5
43	F50%B50%O0%	19.4	87.7	6.7	8.9	65.0	30.4	17.9	67.4	1.9	5.3	215.2
44	F75%B0%O25%	27.0	86.7	6.5	7.8	88.5	31.8	7.3	45.3	2.4	3.9	183.0
45	F75%B5%O20%	25.7	87.3	6.5	8.2	88.7	32.6	7.7	44.5	2.4	3.9	184.4
46	F75%B10%O15%	24.0	89.1	6.6	8.3	89.0	33.5	8.2	43.6	2.3	3.9	185.4
47	F75%B15%O10%	24.5	84.4	6.7	8.4	89.3	34.3	8.7	42.8	2.2	3.9	187.5
48	F75%B20%O5%	23.6	84.8	6.7	8.3	89.5	35.1	9.2	42.0	2.1	3.9	190.2
49	F75%B25%O0%	22.3	83.6	6.8	8.7	89.8	36.0	9.6	41.2	2.0	3.9	185.0

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2022:30

Black soldier fly larvae composting is a biowaste treatment that follows principles of circular economy. That, makes the technology a promising option for biowaste management in cities in low and middle-income countries. However, fiber-rich biowaste such as fruits waste with low protein reduce the biomass conversion efficiency (BCE). This thesis present ways that increased the BCE of banana and orange peels up to 100% using pre-treatments and up to 360% increase on blending more than one substrate with fish waste.

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Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-7760- 935-3
ISBN (electronic version) 978-91-7760- 936-0