Process efficiency in black soldier fly larvae composting of plant-based food industry waste

Impact of pre-treatment

Lovisa Lindberg

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

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

Black soldier fly larvae (BSFL) composting, in which biodegradable wastes are converted into animal-feed protein, is a technology that meets circular economy principles. The greatest potential BSFL composting is for mixed food waste, but only plant-based waste is permitted as feed for the larvae. It has lower biomass conversion efficiency (BCE), but this could be improved by pre-treatment.

This thesis investigated process efficiency and GHG and ammonia emissions from BSFL composting using orange peel and broccoli and cauliflower, with ammonia or fungi pre-treatment. The impact of enzyme and ammonia pre-treatment time on process efficiency when using mixed lettuce and cabbage waste was also assessed.

Following two weeks of substrate pre-treatment with ammonia and fungi, direct emissions of GHG and ammonia were evaluated. Lettuce and cabbage was pre-treated with enzymes or ammonia for 0-8 days prior to BSFL composting.

BCE on a volatile solids (VS) basis was greater overall for food waste and lettuce and cabbage (~20%) than for orange peel and broccoli and cauliflower (~7%). The BCE was low (6%) in the orange peel control and even lower in both orange peel pre-treatments. Direct addition of enzymes at the start of BSFL composting gave 22% higher BCE compared with the control.

Total emissions of N_2O and CH_4 were almost four-fold larger for the broccoli and cauliflower control than when pre-treated, indicating that ammonia pre-treatment significantly reduced total GHG emissions with no negative impact on BCE during BSFL composting, but with increased ammonia emissions.

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

Author's address: Lovisa Lindberg, Swedish University of Agricultural Sciences, Department of Energy and Technology, Uppsala, Sweden

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Sammanfattning

En avfallsbehandling som baseras på principer för cirkulär ekonomi är fluglarvskompostering med den amerikanska vapenflugans larver, där biologiskt nedbrytbart avfall omvandlas till animaliskt foderprotein. Den största potentialen med fluglarvskompostering är vid behandling av matavfall, i dagsläget är endast växtbaserat avfall tillåtet som foder för larver, vilket resulterar i en mindre biomassaomvandlingseffektivitet men som skulle kunna förbättras genom förbehandling.

Syftet med denna avhandling var att undersöka processeffektiviteten och utsläppen av växthusgaser och ammoniak från fluglarvskompostering vid användning av apelsinskal och en blandning av broccoli och blomkål, med förbehandling av ammoniak eller svamp, samt förstå effekten av enzym- och ammoniak-förbehandlingstid på processeffektiviteten vid behandling av sallad och vitkål.

Två veckors förbehandling av substrat med ammoniak och svamp undersöktes samt direkta utsläpp av växthusgaser och ammoniak från behandlingarna utvärderades. Sallad och vitkål förbehandlades med enzymer eller ammoniak i 0-8 dagar.

Bioomvandlingseffektiviteten av organiska ämnen (VS) var totalt sett större för matavfall samt för sallad och vitkål (~20 %) än för apelsinskal och broccoli och blomkål (~7 %). Bioomvandlingseffektiviteten var låg (6 %) i apelsinskalskontrollen och ännu lägre i förbehandlingarna, vilket tyder på att detta substrat är olämpligt för fluglarvskompostering. Direkt tillsats av enzymer resulterade i en 22 % högre biomassaomvandlingseffektivitet jämfört med kontrollen. De totala utsläppen av N_2O och CH_4 var nästan fyra gånger högre för kontrollen än för förbehandlad broccoli och blomkål vilket indikerar att förbehandling med ammoniak minskade de totala växthusgasutsläppen utan att påverka bioomvandlingseffektiviteten negativt.

Author's address: Lovisa Lindberg, Swedish University of Agricultural Sciences, Department of Energy and Technology, Uppsala, Sweden

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. Lindberg, L., Ermolaev, E., Vinnerås, B. and Lalander, C. Process efficiency and greenhouse gas emissions in black soldier fly larvae composting with orange peel and vegetables (Submitted)

II. Lindberg, L., Vinnerås, B. and Lalander, C. Process efficiency in relation to enzyme pre-treatment time in black soldier fly larvae composting (Submitted)

Abbreviations

FW	Food waste
OP	Orange peel
BC	Broccoli and cauliflower
LC	Lettuce and cabbage
GHG	Greenhouse gas
TS	Total solids
VS	Total volatile solids
WW	Wet weight
BCE	Biomass conversion efficiency

1. Introduction

In Sweden, 40% of biodegradable waste is treated biologically, mainly by anaerobic digestion. Anaerobic digestion is a costly process but it is heavily subsidised, making it economically viable in Sweden today (Westlund et al., 2019). However, the future goal is to biologically treat 75% of biodegradable waste by 2023 (Anderzén, 2021) and therefore other more economically viable treatment methods for biodegradable waste allowing material re-use are needed (European Union, 2016). In 2015, the European Commission (EC) launched an action plan for a transition to a circular economy in the European Union (EU). This action plan identifies reuse of resources, including organic waste, as an important step in the transition from a linear to a circular economy model (European Commission, 2019). A promising method for reuse of biodegradable wastes is fly larvae composting, using the larvae of the black soldier fly (*Hermetia illucens* L. (Diptera: Stratiomyidae)) (Tomberlin et al., 2015), in which biodegradable wastes are converted into larval biomass (Čičková et al., 2015). The black soldier fly larvae (BSFL) biomass produced can be used in animal feed (Hartinger et al., 2021; Li et al., 2021) and the composting residues can be used as fertiliser (Beesigamukama et al., 2020).

Food waste is one of the substrates that can achieve high process efficiency in BSFL composting (Lalander et al., 2019), but regulation (EC) No 1774/2002 prevents use of food waste as substrate for larvae. Within the EU, only plant-based biodegradable streams are permitted for use as feed for larvae, and the larvae produced are permitted for use as feed for aquaculture and non-production animals within the EU (EU No 2017/893). The plant-based stream represents less than 15% of food waste from the whole Swedish food chain and primarily consists of side-streams in primary production (Westöö and Jensen, 2018). Biomass conversion efficiency (BCE) when

using single-source plant-based biodegradable waste in BSFL composting is lower than when using food waste (Lalander et al., 2019). One possible reason for the low BCE is the nutritional content of plant-based waste, characterised by low protein content in relation to high carbon content and low availability of lignin and hemicellulose (Gold et al., 2018; Kumar et al., 2018; Meneguz et al., 2018b; Nguyen et al., 2015; Nyakeri et al., 2017). Improved BCE has been achieved using vegetable canteen waste (Gold et al., 2020), which could be a result of using mixed vegetables instead of single-source plant-based waste. Nutritional features of the substrate considered to be important for larval development during composting are high protein content (Beniers and Graham, 2019; Lalander et al., 2019), non-fibre carbohydrate content similar to the protein content and low lipid and fibre content (Gold et al., 2020).

One way of increasing the process efficiency of plant-based biodegradable substrates could be to try adjust the unbalanced levels of protein and readily available carbohydrates through different pre-treatments. It has been shown that selected pre-treatments can increase the process efficiency in BSFL composting and improved the digestibility of the substrates low in protein and high in fibre to the larvae (Isibika et al., 2019). One way of increase the protein content in the substrate is by adding non-protein nitrogen (e.g. ammonia) increase the protein content through microbial assimilation (Tadele and Amha, 2015), whereas a fungi pre-treatment could be used to degrade fibre into to more easily available carbohydrates that that can be readily digested by the larvae (Godliving, 2009; Sánchez, 2009).

Another possible way to degrade the fibres into easily available carbohydrates, but which has yet to be tested with BSFL composting, is adding an enzyme cocktail and performing enzymatic hydrolysis. Previous studies have identified a need for high substrate water content in enzyme hydrolysis (Baksi et al., 2019; Izaguirre et al., 2019), which is not optimal for achieving dry separation of larvae and residues in BSFL composting (Lalander et al., 2020). Moreover, the optimal duration of enzymatic hydrolysis and of other pre-treatments in terms of process efficiency for BSFL composting is unknown.

Pre-treatment duration of food waste before anaerobic digestion has been shown to be influential, with shorter pre-treatment duration being more efficient in terms of energy and costs (Arelli et al., 2020; Fisgativa et al.,

2018). Therefore, it is important to understand the pre-treatment duration needed for BSFL composting from different perspectives.

Emissions of greenhouse gases (GHG) have been found to be considerably lower in BSFL composting than in conventional windrow composting (Ermolaev et al., 2019; Mertenat et al., 2019). However, there is a lack of data on GHG emissions from BSFL composting of plant-based biodegradable substrates and from the pre-treatment phase. Therefore it is important to investigate direct emissions from pre-treatment and BSFL composting, in order to enable comparisons of the environmental impact with alternative treatment options.

2. Objectives

The overall aim of the work described in this thesis was to evaluate the impact on lack soldier fly larvae composting process efficiency of a chemical, biological and bio-chemical pre-treatments treating plant-based waste sources, and to understand the greenhouse gas emissions associated to the chemical and biological pre-treatments.
Specific objectives were to:

(1) examine the impact on process efficiency and greenhouse gas emissions from BSFL composting when applying two pre-treatments: 1) chemical pre-treatment using ammonia solution; 2) biological pre-treatment using fungi, and to assess the associated direct emissions of greenhouse gases and ammonia.

(2) assess a chemical (addition of ammonia) and a bio-chemical (addition of enzymes) pre-treatment and examine the impact of pre-treatment time on process efficiency of BSFL composting of lettuce and cabbage. The hypothesis of chemical pre-treatment, addition of ammonia, was that a longer pre-treatment time would increase the protein content of the substrate through microbial assimilation.

3. Background

3.1 Black soldier fly

Black soldier fly (BSF; *Hermetia illucens* L. (Diptera: Stratiomyidae)) is a tropical fly that is widely distributed between latitudes 40°S and 45°N (Dortmans et al., 2017). The eggs are laid close to decomposing organic matter, in clusters comprising 400-800 eggs that hatch after approximately four days (Dortmans et al., 2017). Under optimal conditions in terms of temperature, food quality and quantity, the larval growth period is 14-16 days, but the insect can extend its life cycle under unfavourable conditions. Feeding occurs during the larval stage and stops in the sixth and final instar (Dortmans et al., 2017). As it does not feed in the fly stage, BSF is not a vector for disease transmission (Nguyen et al., 2015). After the fly emerges, it lives for 1-2 weeks. An abundance of light and a temperature of 25-32°C in a humid environment are required for the adult flies to mate (Dortmans et al., 2017).

3.2 BSFL composting

Fly larvae composting is a technology that meets the principles of a circular economy (European Union, 2016), as the larvae convert food waste into larval biomass (Čičková et al., 2015). The use of BSFL composting in biodegradable waste management has the potential to add value to non-utilised biodegradable waste, generating additional income for waste management companies (Lohri et al., 2017).

3.2.1 Products of BSFL composting

The products from BSFL composting are larvae and substrate residues. The protein content of the larvae is around 40% on a total solids (TS) basis (Lalander et al., 2019; Sealey et al., 2011; Surendra et al., 2016). The fatty acid profile and fat content of the larvae depend on the substrate, with the fat content varying between 16 and 58 % of dry matter (Ewald et al., 2020; Meneguz et al., 2018b). The residues are not a mature compost since the carbon:nitrogen (C/N) ratio is above 20 and the pH is below 7 (Muktadirul Bari Chowdhury et al., 2013). However, nitrogen and phosphorus concentrations are higher than those in organic compost (Chiam et al., 2021).

3.3 BSFL composting of different wastes

Important factors in BSFL composting are; larval density and feeding frequency (Paz et al., 2015). Depth of the substrate is also important, since if it is too deep anaerobic zones will develop making conditions unsustainable for the larvae, and the substrate will be left unprocessed (Čičková et al., 2015; Dortmans et al., 2017),.

One of the advantages with BSFL composting is the variety of substrates that can be treated, such as food waste (Gold et al., 2020; Lalander et al., 2019; Lalander et al., 2020; Liu et al., 2020), abattoir waste (Lalander et al., 2019), human faeces (Gold et al., 2020; Lalander et al., 2019), fruit and vegetables (Gold et al., 2020; Lalander et al., 2019; Paz et al., 2015) and sludge (Lalander et al., 2019; Liu et al., 2020). Biomass conversion efficiency has been found to be 14-27% with food waste, 11-23% with human faeces, 4-23% with fruit and vegetables, 15% with abattoir waste and 0.2-2.3% with sludge (Gold et al., 2020; Lalander et al., 2019; Lalander et al., 2020; Liu et al., 2020; Paz et al., 2015). The material reduction achieved in those studies was 13-63% with sludge, 47-58% with fruit and vegetables, 22-55% with food waste, 39-49% with human faeces and 46% with abattoir waste (Gold et al., 2020; Lalander et al., 2019; Liu et al., 2020; Paz et al., 2015). Mixing vegetables and legumes has been shown to increase BCE to a similar level to that obtained when using mixed food waste (Gold et al., 2020). However, Gold et al. (2020) also found that protein conversion efficiency was lower for vegetable canteen waste than for food waste, and suggested that the higher C/N ratio in vegetable canteen waste generated larvae with a higher fat content than larvae fed on food waste. Similarly,

Lalander et al. (2019) found that feeding larvae a mix of abattoir waste and fruit and vegetable waste gave higher protein conversion efficiency than when using the substrates separately. Lalander et al. (2019) attributed this to the mixture being more balanced in terms of available protein to carbohydrates ratio, resulting in more efficient utilisation of the substrate by the larvae.

3.4 Nutritional needs of BSFL

The nutritional parameters that are considered to be of greatest importance to the development of BSFL are protein, non-fibre carbohydrates (NFC), fibre, lipids and macronutrients (Barragán-Fonseca et al., 2018a; Barragán-Fonseca et al., 2018b; Gold et al., 2018; Lalander et al., 2019). Unfavourable ratios of nutrients in the substrate can result in prolonged larval development and efficiency limitations in biomass production and waste reduction (Danielsen et al., 2013). The most important nutritional characteristics of the substrate have been shown to be protein content and the content of easily available carbohydrates (Beniers and Graham, 2019; Lalander et al., 2019). Depending on the intended use of the larvae, keeping the NFC content in the substrate at a similar level to the protein content (protein-to-carbohydrate ratio 1:1) has been shown to be important, since a surplus of NFC results in larger larvae but no increase in protein content (Gold et al., 2020). Higher protein content is favourable when the larvae are intended for use as a protein source in animal feed, as it increases the protein content in the larvae (Lopes et al). In addition, maintaining a lower content of lipids and fibres in the substrate is of interest since a high fibre content, in combination with low protein and NFC content, results in low BCE, material reduction and larval weight (Gold et al., 2020). Fruit and vegetable wastes are typically low in protein and lipid and high in fibre, i.e. they do not coincide with the nutrient requirements of BSFL.

3.5 Pre-treatment

High BCE in BSFL composting is desirable due to the economic value of the larvae as animal feed. Increased material reduction is also desirable, since it decreases the volume of the residues. From a waste management perspective, it is desirable to achieve high material reduction simply in terms of reducing

the volume of waste to de disposed of. Combining different pre-treatments based on fungi and ammonia before using banana peels as substrate in BSFL composting has been found to increase the BCE, from 7 to 15 % on a VS basis (Isibika et al., 2019). Other pre-treatments tested prior to BSFL composting include addition of bacteria (Ermolaev et al., 2019; Isibika et al., 2019; Liu et al., 2021; Yu et al., 2011), heating, combined bacterial and thermal treatment (Isibika et al., 2019) and fermentation (Gao et al., 2019).

3.5.1 Ammonia pre-treatment

The concept behind using ammonia solution as a pre-treatment is to stimulate the microorganisms in the substrate to use the ammonia in order to degrade complex molecules (Tadele and Amha, 2015). For example, ruminants have a symbiotic relationship with rumen microorganisms whereby low quality protein is transformed into high quality protein (Wang and Tan, 2013). All forms of nitrogen sources are degraded in the rumen to peptides, amino acids or ammonia (NH_3). Ammonia nitrogen cannot be used directly by the ruminants, but the microorganisms in the rumen have the ability to assimilate it (Wang and Tan, 2013) and use it in production of amino acids and protein (Nadeem et al., 2014). The hypothesis in ammonia pre-treatment prior to BSFL composting in this thesis was that adding non-protein nitrogen to the substrate would help increase degradation and protein production in substrates with low available nitrogen. However, high concentrations of ammonia are toxic to plants and organisms, and therefore it is important that ammonia assimilation by the microorganisms is sufficiently fast (Lea, 1985). The assimilation is carried out by two enzymes, glutamine synthetase and glutamate synthase.

3.5.2 Fungi pre-treatment

Pre-treatment of plant-based waste with fungi has been used in BSFL composting (Isibika et al., 2019), but also as a pre-treatment for composting (Godliving, 2009; Nakasaki et al., 2015) and anaerobic digestion (Elissen et al., 2021; Kainthola et al., 2021) due to the ability of fungi to degrade complex molecules (Sigoillot et al., 2012). The hypothesis in this thesis was that the fungal enzymes would degrade more complex molecules into carbohydrates, which can be easily digested by the larvae. Since it is based on the action of fungal enzymes, fungi pre-treatment is a biological enzymatic pre-treatment method. The enzymes are used to degrade

lignocellulosic material creating a hydrolytic system that degrades polysaccharides, and a ligninolytic system that degrades lignin and opens phenyl rings. Only a few fungi species, such as white-rot fungi and soft-rot fungi, have developed the ability to degrade lignin and cellulose (Godliving, 2009; Sánchez, 2009). Water is essential in fungal growth for the transfer of nutrients (Monlau et al., 2013; Reid, 1989), but high moisture levels limits oxygen circulation and substrate loading per unit volume. However, high moisture content can enhance fungal enzyme production (Monlau et al., 2013). The concentrations of nitrogen and crude protein per unit mass of pre-treated dry matter have been demonstrated to increase during fungal pre-treatment (Jalc et al., 1997; Zeng et al., 2011). Even when there is a reduction in substrate dry matter, there is no loss of nitrogen during pre-treatment while adding fungal biomass to the substrate can increase the protein content (Jalc et al., 1997).

3.5.3 Enzyme pre-treatment

In enzyme pre-treatment, a solution containing a mixture of enzymes is used with the aim of releasing easily available carbohydrates. Enzymatic bio-chemical pre-treatment could be an option to avoid the unwanted material degradation that can occur in fungi pre-treatment (Isibika et al., 2019). In addition, using enzymes as a pre-treatment could be less time-consuming than fungi pre-treatment, which is estimated to take between 7 and 14 days (Isibika et al., 2019), as the enzymes would be added directly, rather than having to wait for the fungi to grow and produce the enzymes. The hypothesis with using enzymes in this thesis was the same as using fungi, i.e. that the enzymes would degrade more complex molecules into carbohydrates that can be easily digested by the larvae, but during a shorter time period without consuming the substrate along the way. To the best of our knowledge, enzyme pre-treatment has not yet been implemented prior to BSFL composting. However, it has been used prior to other biological waste treatments, such as anaerobic digestion based on saw dust (Baksi et al., 2019), municipal waste (Izaguirre et al., 2019) and tomato plant waste (Moreno et al., 2021). Baksi et al. (2019) investigated two different concentrations of enzymes added to saw dust (2.56% and 26.5% enzymes in the substrate) and found that these generated a solution with a glucose concentration of 15 g L^{-1} and 17-21 g L^{-1} respectively, after 10-20 h at 50°C, where the concentration of glucose provides an indication of how well the

enzymes degraded the substrate. Izaguirre et al. (2019) added enzymes at a rate of 13.5% of substrate to municipal waste and kept the mixture at 50°C for 48 h, achieving a glucose concentration of 19 g L^{-1}. Moreno et al. (2021) added 0.08% enzyme blend (Cellic CTec2) to a solution with 5% dry weight tomato plants at 50°C and achieved a peak in glucose concentration of 8 g L^{-1} after 24 h, after which the glucose concentration levelled out. However, all these studies were performed on a small scale.

According to Galbe et al. (2011), enzymatic hydrolysis is a slow process that can take 72 h, while others have found that it takes only 24 h (Pleissner et al., 2013; Wang et al., 2020). After enzymatic hydrolysis, up to 80% of carbohydrates in the substrate are more easily available (Wang et al., 2020). Complete hydrolysis of cellulose depends on the synergetic reactions of the enzymes in terms of whether they hydrolyse the reducing or non-reducing end of the cellulosic chain, with interactions of both enzymes being necessary for complete hydrolysis (Teeri, 1997; Zhang et al., 2018). Vegetables often have a high content of cellulose with a complex and heterogeneous structure (Teeri, 1997). Therefore, a mixture of enzymes can be expected to be more efficient than one specific enzyme in improving the degradation of vegetables, since adding hemicellulases degrades hemicellulose and results in more efficient degradation of cellulose (Galbe et al., 2011). Cellulolytic enzymes uses endo- and exo-types of actions, e.g. endoglucanase cleaves cellulosic bonds along the length of the cellulose chains yielding shorter chains that eventually are as short as glucose monomers (Teeri, 1997). Other types of enzymes taking part in hydrolysis are glucanase, lipase and protease, which degrade complex sugars, lipids and proteins, respectively (Fernandes, 2010).

3.6 Gas emissions from BSFL composting

Most studies on the GHG emissions from BSFL composting has been conducted on laboratory scale and focused on substrates such as food waste, agricultural waste and manure (Chen et al., 2019; Ermolaev et al., 2019; Pang et al., 2020; Parodi et al., 2020). Mertenat et al. (2019) used a life cycle assessment approach the global warming potential (GWP) by measuring GHG emissions in a BSF waste treatment facility and compared it with values from an open windrow composting facility. The results show that BSFL composting has less GWP than composting (Mertenat et al., 2019).

Overall methane (CH_4) and nitrous oxide (N_2O) emissions in those studies ranged from 0.03 to 8.6 g/kg initial ww, while ammonia (NH_3) emissions ranged from 0.05 to 0.5 g/kg initial ww (Chen et al., 2019; Ermolaev et al., 2019; Pang et al., 2020; Parodi et al., 2020). In one previous study, Ermolaev et al. (2019) investigated GHG and ammonia emissions during BSFL composting after bacterial pre-treatment of food waste and found that the bacterial pre-treatment had no impact on the emissions. However, the GHG were not measured during pre-treatment. In order to have a full understanding of the environmental impact of BSFL composting of plant-based waste, the GHG emissions also during pre-treatment has to be known.

4. Materials and Methods

4.1 Materials

The plant-based waste used in the work described in this thesis was provided by the fruit and vegetable wholesaler Grönsakshallen Sorunda (Stockholm, Sweden). The waste fractions studied were peel from organically grown oranges, broccoli and cauliflower trimmings, lettuce waste and cabbage waste. All wastes were stored for up to 9 days at 8-15°C before milling (Robot-Coupe, model Blixer 4 V.V, France). The orange peels were used in the treatments directly after milling, while the other fractions were stored for up to 10 days at -18°C before use.

Source-segregated household food waste was provided by the recycling plant Lilla Nyby (Eskilstuna & Strängnäs Energi och Miljö, Eskilstuna, Sweden), and collected and minced prior to transportation to the BSFL composting facility at the Swedish University of Agricultural Sciences (SLU, Uppsala, Sweden). The minced food waste was used in the experiment directly upon arrival.

The BSFL used in experiments were 5 days old, >0.2 cm long and had an average weight of 0.55 mg per larva when added to the treatments. The larvae were provided by the BSF colony at the Department of Energy and Technology, SLU, which has been running continuously since 2015 (Uppsala, Sweden).

4.2 Experimental set-up

In Paper I, which will be called Study 1, two pre-treatments were evaluated: 1) chemical ammonia pre-treatment was applied with the intention of increasing the substrate protein level through microbial assimilation of the ammonia into amino acids; and 2) biological pre-treatment using fungi (*Trichoderma reesei*) was applied with the intention of converting complex hydrocarbons into readily available carbohydrates. A control with no pre-treatment was also assessed. The associated direct emissions of GHG and ammonia during the two steps of the treatment were also evaluated, in order to assess the potential emissions from pre-treating plant-based waste. Orange peels and a mix of broccoli and cauliflower trimmings were used in three treatments: no pre-treatment (c), pre-treatment with the fungal species *Trichoderma reesei* (t) and pre-treatment with ammonia solution (a). A control using food waste with no pre-treatment was also assessed (Table 1).

In Paper II, which will be called Study 2 due to some additional data, the impact on process efficiency in BSFL composting of mixed lettuce and cabbage waste of bio-chemical pre-treatment duration using enzymes and ammonia was assessed. A longer pre-treatment time was expected to generate more readily available carbohydrates or increased protein content, respectively. Each treatment contained equal amounts of lettuce and cabbage, which were pre-treated with either enzyme or ammonia solution for 0 to 8 days. A control with no pre-treatment was also assessed (Table 1).

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

Treat-ment code	Substrate	Pre-treatment time [days]	BSFL composting time [days]	BSFL VS load [g VS/larva]	Total initial material [kg ww/ treatment]	Fibre content[i] [g/kg ww]	Protein content[i] [g/kg ww]	Carbohydrate content[i] [g/kg ww]
No pre-treatment								
FW-c	Food waste	-	17	0.217±0.004	15.4 ±0.2			
OP-c	Orange peel	-	27	0.203±0.003	14.6 ±0.1	37	15	218
BC-c	Broccoli & cauliflower	-	28	0.076±0.000	15.7 ±0.1	32	31	25
LC-c	Lettuce & cabbage	-	14	0.219±0.000	0.9 ±0.1	13	9.7	36
Ammonia pre-treatment								
OP-a	Orange peel	16	35	0.177±0.003	14.4 ±0.1			
BC-a	Broccoli & cauliflower	16	30	0.067±0.004	15.9 ±0.6			
LC-a0	Lettuce & cabbage	0	14	0.218±0.000	0.9 ±0.1			
LC-a2	Lettuce & cabbage	2	15	0.206±0.001	0.9 ±0.1			
LC-a4	Lettuce & cabbage	4	14	0.209±0.003	0.9 ±0.1			
LC-a8	Lettuce & cabbage	8	15	0.217±0.005	1.0 ±0.1			
Trichoderma reesei *pre-tretment*								
OP-t	Orange peel	16	31	0.147±0.015	15.4 ±0.1			
BC-t	Broccoli & cauliflower	14	23	0.025±0.004	15.8 ±0.5			
Enzyme pre-treatment								
LC-e0	Lettuce & cabbage	0	14	0.218±0.000	0.9 ±0.1			
LC-e2	Lettuce & cabbage	2	14	0.243±0.000	1.2 ±0.1			
LC-e4	Lettuce & cabbage	4	13	0.219±0.000	1.2 ±0.1			

[i] Theoretically calculated based on values from (Livsmedelsverket).

4.2.1 Pre-treatments

Fungi pre-treatment: Trichoderma reesei used was pre-cultured for 7 days at 28°C and then harvested by scraping the fungi off the agar plate with a 10-µL inoculation loop and mixing it into sterile 0.9% sodium chloride (NaCl) solution. The fungal mixture had a density of 10^7 g/mL and was commingled into the substrates to 1% (w/w) concentration.

Ammonia pre-treatment: 24.5% ammonia solution (Nitor, Sweden) was added to the substrate to reach a total NH_3-N concentration of 0.7% (w/w) in Study 1 and 0.2% (w/w) in Study 2. After the pre-treatment (Table 1), the pH was adjusted to pH 7.5±0.5 using stepwise addition of concentrated sulphuric acid (H_2SO_4) (Fisher Chemicals, Uppsala, Sweden). The pre-treated substrate was sealed in plastic bags to prevent ammonia from evaporating during the different durations of pre-treatment, which was performed at 30°C in Study 1 and 28°C in Study 2. The different pre-treatment durations chosen in Study 2 were 0, 2, 4 and 8 days.

Enzyme pre-treatment: An enzyme cocktail (SAE0020 Sigma-Aldrich), consisting of cellulases, ß-glucosidases and hemicellulose, was added to the substrate to reach a concentration of 1% (w/w). During the pre-treatment, the substrate was placed in a bucket and a drill rotating on the lowest setting, fixed on a stand, stirred the material in the bucket for the required duration (0, 2 and 4 days, Figure 1a). In Study 1, the ingoing material into the pre-treatment was fixed to 15 kg and what was left after completion of the pre-treatment was divided into three feeding portions for BSFL composting. In Study 2, a surplus of substrate was pre-treated so that at the start of the BSFL composting, the amount of VS per larvae was 0.2 g. The different pre-treatment durations chosen in Study 2 were 0, 2 and 4 days.

All pre-treatments were performed separately for each feeding occasion and for each triplicate, with the exception of the enzyme pre-treatment, which was performed separately for each feeding occasion only.

Figure 1. a) Picture of the enzyme pre-treatment set-up. b) Picture of the experimental set-up for fungi and ammonia pre-treatment and BSFL composting placed, inside a tent.

4.2.2 BSFL composting

The BSFL composting step in Study 1 was performed on triplicate samples in a greenhouse with the temperature regulated to 30±3°C. In Study 2, all treatments were performed in a growth tent with dimensions 120 cm x 120 cm x 200 cm (Secret Jardin, Hydro Shoot 120). A car heater connected to a temperature regulator (Trixie) and a fan for circulating the air, both placed on the floor in the tent, maintaining an average temperature of 28.8±0.8°C over the course of the treatment. The treatment boxes (Study 1: 36.5 cm x 56.2 cm x 11.5 cm, Study 2: 21 cm x 17 cm x 11 cm) were kept in a rack,

with 3 or 8 cm between the boxes (Figure 1b). The larval feeding rate was calculated based on the total amount of initial material in Study 1 and adjusted during the treatment to a VS amount of 0.2 g per larvae in Study 2 (Table 1). In Study 1, 15.000 larvae were added, giving a density of 6.25 larvae/cm^2. These were fed with approximately 15 kg of substrate in total and the experiment was terminated when the first prepupae were observed, but lasted for a minimum of 17 days (Table 1). The larval density in Study 2 was adjusted to avoid exceeding 5 cm depth of substrate in the boxes, resulting in addition of 300 larvae, giving a density of 1.5 larvae/cm^2 based on VS per larvae needed. These were fed with approximately 1 kg of substrate in total (Table 1). The BSFL composting was kept going until 7 days after the last feeding (Table 1). In both studies, larvae were fed on days 0, 3 and 6 of the treatment by adding new substrate on top of the material without stirring.

4.3 Sampling

The total amount of substrate was weighed before the pre-treatment step and before BSFL composting. Larvae and residues were separated at the end of treatment by sieving into two fractions that were weighed separately (dry separation). In Study 2, water was added to flush down the residues through the sieves, a process called wet separation. The average larval weight was estimated by weighing three sub-samples of 100 larvae. The total number of larvae remaining at the end of the treatment was calculated by dividing the total weight of all larvae by the average weight per larva. The material in each replicate was sampled in triplicate for analyses of total solids and total volatile solids and, in Study 1, also for total nitrogen and pH (Level one pH meter, Inolab, with a Sentix electrode, PHM210, MeterLab®, Radiometer, Copenhagen). Samples for determination of GHG emissions in Study 1 were taken during the pre-treatment and BSFL composting steps.

4.3.1 Physical-chemical sampling

Samples for TS, VS, pH and nitrogen analysis were taken before the start of pre-treatment, before the start of BSFL composting and after BSFL composting. Three TS and VS samples were taken from each replicate and each sample contained substrate from five random locations in the treatment box (10-15 g per sample). The same procedure was followed for pH

measurements and sampling for nitrogen analysis, but with only one sample from each replicate (10 g per sample). The substrate removed for sampling was taken into account when calculating the feeding rate, BCE and material reduction.

4.3.2 Gas emissions sampling

Gas measurements were performed as soon as the substrate was placed in the treatment box, then after 1 week and 2 weeks of pre-treatment. The final gas emissions measurement in the pre-treatment step coincided with the first measurement in the BSFL composting step, during which gas measurements were performed four times in total (before each of the three feeding occasions and at the end of BSFL composting).

The gas emissions were measured using a chamber technique. Gas samples were collected over a period of approximately one hour to obtain the gas emission rates (Ermolaev et al., 2019). The boxes were placed inside a plastic chamber for these measurements, as described in more detail in Paper I. For each emissions measurement, gas samples were extracted on three occasions: directly upon sealing the box, after 20 min and after 1 h, with the exact times and volumes removed recorded. Linear regression was used to calculate the emission rates. Dilution was accounted for following the method described in Ermolaev et al. (2019).

4.4 Physical and chemical analysis

For TS analyses, samples were dried for a minimum of 48 h at 60-70°C, to avoid evaporation of volatile solids (Vahlberg et al., 2013). For VS analysis, samples were combusted at 250°C for 2 h and at 550°C for 4 h (modified ISO 18122:2015). Total nitrogen was measured as described in Lalander et al. (2015). In brief, 0.5 g samples were boiled in 15 mL concentrated sulphuric acid for 20 min and then diluted 1:50. All forms of nitrogen was oxidised to nitrate (NO_3) using a Crack-test 10 (114544) and nitrate concentrations were measured using Spectroquant© kit number 114764, following the instructions given by the provider.

4.5 Gas analysis

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

4.6 Calculations

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

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

where mVS$_{larvae}$ and mVS$_{initial}$ is the total mass of volatile solids of larvae and initial substrate given to larvae, respectively.

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

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

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

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

4.7 Statistical analysis

The *Linest* function in Excel (Microsoft version 16, USA) was used for calculating gas emission rates. Two-sided ANOVA (with 95% confidence interval) was used to evaluate whether the treatments were significantly different during pre-treatment and BSFL composting in terms of biomass conversion efficiency (BCE), material reduction and gas emissions. Tukey's Honest Significant Difference (Tukey's HSD) was used for confirmation when a significant difference was established. Normality was verified in the model residuals using Shapiro-Wilk test. Where normality was not verified, the non-parametric Kruskal-Wallis test was used and followed by Wilcoxon rank sum test. Paired t-test with a variance of 95% confidence interval was used to compare TS and VS values in different stages of each treatment. General linear regression with 95% confidence interval was used to assess correlations between response variables and substrate properties. ANOVA, regression analyses, non-parametric tests and graphical representations were all made in R (R Core Team, 2019).

5. Results

Larval total solids (TS) content in enzyme pre-treated material decreased with increasing pre-treatment time except following pre-treatment with ammonia, where a longer pre-treatment resulted in higher TS in the larvae (Table 2). Several of the TS and VS values were not normally distributed and resulted in only significantly different values between the lowest and highest value.

Table 2. Total solids (TS) and total volatile solids (VS) of initial substrate, substrate after pre-treatment, larvae and residues from black soldier fly larvae (BSFL) composting and larval VS load during the treatment. Values presented are mean ± SD (n=3). Significant differences ($p<0.05$) in TS and VS from initial substrate and after pre-treatment are denoted with *. Different letters within columns indicate significant difference ($p<0.05$) in initial substrate, after pre-treatment, and in BSFL and residue for TS and VS values including both studies. Pre-treatment codes: c = control (no pre-treatment); t = *Trichoderma reesei* pre-treatment; a, a0, a2, a4, a8 = ammonia pre-treatment for 0, 2, 4, 8 days; e0, 2, e4 = enzyme pre-treatment.

	Initial substrate		After pre-treatment		BSFL		Residue	
Code	TS [%]	VS[i] [%]	TS[i] [%]	VS[i] [%]	TS [%]	VS[i] [%]	TS[i] [%]	VS[i] [%]
No pre-treatment								
FW-c	23.5^{a} ± 0.2	88.6^{ab} ±0.3			40.6*^{a} ±0.9	83.6*^{a} ±0.1	69.2*^{a} ±3.0	82.4*^{abc} ±0.2
OP-c	21.5^{bc} ± 0.4	96.8^{ab} ±0.4			25.4*^{be} ±1.2	90.0*^{a} ±0.2	31.8*^{ab} ±1.4	95.0*^{a} ±0.1
BC-c	8.2^{d} ± 0.1	87.4^{b} ±1.1			19.5*^{c} ±1.4	83.0*^{a} ±1.6	29.7*^{ab} ±5.6	70.5*^{abc} ±0.6
LC-c	8.6^{de} ± 0.4	89.0^{ab} ±0.5			26.1*^{be} ±0.8	84.7*^{a} ±0.4	3.9*^{ab} ±0.8	66.6*^{abc} ±5.9
Ammonia pre-treatment								
OP-a	21.6c ± 0.1	96.9^{a} ±0.1	19.2*^{ab} ±0.2	96.5*^{a} ±0.1	24.3*^{be} ±2.0	88.0*^{a} ±0.8	52.8*^{ab} ±4.2	94.2*^{ac} ±0.2
BC-a	8.6^{de} ± 0.1	89.5^{ab} ±0.3	7.3*^{b} ±0.2	88.0*^{abc}±1.3	23.9*^{bc} ±2.7	86.8^{a} ±0.9	15.0*^{ab} ±5.1	76.9*^{abc} ±1.4
LC-a0	8.6^{de} ± 0.4	89.0^{ab} ±0.5			24.4*^{be} ±0.4	84.6*^{a} ±0.1	3.4*^{ab} ±0.2	66.9*^{abc} ±0.6
LC-a2	8.8^{e} ± 0.1	88.6^{ab} ±0.6	8.1*^{ab} ±0.7	87.7*^{abc}±0.8	22.5*^{bc} ±2.2	84.8*^{a} ±0.5	3.5*^{ab} ±0.1	68.4*^{abc} ±1.2
LC-a4	8.8^{e} ± 0.1	88.6^{ab} ±0.6	7.8*^{ab} ±0.4	87.1*^{abc}±0.7	24.3*^{be} ±0.6	84.7*^{a} ±0.2	3.8*^{ab} ±0.3	69.7*^{abc} ±0.7
LC-a8	8.8^{e} ± 0.1	88.6^{ab} ±0.6	7.0*^{ab} ±0.9	85.1*^{abc}±2.6	26.6*^{be} ±0.9	85.3*^{a} ±0.4	3.2*^{ab} ±0.1	66.4*^{abc} ±1.2
Trichoderma reesi *pre-treatment*								
OP-t	21.1^{b} ± 0.4	96.6^{ab} ±0.1	29.8*^{a} ±2.9	94.1*^{ac} ±0.9	23.6*^{bc} ±1.0	87.7*^{a} ±1.7	37.5*^{ab} ±3.8	92.5^{abc} ±0.2
BC-t	8.2^{d} ± 0.2	87.4^{ab} ±1.5	11.3*^{ab} ±1.5	70.8*^{b} ±2.4	35.3*^{d} ±2.6	77.3*^{a} ±2.1	86.9*^{a} ±2.0	57.5*^{bc} ±1.2
Enzyme pre-treatment								
LC-e0	8.6^{de} ± 0.4	89.0^{ab} ±0.5			28.9*^{e} ±1.6	86.8*^{a} ±0.3	2.2*^{b} ±0.3	54.0*^{b} ±1.0
LC-e2	7.5^{f} ± 0.2	88.7^{ab} ±1.0	8.0*^{ab} ±0.1	88.7^{abc} ±0.1	27.1*^{be} ±0.7	88.9^{a} ±0.2	3.6*^{ab} ±0.2	73.2*^{abc} ±2.9
LC-e4	7.5^{f} ± 0.2	88.7^{ab} ±1.0	7.4^{b} ±0.1	85.9*^{bc} ±0.1	24.4*^{be} ±2.3	88.5*^{a} ±0.5	3.5*^{ab} ±0.5	71.9*^{abc} ±3.3

[i] Non-normal distribution, non-parametric Kruskal-Wallis test was used.

Biomass conversion efficiency in the different treatments with lettuce and cabbage was not significantly different on a VS basis, with the exception of direct enzyme pre-treatment of the larvae (treatment LC-e0), which was not significantly different from the treatment with food waste (Table 3). The BCE_{VS} values obtained for the treatments with orange peel and broccoli and cauliflower trimmings were significantly lower than for the treatments with lettuce cabbage. The material reduction on a VS basis for the entire process was significantly higher for treatment LC-e0 and for *Trichoderma reesei* treatment with broccoli and cauliflower (BC-t). The lowest material reduction was achieved in the treatments with orange peel (OP-t, OP-a).

Table 3. Material reduction (Red) and biomass conversion efficiency (BCE) on a volatile solids (VS) and wet weight (WW) basis during pre-treatment, black soldier fly larvae (BSFL) composting and the entire process. Values presented are mean ± SD (n=3) except in the enzyme pre-treatment (n=1). Different letters within columns indicate significant difference ($p<0.05$) in pre-treatment, BSFL composting and the entire process for material reduction and biomass conversion efficiency on a VS or WW basis including both studies. Pre-treatment codes: c = control (no pre-treatment); t = *Trichoderma reesei* pre-treatment; a, a0, a2, a4, a8 = ammonia pre-treatment for 0, 2 4, 8 days; e0, e2, e4 = enzyme pre-treatment for 0, 2, 4 days.

	Pre-treatment	BSFL composting		Entire process				Larval size	Survival rate
Code	Red[i] [%]	Red_{VS}[ii] [%]	BCE_{VS} [%]	Red_{VS} [%]	BCE_{VS} [%]	Red_{WW}[ii] [%]	BCE_{WW} [%]	[g/100 larvae]	[%]
No pre-treatment									
FW-c		63.5^{65}_{63}	22.7^{cd} ±0.9	63.9^{de} ±0.9	22.7^{c} ±0.9	86.7^{87}_{86}	14.2^{d} ±0.8	14.1 ±0.5	104± 6
OP-c		37.0^{42}_{37}	5.7^{a} ±1.7	38.7^{a} ±3.1	5.7^{a} ±1.7	57.7^{59}_{57}	5.2^{afg} ±1.2	5.0 ±1.2	100
BC-c		81.2^{82}_{81}	14.4^{b} ±1.5	81.2^{bc} ±0.5	14.4^{b} ±1.5	93.2^{95}_{92}	6.4^{abc} ±0.1	10.8 ±0.8	63± 4
LC-c		78.5^{79}_{78}	19.7^{e} ±0.2	78.4^{b} ±0.3	19.7^{e} ±0.2	39.9^{42}_{22}	6.8^{abc} ±0.2	21.3 ±0.9	69± 4
Ammonia pre-treatment									
OP-a	10.9^{cd}±0.02	51.6^{55}_{49}	4.9^{ag}±0.6	57.5^{e} ±2.7	4.4^{a} ±0.6	83.7^{84}_{83}	3.9^{f} ±1.2	4.3 ±0.1	96± 5
BC-a	15.3^{ac} ±0.2	59.5^{62}_{55}	15.0^{b} ±0.5	66.2^{df} ±2.8	12.3^{b} ±0.9	75.8^{84}_{69}	4.5^{fg} ±0.5	9.5 ±2.6	56±25
LC-a0		78.0^{79}_{77}	18.5^{e} ±0.2	78.1^{bc} ±1.0	18.5^{e} ±0.2	27.6^{30}_{25}	6.7^{abc} ±0.2	17.3 ±0.3	85± 1
LC-a2	7.8^{d} ±1.7	78.4^{80}_{77}	19.9^{e} ±0.2	79.3^{bc} ±1.4	19.1^{e} ±0.2	37.3^{38}_{33}	7.6^{b} ±0.8	19.9 ±1.1	86±12
LC-a4	11.5^{cd}±1.0	76.6^{78}_{76}	21.1^{cef}±0.8	77.8^{bc} ±0.7	19.5^{e} ±0.7	38.0^{43}_{30}	7.1^{bc} ±0.1	22.5 ±1.1	75± 2
LC-a8	15.0^{ac} ±2.2	79.5^{80}_{77}	19.0^{e} ±0.4	80.1^{bc} ±1.6	17.3^{e} ±0.4	29.3^{31}_{27}	5.7^{acg} ±0.1	19.3 ±1.3	76± 6
Trichoderma reesi *pre-treatment*									
OP-t	24.8^{a} ±8.0	45.3^{48}_{35}	2.6^{g} ±0.7	60.1^{de} ±4.2	1.8^{d} ±0.4	82.0^{83}_{82}	1.3^{e} ±0.3	1.8 ±0.4	100
BC-t	65.4^{b} ±4.9	51.7^{61}_{44}	16.3^{b} ±1.0	84.4^{bg} ±1.3	5.4^{a} ±0.8	97.8^{98}_{97}	1.4^{e} ±0.3	6.1 ±2.3	28±15
Enzyme pre-treatment									
LC-e0		89.7^{90}_{89}	24.1^{de} ±0.2	89.4^{g} ±0.6	24.1^{c} ±0.2	29.2^{45}_{25}	7.2^{bc} ±0.5	23.4 ±1.0	68± 7
LC-e2	9.4^{cd}	68.3^{76}_{68}	20.2^{ce} ±0.8	73.6^{cf} ±4.1	18.2^{e} ±0.7	33.3^{39}_{33}	4.9^{fg} ±0.3	27.3 ±0.4	56± 4
LC-e4	14.8^{acd}	70.6^{75}_{61}	23.4^{df} ±1.9	74.6^{c} ±5.8	19.1^{e} ±1.5	32.6^{37}_{32}	5.9^{abcg} ±1.0	23.9 ±1.9	76±17

i) Normality achieved with Box-Cox transformation (two variables);

ii) Non normal distribution, non-parametric Kruskal-Wallis test was used, $median^{highest}_{lowest}$ value.

The distribution of added VS in the treatments was divided into four fractions: respiration in pre-treatment, respiration in BSFL composting, larvae and residues (Figure 2). The treatment with the largest proportion of total respiration and pre-treatment respiration was that with broccoli and cauliflower pre-treated with *Trichoderma reesei*. The orange peel control had the smallest respiration proportion of initial VS and the largest proportion of residues. The variation in the proportions was smaller for the treatments in Study 2 than for the treatments in Study 1. The two controls in Study 1 and Study 2 had a similar proportion of larvae production, but the food waste control treatment produced more residues than the control for lettuce and cabbage (Table 3).

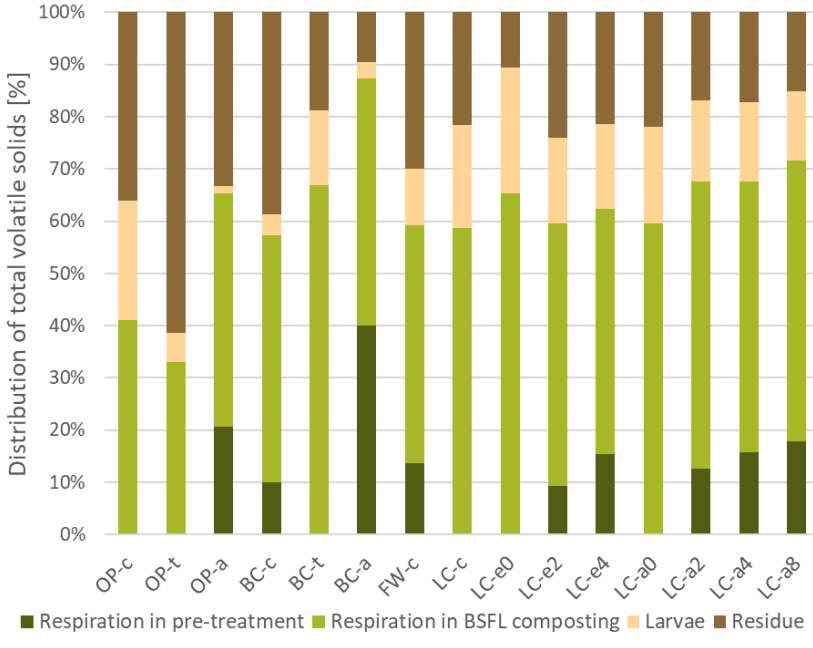

Figure 2. Mass balance (volatile solids (VS) basis) during treatment with black soldier fly larvae (BSFL) composting, of substrates comprising: food waste (FW), orange peels (OP), mixed broccoli and cauliflower trimmings (BC) and mixed cabbage and lettuce (LC) following different pre-treatments: c = control (no pre-treatment); t = *Trichoderma reesei* pre-treatment; a, a0, a2, a4, a8 = ammonia solution pre-treatment for 0, 2 4, 8 days; e0, e2, e4 = enzyme pre-treatment for 0, 2, 4 days. Distribution of initial VS during treatment is divided into four fractions: respiration in pre-treatment, respiration in BSFL composting, larvae and residues.

Total CH_4 emissions in Study 1 varied more for treatments that had higher emissions, due to inconsistent emission pattern (Figure 3a). Total emissions of N_2O were higher in treatments with broccoli and cauliflower trimmings than with orange peels (Figure 3b). Pre-treatment of broccoli and cauliflower trimmings reduced the total amount of N_2O emitted. Total N_2O emissions were not significantly different from zero in any of the orange peel treatments, while ammonia pre-treatment increased the NH_3 emissions (Figure 3c). Total CO_2 emissions from the entire process in Study 1 were similar across all treatments (Figure 3d). Total emissions of CH_4 and N_2O expressed in CO_2-equivalents over a 100-year period (IPCC, 2013) on a VS basis were very low compared with the direct CO_2 emissions (Figure 3e). Total emissions from the treatments were similar, with only the controls of trimmings and peels being significantly different from each other (Figure 3f).

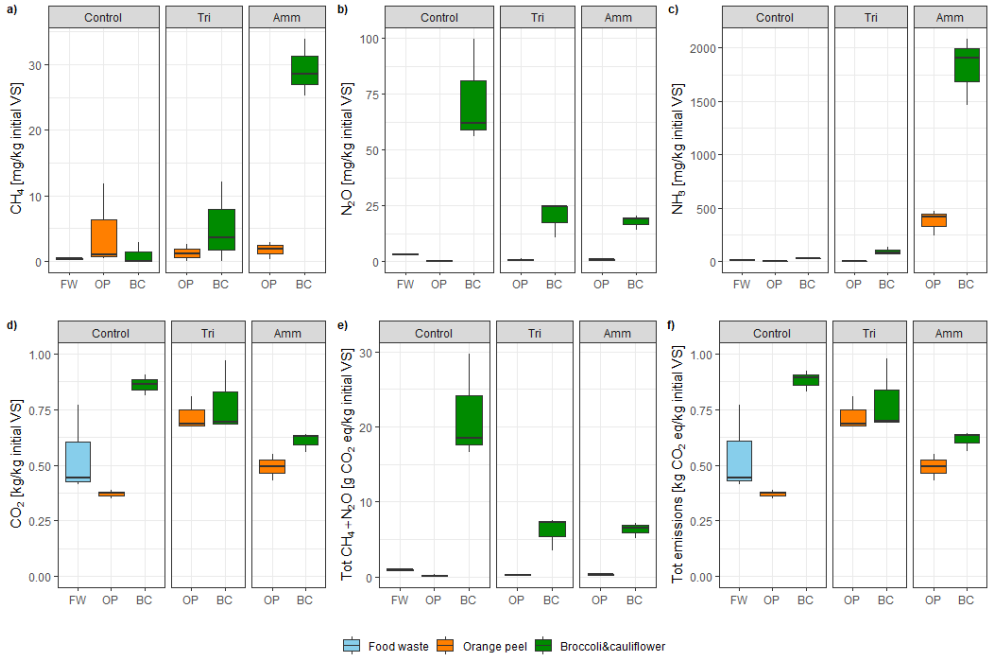

Figure 3. Total emissions, comprising emissions during black soldier fly larvae (BSFL) composting and during pre-treatment, calculated per kg of initial volatile solids (VS) for the control, ammonia (amm) and *Trichoderma reesei* (Tri) pre-treated substrates based on food waste (FW), orange peels (OP) and broccoli and cauliflower trimmings (BC): a) CH_4 [mg/kg VS]; b) N_2O [mg/kg VS]; c) NH_3 [mg/kg VS]; d) total CO_2 [kg/kg VS]; e) CO_2 equivalents [g CO_2-eq/kg VS] of CH_4 and N_2O emissions; and f) CO_2 equivalents [kg CO_2-eq/kg VS] of all emissions.

The production of larvae was greatest in the treatment with food waste, followed by ammonia-treated (2 days) lettuce and cabbage, and smallest in the orange peels pre-treated with *Trichoderma reesei* (Table 4). The percentage change compared with food waste and other non-pre-treated substrates was only negative compared with the control of the same substrate meaning that the control produced a higher value. For lettuce and cabbage, half of the treatments increased production of larvae and residues, compared with the control. The highest production of residues was achieved in lettuce and cabbage pre-treated with ammonia for 0 days and lowest production was achieved in broccoli and cauliflower pre-treated with *Trichoderma reesei.*

Table 4. Production of pre-treated substrate, larvae and residues from an initial quantity of material of 1 ton wet weight substrate in black soldier fly larvae (BSFL) composting. Values presented are mean ± SD (n=3) except in the enzyme pre-treatment (n=1). Different letters within columns indicate significant difference (p<0.05) in pre-treatment, larvae and residues, including both studies. Pre-treatment codes: c = control (no pre-treatment); t = *Trichoderma reesei* pre-treatment; a, a0, a2, a4, a8 = ammonia solution pre-treatment for 0, 2 4, 8 days; e0, e2, e4 = enzyme pre-treatment for 0, 2, 4 days.

	1 ton substrate, wet weight (WW) basis				
	After pre-treatment[iii] [kg]	Larvae[iii] [kg]	Change to control [%]	Residue[ii] [kg]	Change to control [%]
Food waste (FW)					
FW-c		142^{e} ± 7.8	0	134^{ab}± 8.2	0
Orange peel (OP)					
OP-c		51.8^{ah} ± 12	-64^{i}	422^{ab}± 11	214^{i}
OP-t	651^{a}± 4	13.7^{f} ± 2.7	-73^{ii}	185^{ab}± 5.0	-56^{ii}
OP-a	612^{c}± 0.2	24.4^{g} ± 1.2	-53^{ii}	103^{ab}± 3.5	-76^{ii}
Broccoli & cauliflower trimmings (BC)					
BC-c		64.5^{abcd}± 0.6	-55^{i}	66^{ab}± 12	-51^{i}
BC-t	329^{b}±10	14.4^{f} ± 2.7	-78^{ii}	23^{a} ± 1.2	-66^{ii}
BC-a	534^{d}± 0.6	25.5^{g} ± 3.1	-60^{ii}	134^{ab}± 43	103^{ii}
Lettuce & cabbage (LC)					
LC-c		67.8^{abd} ± 2.4	0	654^{ab}±107	0
LC-e0		72.3^{bd} ± 4.6	6.6	669^{ab}±103	2.3
LC-e2	822^{h}	48.7^{ch} ± 2.6	-28	651^{ab}± 28	-0.5
LC-e4	856^{i}	59.2^{abdh}± 8.2	-13	662^{ab}± 23	1.3
LC-a0		67.5^{abd} ± 1.8	0.5	724^{b} ± 25	11
LC-a2	972^{e}± 2	75.5^{b} ± 8.0	11	640^{ab}± 26	-2.1
LC-a4	968^{f}± 1	71.0^{bd} ± 1.3	4.7	630^{ab}± 65	-3.7
LC-a8	963^{g}± 1	57.4^{dh} ± 0.6	-15	711^{ab}± 18	8.7

i) Percentage change in comparison with food waste;

ii) Percentage change in comparison with the control of the same substrate;

iii) Normality achieved when using Box-Cox transformation, two variables;

iv) Non normal distribution, non-parametric Kruskal-Wallis test was used.

6. Discussion

6.1 Impact of increasing nitrogen content – Ammonia pre-treatment

6.1.1 Chemical pre-treatment with ammonia solution

The intention with using ammonia solution as a pre-treatment, and thus increasing the nitrogen content in the substrate was to stimulate the microorganisms in the substrate to use the ammonia and degrade complex molecules. Ammonia pre-treatment did not result in BCE, but it did increase the material reduction in the orange peel treatment (Table 3). It also increased total CO_2 emissions in comparison with the control (Figure 3), which means that the added nitrogen stimulated the microorganisms and they used the nutrients for their own respiration. In the other pre-treatments, neither BCE nor material reduction was increased by the added nitrogen compared with the control. This could be a result of not adding enough nitrogen to these substrates since a three-fold higher addition of nitrogen resulted in increased BCE in a study by Isibika et al. (2019) using banana peel as substrate. Another reason could be the high C/N ratio in banana peels, which is quite similar of that in orange peels (63, theoretically calculated (Livsmedelsverket)). Adding nitrogen might have stimulated some protein production in the banana peel substrate, resulting in a protein to carbohydrate ratio closer to 1, which has been concluded to be an optimal ratio (Gold et al., 2020).

In contrast to the ammonia pre-treatment for BSFL composting. which resulted in no significant difference in Study 1 and 2, ammonia pre-treatment for 24 h prior to anaerobic digestion has been shown to result in significantly

higher yield of methane after 39 days of treatment in the digester (Wang et al., 2018). However, addition of ammonia alone in that study did not result in a significant improvement in methane yield compared with the control (Wang et al., 2018). The factor found make a significant difference to the methane yield with the different concentrations of added ammonia was an adjusted and controlled pH of 9 and above during the treatment (Wang et al., 2018). Those authors concluded that ammonia pre-treatment released soluble organic matter and that levels of free ammonia above 0.1 g/L improved the biodegradability. This is a much lower ammonia concentration than used in Study 1 (7 g/L) and Study 2 (2 g/L). Since pH up to 10 at the start of BSFL composting has not been shown not to give a significant difference in larval weight or development time (Ma et al., 2018; Meneguz et al., 2018a), maintaining high pH during both pre-treatment and BSFL composting could be an option to get higher biomass conversion efficiency and a greater material reduction. To achieve a high pH, less time could be spent on ammonia pre-treatment of BSFL composting substrate, to achieve similar results as without adjusting the pH.

6.2 Impact of increasing readily available carbohydrates – Fungi and enzyme pre-treatments

6.2.1 Biological pre-treatment with fungi

The intention with using fungi for pre-treatment was that the fungal enzymes will degrade fibres into carbohydrates and make them easily digestible to the larvae. It was found that BCE did not increase with the fungi pre-treatment compared with the control. However, the material reduction increased for the treatments with orange peel and broccoli and cauliflower trimmings, accompanied by increased total CO_2 emissions (Figure 3) suggesting that the material reduction and respiration were performed by the fungi, and perhaps the present bacterial community, during pre-treatment and BSFL composting. The decreased BCE could be a result of the fungi consuming easily digested nutrients before the larvae were added and not contributing to release of any more nutrients during the BSFL composting step. Isibika et al. (2019) achieved a significant increase (of 61-100%) in BCE with banana peel substrate using fungi pre-treatment (Table 5), which was probably associated to a reduction in fibre content in both fungi pre-treatments. The

fibre content in the banana peel substrate was higher than that theoretically calculated for orange peels and broccoli and cauliflower trimmings, creating a larger resource for the fungal cells, which might have resulted in them not consuming all the readily available carbohydrates and leaving some that the larvae could consume.

The fungi pre-treatment in Study 1 resulted in a product that could not be separated in the treatment with orange peel due to a hard crust and moist material underneath. The substrate might have needed mixing during the pre-treatment and BSFL composting steps to prevent a hard crust from developing and a longer pre-treatment to let the substrate dry out slightly. The pre-treatment time used for fungi pre-treatment in previous studies varied greatly, from 4 h to 21 days (Pennacchio et al., 2021; Szűcs et al., 2021; Wang et al., 2021). Pennacchio et al. (2021) used a fungi pre-treatment time of 4 h, followed by 72 h enzymatic hydrolysis. They found that a longer pre-treatment with fungi made no significant difference to the results, but that the fungus (*Trichoderma harzianum*), increased the availability of cellulose for hydrolysis (Pennacchio et al., 2021). It has been shown that the biosurfactant properties of *Trichoderma harzianum* (Pitocchi et al., 2020) can explain its ability to loosen cellulose and render it more available to enzymatic hydrolysis. Combined fungal and enzyme pre-treatment could decrease the pre-treatment time needed, since enzyme activity with fungi pre-treatment decreases with time (Szűcs et al., 2021), and renders the vegetable substrate more available to the larvae. However, Szűcs et al. (2021) suggest that 10 days of fungi pre-treatment is necessary to achieve higher biogas production yield for cellulose-rich substrates. Instead of longer fungi pre-treatment with orange peel, a combination of fundi with added enzymes might help degrade the carbohydrates and fibre.

6.2.2 Bio-chemical pre-treatment with enzymes

The intention with using enzyme solution as a pre-treatment was to achieve readily available carbohydrates by using a mix of enzymes. It was found that BCE was improved by 22% on a VS basis following enzyme pre-treatment in comparison with the control (Table 3). For example, the BCE in Study 2 was 21% in the control and 25% with direct enzyme (0 days) pre-treatment, indicating that addition of enzymes improved the digestibility of the substrate to the larvae. However, the fibre content was not very high in the mixed lettuce and cabbage waste (20%), considerably lower than that of the

banana peel (70%) studied in Isibika et al. (2019). Isibika et al. (2019) found that fungi pre-treatments (using *Rhizopous oligosporus* and *Trichoderma reesei*) for 14 days led to significant improvements in BCE compared with non-pre-treated banana peel (Table 5). The improvement in BCE was probably linked to a reduction in fibre content in the pre-treatment in both cases but particularly in the *Trichoderma reesei* pre-treatment, in which a 42% reduction occurred. This suggests that enzyme pre-treatment could result in a larger difference in BCE compared with the control when using substrate with a higher fibre content. It also suggests that enzyme pre-treatment is preferable to fungi pre-treatment when using substrates with low amount of fibre since the enzymes do not consume what they degrade.

Adding enzymes directly to the larvae (treatment LC-e0), rather than longer duration pre-treatment, resulted in higher BCE compared with the control with lettuce and cabbage (Table 3). Several previous studies have reported that the time allowed for enzymatic hydrolysis is important and claims that 24-72 h are needed to make a difference (Galbe et al., 2011; Izaguirre et al., 2019; Luo et al., 2019; Pleissner et al., 2013; Wang et al., 2020). In those studies, however, the structure of the substrate after enzymatic hydrolysis was not important and the residues were intended for use in extraction of bioplastics (Izaguirre et al., 2019), biogas (Luo et al., 2019), ethanol (Galbe et al., 2011) and biofuels (Baksi et al., 2019; Pleissner et al., 2013; Wang et al., 2020). The structure of the substrate is important in BSFL composting, to enable aeration so that the larvae can actively move through the substrate without disturbing respiration through their spiracles (Barros et al., 2019). In Study 2, the structure of the substrate was visibly reduced with longer pre-treatment times, which could explain why the BCE did not improve. Another possible reason for this outcome is that movement of the larvae may have achieved better mixing of the substrate than the blender used during the pre-treatment. Further, material reduction (9-15%) was observed during the pre-treatment indicating microbiological activity that might have consumed the easily available components for the larvae. However, none of those reasons explain why the BCE and material reduction were higher in the treatment with directly added enzymes (treatment LC-e0) compared with the control. Odnell et al. (2016) found that cellulase was immediately inactivated when added to digester sludge liquid and concluded that this was not explained by either pH or proteolysis. Therefore, some inhibitory substances must already be present in digester sludge, which is

reasonable due to the large number of metabolites usually present in the anaerobic degradation process. In a study by Hou et al. (2021), polysaccharide concentration increased only during the first 12 h of enzyme pre-treatment. However, as there was no addition of cellulose, this indicates that addition of protease stimulated the microorganisms and increased degradation of the material (Hou et al., 2021). Since the polysaccharide concentration only increased during the first 12 h (Hou et al., 2021), that could be the reason why a longer pre-treatment did not generate a higher BCE or material reduction in Study 2.

It is not known how much the enzymes reduced the fibre content, since this was only theoretically calculated in Study 2. However, it is clear that an improvement in digestibility to the larvae can be achieved with a reduction in the fibre content of the substrate, which leads to increased BCE.

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

	Total N [% of ww]	C/N	Pt/CH	Fibre [% of TS]	Feed rate [mg DM/larva per day]	BCE_{TS}	Reference
Lettuce and cabbage, control	0.16[iii]	27.4[ii]	0.3[ii]	19.9 [ii]	18	20.7	Study 2
Lettuce and cabbage, Enz-0d	0.16[iii]				18	24.7	Study 2
Lettuce and cabbage, NH_3 2d	0.35[iv]				17	20.1	Study 2
Banana peel	0.11	53.8	0.4	68.6	40	7.2[i]	Isibika et al. (2019)
Banana peel $Rhiz_{14d}$		49.3	0.03	61.9	40	15.0[i]	Isibika et al. (2019)
Banana peel $Trich_{14d}$	0.04	83.2	0.2	40.8	40	11.6[i]	Isibika et al. (2019)
Banana peel w/ 1% NH_3 7d	0.35	13.2	2.2	72.1	40	9.6[i]	Isibika et al. (2019)
Banana peel w/ 0.8% NH_3 14d	0.37	16.3	3.1	61.7	40	6.0[i]	Isibika et al. (2019)
Mill by-products (1)	0.70	22.5	0.6	51.7	27	14.9	Gold et al. (2020)
Canteen waste (2)	1.34	10.0	4.3	36.2	27	15.3	Gold et al. (2020)
Cow manure (3)	0.23	25.2	6.2	58.4	27	3.8	Gold et al. (2020)
Vegetable canteen waste (4)	0.33	25.9	0.8	31.5	27	22.7	Gold et al. (2020)
Combined (1-4)	0.41	22.1	1.1	48.7	27	20.9	Gold et al. (2020)

i) Biomass conversion efficiency on a VS basis.

ii) Theoretically calculated based on values from (Livsmedelsverket).

iii) Theoretically calculated protein content/6.25

iv) Theoretically calculated based on addition of 0.2% NH3-N = 2 g/kg

6.3 Impact of nutritional composition of the substrates on BCE

Lettuce and cabbage in Study 2 yielded the highest BCE, after the food waste used as the control in Study 1. The value obtained was similar to that reported for vegetable canteen waste and combined waste by Gold et al. (2020) (Table 5). The total nitrogen content was similar to that of food waste (Table 2 in Paper I), vegetable canteen waste and combined waste (Table 5), but twice the total nitrogen content in lettuce and cabbage. The C/N ratio was similar for the lettuce and cabbage, vegetable canteen waste and combined waste substrates (Table 5), but it is unknown how the enzyme pre-treatment affected this ratio.

Black soldier fly larvae composting of vegetable canteen waste (Gold et al., 2020) had a similar C/N ratio to that calculated for the lettuce and cabbage used in Study 2, but a protein to carbohydrate ratio closer to 1 and a higher fibre content, resulted in similar BCE (Table 5). Higher feeding rate in the treatments by Gold et al. (2020) had no effect on BCE indicating that protein to carbohydrate ratio and fibre content are of higher importance (Table 5). The combined substrate containing vegetable canteen waste in that study had a protein to carbohydrate ratio close to 1 and higher fibre content than the treatment with only vegetable canteen waste (Gold et al., 2020). However, BCE was higher in the treatment with only vegetable canteen waste than in the combined substrate treatment (Gold et al., 2020), indicating that a protein to carbohydrate ratio closer to 1 is not enough to improve BCE if the fibre content is high. Even though this combination of different wastes did not increase BCE compared with using individual wastes, Gold et al. (2020) has showed that other combinations can be successful and therefore co-composting is an option of interest.

The total nitrogen content in the treatment with ammonia in Study 2 was similar to that in the ammonia treatments applied by Isibika et al. (2019) (Table 5), the ammonia treatment in Study 2 resulted in higher BCE, most likely due to the lower fibre content of the substrate used. However, BCE in the ammonia treatment in Study 2 was not significantly different to the control for lettuce and cabbage (Table 5). Assuming that the protein to carbohydrate ratio for the treatment with ammonia in Study 2 was similar ratio to that in the other treatments with lettuce and cabbage, the protein to carbohydrate ratio was closer to 1 than in the ammonia pre-treatment with banana peel. According to in Gold et al. (2020), a protein to carbohydrate

ratio of 1:1 is optimal for achieving high BCE in BSFL composting and in this case it might have been a factor resulting in higher BCE in the treatment with ammonia pre-treatment in Study 2. As concluded in previous studies (Beniers and Graham, 2019; Gold et al., 2020; Lalander et al., 2019), high content of protein and carbohydrates, together with a low content of fibre, increases the process efficiency, which better reflects the nutritional content in the ammonia treatment in Study 2 than that of the banana peels in Isibika et al. (2019). In their study, only ammonia pre-treatment of banana peel for seven days resulted in an improvement compared with non-pre-treated banana peel (33% increase in BCE) (Table 5). The C/N ratio was lower and the protein to carbohydrate ratio was closer to 1 for their 7 days pre-treatment compared with the 14 days pre-treatment (Isibika et al., 2019).

6.4 Impact of larval VS-load

The VS content after the pre-treatments was significantly lower than in the initial substrate in all but one of the treatments (Table 2). This decrease in VS can be related to microbial respiration during the pre-treatment step. The largest decrease in VS content was seen in the broccoli and cauliflower fungi pre-treatment, where combined fungi and bacterial respiration was evident in the increased CO_2 emissions during pre-treatment (Figure 1 in Paper I). The increased degradation resulted in a lower VS load per larva in both the ammonia pre-treatments and fungi compared to the enzyme pre-treatment. It has been demonstrated that a lower larval load leads to lower larval growth (Paz et al., 2015).

A high material reduction has not been seen in previous studies involving treatments with orange peel or banana peel (Isibika et al., 2019), indicating that broccoli and cauliflower wastes have a nutritional content which is more easily degradable for the bacterial community than orange peel and banana peel.

The ammonia pre-treatments resulted in no significant difference compared with the control in either biomass conversion efficiency (BCE) or material reduction. However, significantly lower material reduction was observed compared with the control. This was despite the fact that the VS load did not meet the required level of 0.2 g per larva in all treatments, which in a pre-study (data not shown) resulted in a significant improvement in BCE when pre-treating for a longer period. It seems that the importance of pre-

treatment is greater when the larvae do not have enough VS, which results in a significantly different BCE. However, the ammonia pre-treatment gave no significant difference in process efficiency when the feed demand was met. In the previous study by Isibika et al. (2019), there was also no significant difference in BCE or material reduction with ammonia pre-treatment for 7 days and 14 days with banana peels. This could be a result of the added ammonia not stimulating protein production and giving no added benefits for the larvae, and therefore resulting in lower BCE. Based on the same material reduction as in the control and since the respiration was still high, it can be concluded that the larvae did not grow more and the added ammonia instead stimulated microbial respiration.

6.5 Parameters impacting upon BSFL composting at larger scale

6.5.1 Economic and waste management perspective

Desirable process parameters in BSFL composting, irrespective of the intended end-use, are: operational feasibility of larvae separation from residues (time consumption and separation efficiency) and low emissions compared with other biological treatments (Table 6). Compared with BSFL composting, the emissions of CH_4, N_2O and NH_3 are up to 100-fold higher, and up to for emissions of CO_2 up to two-fold higher, in conventional composting (Ermolaev et al., 2015; Mertenat et al., 2019; Zhang et al., 2016). However, the residues from BSFL composting are not mature and N_2O emissions often occur during late maturation, creating a risk of further emissions during storage (Chen et al., 2019).

From an economic point of view, the value of the products is most important and therefore high BCE is of interest. From a waste management perspective, a high through-put of waste is of importance and therefore a high material reduction is wanted. The duration of the treatment is of importance from an economic perspective; however if improving the total biomass conversion or material through-put with longer treatment time, the cost of increased treatment time may be acceptable. Substrates that can be excluded from being attractive treatment options are untreated orange peel and orange peel pre-treated with fungi. Dry separation of broccoli and cauliflower pre-treated with ammonia was not possible after five weeks of

BSFL composting (Table 1). No wet separation method was evaluated or tested and thus it is unclear whether wet separation is a more manageable approach, although the texture of the material suggests that this option would not be attractive.

The BSFL composting time for the substrates pre-treated with ammonia was very different in the two studies. In Study 1, composting proceeded until dry separation was possible, which was more than twice as long as in the treatment time in Study 2 (Tables 1 and 6). In Study 2, wet separation was successfully used after half the treatment time needed in Study 1, achieving no significant difference in either BCE or material reduction compared with the control (Tables 3 and 6). With no significant improvement in process efficiency from pre-treating for double the amount of time, the reason for longer BSFL composting duration is questionable (Table 3).

However, in Study 1 there was no significant difference in BCE or material reduction but total emissions of the potent greenhouse gases CH_4 and N_2O from the ammonia pre-treated broccoli and cauliflower were significantly lower than for the control (Table 6). Instead, the ammonia pre-treatment resulted in higher emissions of NH_3, representing 0.12-10.0% of NH_3 emissions reported during conventional composting (Zhang et al., 2016). In all, a shorter ammonia pre-treatment could be an option to treat vegetable waste, in order to emit less greenhouse gases. The substrate seems to be the most important factor in predicting whether a treatment results in high BCE or high material reduction.

From an economic point of view, all treatments with high BCE are of interest, in combination with a successful separation and short treatment duration. This means that the treatments with food waste, enzyme pre-treated lettuce and cabbage, and the lettuce and cabbage control are of interest.

From a waste management perspective, all treatments with high material reduction in combination with a successful separation, are of interest. The treatments that qualified in this regard were the treatments with food waste, orange peels pre-treated with ammonia and all treatments with broccoli and cauliflower (Table 6). Among these, fungi pre-treated broccoli and cauliflower would be a preferable option to the untreated control, since dry separation was considerably simpler than with the control. If successful separation was less important (meaning that the value of the larvae would not be included), BSFL composting could be used only to reduce the waste volume. That would make BSFL composting suitable even for the treatments

that did not have successful separation, i.e. orange peel control and orange peel pre-treated with fungi. If the VS load had been closer to 0.2 g per larva in all treatments, then the BCE and perhaps the material reduction (i.e. the material through-put) may have been higher. Thus, more larvae could potentially have been generated per tonne waste, alternatively, a higher material through-put per time could have been achieved.

In addition to more functional treatment, the fungi pre-treatment decreased the total GHG emissions in the treatment. As previously mentioned, ammonia pre-treated broccoli and cauliflower could be preferred to the control from a waste management perspective, even though the treatment might take longer than with no pre-treatment. The treatment with orange peel pre-treated with ammonia could be of highest interest from a waste management perspective, since no other treatment with orange peel permitted successful separation. Orange peel is also a challenging substrate to treat in anaerobic digestion (Zema et al., 2018), which leaves options like co-composting for this waste. Isibika et al. (2021) found that co-composting orange and banana peels with 25% low-quality fish waste more than doubled the BCE, from ~5% to 12%, on a VS basis. Yet, that require other available substrates that work well with orange peel. Although Gold et al. (2020) recommends a protein to carbohydrate ratio of around 1, Isibika et al. (2021) found that an increase in protein to carbohydrate ratio to 0.4:1 was sufficient to increase more than double the BCE. In a waste management perspective, that may be sufficient.

Table 6. Comparison of different parameters from Study 1 and 2. The rating (low, medium, high) is in relation to the highest and lowest value for that parameter. Pre-treatment codes: c = control (no pre-treatment); t = *Trichoderma reesei* pre-treatment; a, a0, a2, a4, a8 = ammonia solution pre-treatment for 0, 2 4, 8 days; e0, e2, e4 = enzyme pre-treatment for 0, 2, 4 days.

	BCE_{VS}	Red_{WW}	Separation		Total time	Emissions		
			Dry	Wet	Weeks	CO_2	CH_4 and N_2O-eq	NH_3
Food waste (FW)								
FW-c	High	High	Yes	No	2	Low	Low	Low
Orange peels (OP)								
OP-c	Low	Medium	No	No	4	Low	Low	Low
OP-t	Low	High	No	No	7	Medium	Low	Low
OP-a	Low	High	Yes	No	7	Low	Low	Medium
Broccoli and cauliflower trimmings (BC)								
BC-c	Medium	High	Yes	No	4	High	High	Low
BC-t	Low	High	Yes	No	5	Medium	Medium	Low
BC-a	Medium	High	No	-[i]	7	Low	Medium	High
Lettuce and cabbage (LC)								
LC-c	High	Low	No	Yes	2			
LC-e0	High	Low	No	Yes	2			
LC-e2	High	Low	No	Yes	2			
LC-e4	High	Low	No	Yes	2			
LC-a0	High	Low	No	Yes	2			
LC-a2	High	Low	No	Yes	2			
LC-a4	High	Low	No	Yes	3			
LC-a8	High	Low	No	Yes	3			

i) Was not tested and could not be visually determined.

6.5.2 Economic value in enzyme pre-treatment

From an industrial point of view, it is important to consider the costs and assess whether pre-treatment of substrate is worth performing to improve product yield. For a treatment to be profitable, the total cost of adding enzymes cannot exceed 0.9 € per kg enzymes (2.52 € per ton incoming waste), including transportation costs, when treating 1 ton substrate a day. In order to estimate the economic viability of enzyme pre-treatment, the company Creative Enzymes Company was asked about the feasibility of supplying a powder cheaper option than the liquid used during the experiments. The residue in all treatments had a high water content, which is not desirable in a compost used directly on soil, and this re-use was therefore excluded from the cost evaluation. A comparison of two different enzyme products (powder, liquid) was used to assess the cost in the enzyme pre-treatment (Table 7). The treatment costs were excluded since they were the same in the control treatment and the treatment with enzyme pre-treatment. The cost of enzyme powder was low, but standard transportation cost were assumed and these costs might differ depending on where the treatment takes place. The calculations were based on treating 1 ton substrate a day, requiring 2.8 kg enzyme powder a month, with a cost of 5.7 € per kg enzymes. It was found that the increase in larval biomass did not generate additional revenue. Since profit margin is difficult to achieve with the enzyme products available today, use of fungal enzymes would be more feasible, even though the treatment time may be longer.

Table 7. Cost evaluation of larvae produced in black soldier fly larvae (BSFL) composting with and without enzyme (Enz) pre-treatment.

	1 ton substrate, wet weight (ww) basis			
	Enzyme cost [€]	BSFL protein [kg]	Value[i] [€]	Total [€]
Control		7.08[a]±0.09	14.5	14.5
Enz, 0 days		8.35[b]±0.10	17.1	
- Liquid	24000[ii]			-
- Powder	16[iii]			1.1

i) World market fish meal prices, average November 2020–April 2021 = 2.05 € kg protein^{-1} (Index Mundi, 2020);

ii) Liquid enzyme (Cellic CTec2) from Sigma-Aldrich, using the conversion 10 SEK = € 1, 1000 u/g;

iii) Enzyme powder for organic waste from Creative Enzymes Company, $390/kg (when ordering 2-20 kg) plus a handling cost of $50 and transportation cost of $120, using the conversion $1 = € 0.8, diluted to 1000 u/g.

7. Conclusions

Ammonia pre-treatment was used with the hypothesis that ammonia would improve process efficiency. No improvements in BCE were however found with this pre-treatment, indicating that the ammonia was not assimilated into protein in a form that the larvae could digest. However, from an economic point of view, a short ammonia pre-treatment appeared to have an impact on microbial respiration and could thus be an option for the treatment of vegetable waste to reduce GHG emissions. Furthermore, the ammonia pre-treatment could also be a suitable option for treating orange peels, if the aim is only to decrease the waste volume.

The aim of the fungi pre-treatment was to degrade complex carbohydrates into simpler, more available carbohydrates that would be available to the larvae. No improvement in the total BCE was found for fungi pre-treatment, likely because the fungi consumed the more available carbohydrates, resulting in a considerably lower VS larval dose, which likely reduced the overall BCE.

For substrates with a high water content, it is more time-efficient to perform wet separation than to wait for the substrate to dry out, since pre-treatment with ammonia gave no improvement in either BCE or material reduction with increased BSFL composting time. From a waste management perspective, fungi pre-treatment could be useful to dry out the substrate faster and enable dry separation, and to reduce waste volume.

The hypothesis that enzyme pre-treatment would increase readily available carbohydrates did, however, appear to hold true. A 22% increase in BCE was achieved in enzyme pre-treatment for 0 days, achieving similar BCE as for food waste. However, adding enzymes to increase the BCE was shown not to be profitable, since enzyme products are currently too expensive. Greater improvements may be obtained if a substrate with higher

fibre content is pre-treated with enzymes or if a combination of fungi and enzymes is used.

8. Future research

Use of higher pH during pre-treatment and BSFL composting when pre-treating with enzymes or ammonia should be examined in future work, to see whether the process efficiency can be increased and create an environment less suitable for microorganisms, letting the larvae take advantage of the easily available nutrients in the substrate.

To get a better overview of what changes happening during enzyme pre-treatment, measuring the content of fibre, carbohydrates and glucose before and after pre-treatment and BSFL composting would be of interest. Using substrates with a higher fibre content than lettuce and cabbage would also be interesting, to see if enzyme pre-treatment has a greater effect on such substrates.

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Black soldier fly larvae (BSFL) composting is a technology that meets circular economy principles. The greatest potential BSFL composting is for mixed food waste, but only plant-based waste is permitted as feed for the larvae. It has lower biomass conversion efficiency (BCE), but this could be improved by pre-treatment. This thesis has proved that direct addition of enzymes at the start of BSFL composting gave 22% higher biomass conversion efficiency compared with the control.

Lovisa Lindberg received his graduate education from the Department of Energy and Technology at the Swedish University of Agricultural Sciences (SLU). Her undergraduate degree was obtained from Uppsala University.

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.

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