UPTEC W 19 012
Examensarbete 30 hp
Februari 2019

UPPSALA UNIVERSITET

SLU

Impact of fly specific bacteria on fly larvae composting

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Abstract

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About one third of all edible food is wasted globally each year. This calls for improvements in resource and waste management. An interesting solution for organic waste is fly larvae composting, which both produces protein in the form of larvae and a nutrient rich residue. The larvae can be used as animal feed while the residue can be applied as an organic fertilizer, thus recycling the nutrients. Fly larvae composting with the black soldier fly (BSF), *Hermetia illucens*, is relatively new, but there is extensive research on how the treatment is affected by different parameters, e.g. temperature, moisture and type of organic waste that is treated. The role of bacteria has only been the topic of a few studies in recent years but has shown promising positive effects on larval growth. This study investigated the impact of bacteria isolated from BSF eggs on fly larvae composting of food waste by BSF larvae. The study was done in two experimental phases. In *phase I*, groups of three bacteria were added to each treatment together with the larvae and only single treatments were executed; in *phase II* triplicates of promising groups of three, two or single bacteria were evaluated.

The results of phase I suggested that selected groupings of bacteria could either decrease or increase the bioconversion ratio and in general decrease the reduction ratio of the food waste, while the survival ratio did not seem to be impacted. However, in phase II no significant difference ($p<0.05$) between the treatments with bacteria and the control were found for any evaluated variables. Interestingly, the variation in resulting bioconversion ratio and reduction ratio (on a VS basis) was found to be reduced when one or more bacteria were present. The coefficient of variation in bioconversion ratio was 9.5% for the control compared to between 2.5% and 6.1% for treatments with bacteria. For the reduction ratio the variation was reduced from 5.6% and to between 0.9% and 4.6% for the bacteria treatments. Hence, seeding with bacteria may improve stability of the process, which is especially interesting when scaling up the process.

Keywords: Fly larvae composting, Black soldier fly, Hermetia illuciens, BSF bacteria, Organic waste, nutrient recycling, Eco-technology

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Referat

Flugspecifika bakteriers inverkan på fluglarvskompostering

Kristina Lundgren

Globalt slängs ungefär en tredjedel av all ätbar mat. Därmed finns ett stort behov av säker och hållbar avfallshantering. En intressant lösning för organiskt avfall är fluglarvskompostering, som både producerar protein i form av larver och en näringsrik behandlingsrest. Larverna kan exempelvis användas som djurfoder medan behandlingsresten kan användas som gödselmedel, vilket innebär att näring kan återvinnas. Fluglarvskompostering med amerikansk vapenfluga (BSF), *Hermetia illucens*, är en relativt ny teknik men det finns redan omfattande forskning på hur olika processparametrar, t.ex. temperatur och vattenhalt, påverkar processen. Bakteriers roll har studerats endast i ett fåtal studier men de indikerar att tillsats av bakterier kan ha en positiv inverkan på larvernas tillväxt. Den här studien har därför undersökt hur tillsats av bakterier isolerade från BSF ägg påverkar fluglarvskompostering av matavfall med BSF larver. Studien gjordes i två experimentella delar. I *fas I* undersöktes effekten av tillsats av bakteriegrupper om tre bakterier till matavfallet samtidigt med larverna; i *fas II* utfördes triplikat av lovande bakteriegrupper med tre, två eller enstaka bakterier.

Resultaten från *fas I* indikerade att olika bakteriegrupper antingen kunde höja eller sänka bioomvandlingskvoten och generellt gav en minskning i materialreduktionskvoten medan överlevnadsgraden inte verkade påverkas i samma utsträckning. I *fas II* observerades däremot ingen signifikant skillnad ($p<0,05$) mellan någon av bakteriebehandlingarna och kontrollen för samtliga processvariabler. Variationen i resulterande bioomvandlingskvot och materialreduktionskvot (på VS basis) var dock lägre för bakteriebehandlingarna jämfört med kontrollen. Variationen i bioomvandlingskvot var 9,5% för kontrollen jämfört med 2,5-6,1% för bakteriebehandlingarna. För materialreduktionskvoten minskade variationen från 5,6% till mellan 0,9% och 4,6%. Detta tyder på att tillsats av bakterier kan förbättra stabiliteten hos fluglarvskompostering, vilket är särskilt intressant vid uppskalning av processen.

Nyckelord: Fluglarvskompostering, Amerikansk vapenfluga, Hermetia Illuciens, BSF bakterier, Organiskt avfall, näringsåterföring, Grön teknik

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Preface

This master thesis corresponds to 30 ECTS Credits and concludes the M.Sc. in Environmental and Water Engineering program at Uppsala University and the Swedish University of Agricultural Sciences (SLU). The thesis work was done at the Department of Energy and Technology at SLU. Supervisor was Cecilia Lalander and reviewer was Björn Vinnerås, both from the Department of Energy and Technology, SLU.

I want to express my gratitude to several people who have contributed to the making of this thesis. A big thanks to Eskilstuna Strängnäs Energi och Miljö AB and especially Benny Björk, who provided food waste for my experiments. For suggesting and helping me plan this project, I want to thank Björn and Cecilia. Especially, I want to thank Cecilia for helpful and interesting discussions as well as excellent support during my thesis work. I also want to thank Giulio and Viktoria for helping out during my experiments in the greenhouse. I am also very grateful to everyone in the Environmental Engineering group at the Department of Energy and Technology, SLU, who have shared their knowledge with me. Finally, I want to thank my family and friends for the encouragement and support, not only during this project but in anything I set out to do.

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Uppsala, February 2019

 UPTEC W 19 012, ISSN 1401-5765. Published digitally at the Department of Earth Sciences, Uppsala University, Uppsala, 2019.

Populärvetenskaplig sammanfattning

Flugspecifika bakteriers inverkan på fluglarvskompostering

Kristina Lundgren

Varje år slängs ungefär en tredjedel av all ätbar mat globalt. Det innebär enorma mängder avfall som behöver hanteras, särskilt med tanke på att den globala befolkningen enligt FNs beräkningar förväntas öka från ungefär 7 miljarder idag till 10 miljarder år 2050. I detta problem finns dock också intressanta möjligheter. Organiskt avfall, så som matavfall, innehåller näring som är möjlig att återvinna från materialet på olika sätt. Många är nog bekanta med kompostering och hur avfallet efter lång tid omvandlas till näringsrik jord som sedan går att odla i. En ny lösning är på framåtmarsch som direkt kan producera protein utan omvägen via åkermark. Tekniken kallas fluglarvskompostering och går ut på att låta fluglarver konsumera organiskt material och därmed producera fett och protein i form av larvbiomassa. Tanken är sedan att larverna kan användas som djurfoder åt till exempel fisk eller kyckling. Det material som blir kvar liknar jord och kan användas som gödselmedel. En vanlig flugart inom fluglarvskompostering är den amerikanska vapenflugan. Många studier finns redan på exempelvis vilken temperatur och typ av organiskt material som den amerikanska vapenflugans larver föredrar. Bakteriers inverkan på larverna har dock inte studerats i någon större omfattning ännu trots att forskning med andra flugarter har visat att bakterier är så pass viktiga att vissa arters larver inte ens överlever i en steril miljö. Den här studien har därför studerat hur bakterier tagna från den amerikanska vapenflugans ägg påverkar fluglarvskompostering av matavfall genom att tillsätta grupper av dessa bakterier till matavfallet tillsammans med larverna.

De initiala resultaten pekade på att bakterierna kunde påverka dels hur mycket larvbiomassa som producerades på en viss mängd matavfall, vilket mäts av bioomvandlingskvoten, och dels hur mycket av materialet som bröts ned, vilket mäts genom materialreduktionskvoten. Efter ytterligare försök visade det sig däremot att skillnaderna inte var tillräckligt stora för att kunna sägas vara annorlunda från fluglarvskompostering utan tillsats av bakterier. Detta var intressant eftersom några få tidigare studier som använt sig av hönsgödsel istället för matavfall, hade sett att larverna blev något större när de tillsatte bakterien *b. subtilis*. Den bakterien användes även i denna studie. Kanske kan bakteriers inverkan bero på vilket material som larverna ska

behandla? Eller kanske är det så att andra faktorer spelar större roll? I den här studien visade sig temperaturen ha stor betydelse för materialreduktionskvoten och påverkade även larvernas storlek i viss mån. Alltså verkar det vara viktigare att kontrollera temperaturen än vilka bakterier som finns närvarande för att få en hög produktion av larver och en hög reduktion av materialet.

Den här studien visade även något annat intressant. Nämligen att när en eller flera bakterier tillsattes så minskade variationen i resulterande bioomvandlingskvot och materialreduktionskvot. Det blev alltså lättare att förutsäga hur mycket fluglarvsbiomassa som skulle produceras från avfallet och hur mycket behandlingsrest som skulle bli kvar. Tillsats av bakterier från flugans ägg till materialet verkar alltså göra fluglarvskomposteringen mer stabil vilket är väldigt intressant, särskilt om man vill använda tekniken på stor skala. Det är mer lockande att investera i och lättare att planera inköp och försäljning om produktionen är jämn än om den varierar från gång till gång.

Försöken i den här studien gjordes på liten skala med knappt ett kilo matavfall per försökslåda. Först utfördes en bred experimentfas där tre olika bakterier tillsattes i varje behandling. Alla möjliga kombinationer av 8 olika bakterier studerades. Därefter utfördes ytterligare experiment med de bakterier och grupper av bakterier som gynnade larverna mest och minst, för att kunna stärka de initiala observationerna. Framtida studier skulle kunna fokusera på att tillsätta bakterier till olika typer av organiskt material, på olika stor skala, för att se om de generellt gör fluglarvskomposteringen mer stabil.

Glossary

Bioconversion ratio	The percentage of substrate that is converted into larvae biomass.
BSF	Black soldier fly.
BSFL	Black soldier fly larvae.
Fly larvae composting	Treatment of organic waste by fly larvae that degrade the organic material.
Mini-larvae	Approximately a week old larvae that were added at the start of a treatment.
Reduction ratio	The percentage of initial substrate that has been digested.
Respiration	The part of the reduction ratio that is not due to an increase in larval biomass. Includes both microbial degradation and the larval respiration.
Response variable	Variables used to measure the performance of the fly larvae composting process. For instance bioconversion ratio, reduction ratio, survival ratio and respiration.
Seeding	Seeding with bacteria refers to the addition of bacteria to a substrate just before adding fly larvae.
Survival ratio	The percentage of larvae that survive a treatment.
Substrate	The organic waste that is used in the fly larvae composting.
TS	Total solids or dry matter is a fraction of the wet weight. TS + fraction of water = 1.
VS	Volatile solids is the organic fraction of the TS. VS + fraction of ash = 1.
WW	Wet weight is the total weight including water.

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1 INTRODUCTION

By 2050 the world population will likely have increased to 10 billion people according to the UN's latest predictions (United Nations, 2017). This introduces challenges for food production, which according to the FAO (2009) has to increase with 70% by 2050. At the same time, it has been estimated that globally about one third of edible food is thrown away annually (FAO, 2011). Hence, the growing population presents challenges in both waste and resource management. The need for more efficient waste management with increased recycling, reuse and prevention of waste, is highlighted in the UN's sustainable development goals from 2015. Furthermore, on EU level there is a strategy in place to move towards a circular economy, which means that resources and energy should be conserved where it is possible (EU, 2015). These goals calls for waste treatments that not only reduce the amount and potential hazardousness of the waste, but that also are able to recycle the energy or nutrients in the waste.

For organic waste one possible treatment is thermophilic composting, which generates a residue that can be applied to fields as a fertilizer or a soil amendment (Ceglie and Abdelrahman, 2014). Anaerobic digestion is another option that both produce biogas that can be used for electricity, heat or may be upgraded into vehicle fuel and produce a digestate that can be used as an organic fertilizer (Wellinger et al., 2013). Both treatments aim to create valuable products from the waste, but due to high capital cost anaerobic digestion is only economically superior to composting on larger scales even though more products are generated (Lin et al., 2018). If the biogas is upgraded to transport fuel the economic gains can potentially increase, but this is, according to Lin et al. (2018), mainly due to government subsidies. A novel treatment alternative emerging in the last decade is fly larvae composting, which produces larvae that can be used as animal feed or for biodiesel production and a nutrient rich residue that can be used as an organic fertilizer (Čičková et al., 2015). Hence, fly larvae composting allows for recycling of nutrients and production of protein (rather than moving down the waste hierarchy to recovery or disposal), both which are in increasing demand with the growing population. A study done in a Swedish context investigated which treatment out of thermophilic composting, anaerobic digestion, fly larvae composting or fly larvae composting combined with anaerobic digestion that produced the most value (Lalander et al., 2018). The most value was gained from the combination of fly larvae composting

and anaerobic digestion, while fly larvae composting came in second. The authors suggested that it was likely that fly larvae composting would be the most economically viable option where a large anaerobic digestion plant does not already exist. This highlights the potential that fly larvae composting has in organic waste management.

This study was focused on fly larvae composting with larvae of the black solider fly (BSF), *Hermetia illucens*. In order to scale up the production of larvae and treatment of waste, knowledge is needed on how to optimize the system (De Smet et al., 2018). Impact of temperature, light and moisture content are some factors that have already been studied (Tomberlin et al., 2009; Tomberlin and Sheppard, 2002; Cheng et al., 2017), but according to De Smet et al. (2018) there is a knowledge gap concerning how the black soldier fly and the composting process is impacted by microorganisms. A few studies have observed an increase in larvae growth when certain bacteria are added to the substrate (Yu et al., 2011; Zheng et al., 2012; Xiao et al., 2018). This project will add to this research in order to better understand if seeding with bacteria could increase efficiency.

1.1 PURPOSE AND GOAL

The aim of this study was to assess how seeding with BSF-specific bacteria impact the survival ratio of the larvae, bioconversion ratio and the reduction ratio of fly larvae composting with BSF. By studying groupings of bacteria together with black soldier fly larvae (BSFL) this project aims to better understand the influence of a single or multiple bacteria consortia.

2 BACKGROUND

2.1 FLY LARVAE COMPOSTING

Fly larvae composting is a way to treat organic material by letting fly larvae digest and facilitate the microbial degradation of the material (Čičková et al., 2015). Not only does it reduce the volume and mass of the organic waste (and does so faster than thermophilic composting), but it also generates larvae (Čičková et al., 2015). The fly larvae can for instance be used as feed for animals or humans (Wang et al., 2017) or be converted into biodiesel (Li et al., 2011a). The nutrient rich residue can be used as a fertilizer, since the process increases the concentration of available nitrogen (Green and

Popa, 2012) or it can be used for biogas production (Lalander et al., 2018). Several fly species can be utilized for fly larvae composting, e.g. the black soldier fly, house fly, blow fly and face fly (Čičková et al., 2015). On a small scale it is possible to let wild fly populations colonize the material (Sheppard et al., 1994), but this makes the process unreliable and susceptible to whether or not flies lay eggs, which is why a more controlled environment is required for ensuring continuous treatment (Čičková et al., 2015). By keeping mating and oviposition separate from the actual fly larvae composting it is possible to have a stable production of eggs and larvae, and thus increasing the systems reliability to treat a certain amount of organic material (Čičková et al., 2015). Today, large scale facilities with treatment capacities around 200 tonnes of organic waste per day exist in e.g. China, USA and the Netherlands (Diener et al., 2015)

2.2 COMPOSTING WITH BLACK SOLDIER FLY

The focus of the study was composting with BSFL, which has several advantages compared to other fly species (Čičková et al., 2015). For instance, it is not a vector of disease transmission since the adult fly does not eat. Furthermore, the BSFL grow much larger compared to many other fly larvae species, which makes them easier to separate from the residue (Čičková et al., 2015). Compared to the house fly, the BSF has a longer larval development time and larger larvae, which means that they can process larger amounts of organic waste per larva (Čičková et al., 2015). The BSF grow to a length of about 13 – 20 mm and originates from America, but has managed to spread across the world (Tomberlin et al., 2002). It favours warm or tropical climates and can therefore be found between 45°N to 40°S latitude (Sheppard et al., 1994).

2.2.1 The BSF Composting Process

When composting with fly larvae, the moisture content of the substrate has been found to decrease over time (Čičková et al., 2015). This is due to increased temperature and aeration as a result of the degradation of the material and movement of larvae (Parra Paz et al., 2015). The pH of the substrate has also been seen to increase from neutral or acetic to alkaline as ammonia is released (Čičková et al., 2015). The process has also been shown in several studies to successfully reduce the concentration of *E. coli*, *Salmonella* spp. and viruses in the residue (Lalander et al., 2015; Liu et al., 2008; Erickson et al., 2004).

In one study, with pig manure, human manure and dog food, it was observed that the total phosphorous concentration as well as ammonium concentration increased in the residue after being processed by BSFL (Lalander et al., 2015). The total nitrogen concentration, however, remained unchanged. The total amounts of both phosphorus and total nitrogen decreased; hence the observed increase in concentration could be attributed to a high material reduction. According to Emerson et al. (1975) ammonia emission depend on both pH and temperature, with increasing emissions at high pH and high temperature. Thus the amount of nitrogen in the residue will depend on these parameters as well.

To what degree the substrate is degraded in fly larvae composting has been seen to vary between substrate. In a study by Diener et al. (2011), black soldier fly larvae reduced food waste with about 70% on a TS basis, even though they were subjected to high zinc concentrations. Somewhat lower mass reductions (on a TS basis) have been observed for other substrates, for instance 57% of dairy manure mixed with soybean curd (Rehman et al., 2017), 36% of chicken manure (Xiao et al., 2018) and about 55% of a mixture of pig manure, human manure and dog food (Lalander et al., 2015). Sheppard et al. (1994) approximated the reduction ratio of chicken manure to 50%, which is much higher than what Xiao et al. (2018) reported. Xiao et al. suggested this might be due to differences in source of manure and environmental conditions. In the study by Diener et al. (2011) low oxygen levels and low temperatures were seen to hamper the larvae feeding rate, thus suggesting that those environmental conditions can impact the degradation. Furthermore, the degradation has been shown to depend on the larval density and the feeding rate (Parra Paz et al., 2015). Parra Paz et al., (2015) found that a feeding rate of 60 mg/larva/day or less resulted in well degraded residues (with pH between 7 and 8), while higher rates had lower pH, which indicate less degraded material according to the authors (as BSFL previously have been shown to increase/stabilize pH). On the other hand, when larvae density was too high in their study, the development treatment time increased due to competition of nutrients. Parra Paz et al. (2015) suggested that the optimum feeding rate was 163 mg/larva/day (on a TS basis) together with a larval density of 1.2 larvae/cm^2, if the primary goal is biomass production.

The bioconversion ratio (how many percent of the total inflow substrate that is converted into larvae biomass) has also been shown to differ between substrates. For example, the bioconversion ratio of food waste was found to be roughly 12% TS by Diener et al. (2011). However, Lalander et al. (2018) demonstrated a substantially higher bioconversion of 35% for food waste. Diener et al. (2011) did, however, suggest that their environment was suboptimal due to high zinc concentrations. Furthermore, they had different experimental set-up. In a recent study Lalander et al. (2019) found that a high protein substrate shortened the development time while the amount of available carbon influenced the size of the larvae. Hence, a balanced substrate seems to be important for a high bioconversion ratio. This is also what Rehman et al. (2017) found in their study with soybean curd residue and dairy manure. If mixed, the bioconversion ratio (on a TS basis) of soybean curd residue and dairy manure increased to up to 15% from roughly 12% for only soy bean curd residue and approximately 7% in only dairy manure.

2.2.2 Products of BSF Composting

Larvae as Feed or Food

Tschirner and Simon (2015) found that the protein content of the larvae is impacted by the substrate. They observed that a high fibre diet resulted in larvae with a protein content of 52% (of TS), a protein diet resulted in 45% protein while the control larvae consisted of 37% protein. De Smet et al. (2018) points out that this is very interesting as it indicates that it is possible, at least to a certain degree, to rear BSFL with certain nutritional properties depending on the final use of the larvae. Liu et al. (2017) found that the fat and protein content in BSF varied over the life cycle and that for pre-pupae the protein content was roughly 40% while the fat content was approximately 30%. Since the BSFL are rich in both protein and fat several studies have looked at replacing part of the conventional protein or fat source in feed with BSFL meal (Schiavone et al., 2017; Cullere et al., 2016; Kroeckel et al., 2012; St-Hilaire et al., 2007). Schiavone et al. (2017) found that based on digestibility of highly or partly defatted BSFL to chicken broilers, both types of BSFL could be used as an ingredient in feed for chicken broilers. Similarly, Cullere et al. (2016) suggested that defatted BSFL can replace part of the soya bean meal and soya bean oil in feed for broiler quail (up to 24% and 100% respectively) without any significant negative effect on slaughter weight, weight gain, feed intake, feed conversion ratio or mortality ratio. Kroeckel et al. (2012) instead

replaced 17 – 76% of the fish meal in feed for turbot (*Psetta maxima*) with BSF pre-pupae and observed somewhat lower growth and feed intake, but no impact on mortality compared to the control. The author's suggested that adding chitin degrading enzymes or bacteria to the feed might decrease the negative effect on growth as chitin otherwise impact the lipid digestibility. Another option, suggested by Huyben et al. (2019), is to use larvae instead of pre-pupae as they contain less chitin. Studies have also been done with rainbow trout (*Oncorhynchus mykiss*). St-Hilaire et al. (2007) replaced 25% of the fish meal with BSF pre-pupae without any significant negative impact on weight gain compared to the control that were fed fish meal. Renna et al. (2017) did not observe a significant impact on rainbow trout growth even at 50% replacement with partially defatted BSF larvae.

Both the flies and the larvae can also be human food even if it is very uncommon today (Wang et al., 2017). This does, however, depend on what the larvae have been fed. For instance, there is a concern about heavy metal accumulation if a substrate with high metal concentration is used (Wang et al., 2017). Several studies have found that cadmium accumulates in BSF larvae (Diener et al., 2015; Purschke et al., 2017; Biancarosa et al., 2018). Regarding other metals and their accumulation, there is some disagreement. For instance, Diener et al. did not observe accumulation of lead in the larvae, while Purschke et al. and Biancarosa et al. did. Different substrates were used in these studies (chicken feed, corn semolina and processed wheat with seaweed), but the difference in results has not been investigated further. Wang et al. (2017) suggested that more studies are needed, but also pointed out that even though the accumulation of certain metals makes the larvae less edible (and thus possibly not suitable as feed) it does remove the metals from the waste, which makes the residue safer to use.

Legislation Concerning BSF as Food or Feed

In the EU insects reared for feed or food production fall under the definition of "farmed animals" and thus must comply with Regulation (EC) 1069/2009. For instance, this means that the insects have to be fed on materials of vegetal origin and that it is prohibited to rear insects on animal bi-products, e.g. manure or catering waste, if they are intended to be used as feed or food. According to Regulation (EC) No 999/2001 it is prohibited to use farmed animal derived proteins in feed for ruminant or monogastric animals. However, whole, live insects or fat derived from insects is not included in the

abovementioned ban and insect protein is allowed in feed for pets and fish (if the insects are reared in vegetable based substrates).

According to Lähteenmäki-Uutela et al. (2017) the use of insects as feed or food is regulated to different degrees in different parts of the world. In China and Mexico, where there is a long history of eating insects, there are several insect products on the market. Meanwhile, in Canada it is legal to use black soldier flies as feed for broiler chickens and some food products are available in both Canada and the US. However, in the EU, Canada, China and Australia insects are generally considered "novel foods" and as such the ingredient needs to be registered. The registration is more or less strict and what actually is defined as a novel food is slightly different as well. For instance, in Canada insects are not considered novel foods if they are traditionally eaten somewhere in the world.

Other Products from Larvae

Instead of using the fat in feed Li et al. (2011a) suggest that it can be extracted and used for production of biofuels. The authors point out that such production might reduce the conflict between food and biofuel production as the larvae grow faster than energy crops and can be produced from waste. After fat extraction Li et al. suggest that the defatted larval biomass should be used as a protein source in animal feed. It is also possible to combine anaerobic digestion with BSF composting as shown by Li et al. (2015) who first treated corncob with anaerobic fermentation to produce biogas and then treated the residue with BSF composting to produce larvae for biodiesel production.

Besides being rich in protein and fat the larvae also contain chitin and different bacteria and enzymes (Newton et al., 2005). The chitin is useful as it, as well as its common derivative chitosan, both are popular biopolymers in the medical and cosmetic industry (Elieh-Ali-Komi and Hamblin, 2016). De Smet et al. (2018) suggest that the bacteria and enzymes in the larvae might be extracted in the future as well. Furthermore, Choi et al. (2012) suggested that methanol extracts from BSFL may be used as an antibacterial substance as they found that it had an effect on gram-negative bacteria: *K. pneumoniae*, *N. gonorrhoeae* and *S. sonnei*.

Treatment Residue

The treatment residue is the other product of BSF composting. One study from Green and Popa (2012) found that BSF composting lead to increased concentrations of ammonia in the residue from food waste. The authors point out that this mineralization of organic nitrogen makes the substrate suitable for fertilization of crops and could therefore reduce fertilization costs. Choi et al. (2009) compared the BSFL residue (of food waste) to commercial fertilizer and observed little difference in either chemical composition or the growth of cabbage grown with the two different nutrient sources. They therefore concluded that the residue was suitable for fertilization of crops. Li et al. (2011b) instead used dairy manure treated by BSF to produce sugar (through hydrolysis) and gained 96.2 g of sugar from 273 g of residue. Another potential use is to produce biogas through anaerobic digestion of the residue (Lalander et al., 2018). The biomethane potential of the residue depends on the substrate, for instance, the potential is higher for food waste residue than for faeces residue according to Lalander et al. (2018).

2.2.3 Life Stages and Influence of Process Parameters

BSF has four distinct life-stages: egg, larva, pupae and fly (Tomberlin and Cammack, 2017) (Figure 1). The larvae go through six stages, finishing with the pre-pupae stage (May, 1961). A pre-pupae is according to Sheppard et al. (1994) darker in colour, has emptied its gut and redesigned its mouth into a hook for increased movability in order to find a suitable place to become pupa. In a study by Tomberlin et al. (2009) the pupation lasted between 15 to 18 days depending on temperature (shorter for warmer climate).

Figure 1. Photos of the different life stages of the black soldier fly. a) Egg packages, b) two larvae of different ages, a pre-pupae and pupae, c) adult fly.

The life span of the fly has been found to be impacted by availability of water (Tomberlin et al. 2002). Tomberlin and colleges observed that at 27°C, flies that were provided with water lived for eight to nine days, while flies without access to water lived for six to nine days. In total, at 27°C, the total lifecycle varied between 40 to 43 days in their trial.

In 2002 Tomberlin and Sheppard studied the effect of light, humidity and temperature on mating and oviposition of BSF. Mating was observed to occur more frequently during morning and early day and required light intensities over 63 μmol/m^2s, but 75% of the mating was observed for intensities over 200 μmol/m^2s. They also found that oviposition mainly occurred at temperatures over 26°C and that 80% of oviposition occurred when the humidity was over 60%. According to Booth and Sheppard (1984) an egg package laid by a single female contains on average 500 eggs, which hatch after approximately four days. However, the time to hatching has been shown to depend on temperature. Holmes et al. (2016) found that BSF eggs did not hatch at all in 12°C and took approximately 15 days to hatch at 16°C.

After the eggs have been hatched, the larvae start digesting the organic material that is available to them. Gligorescu et al. (2018) reared BSFL at 20°C and 27°C on a balanced diet (called Gainesville diet), a protein diet and a carbohydrate diet and found that both diet and temperature impacted the development time. The larvae developed faster in warmer conditions and on a balanced diet. In their study the development time from fifth larvae stage to pre-pupae varied from just over 10 days (in 27°C on the Gainesville diet) to over 50 days (in 20°C on a carbohydrate diet). These results are similar to a study by Tomberlin et al. from 2009, in which BSF were reared at 27, 30 and 36°C. The larvae reared at 36°C were both smaller and had a longer development time than those reared at lower temperatures and only 0.1% survived to adulthood. Meanwhile, they found that at 27°C the larvae weight was increased with approximately 5% compared to at larvae weight at 30°C. However, the larvae development was four days shorter at 30°C. Hence, their study suggest that the optimal temperature for rearing BSF is somewhere between 27 and 30°C with the upper limit between 30 and 36°C. The lower threshold for complete development (from egg to fly) has been suggested to lie between

16°C and 19°C, as in the study by Holmes et al. (2016) the larvae did not survive at 16°C.

In a study by Cammack et al. (2017), the larvae were found to prefer a substrate with more than 40% water content. In their study the impact of diet and substrate moisture on the BSF and BSFL was investigated. Substrate moisture levels of 40, 55 and 70% were used and three diets were evaluated, in which protein and carbohydrate proportions were set to 7% and 35%, 21% and 21% or 35% and 7%, respectively. The larvae were found to develop faster and grow larger at 70% moisture and failed to develop at all in 40% moisture (Cammack et al., 2017). Of the diets, it was the balanced diet (21% protein and 21% carbohydrates) that generated the highest survival ratio and fastest development, which is in accordance with what Gligorescu et al. also found in their study from 2018 (as previously mentioned).

Regarding the initial pH of the substrate, Ma et al. (2018) found that survival ratio to pre-pupa was lower when the pH was 2, 4 or 10 compared to 6, 7 or 8. While Meneguz et al. (2018) did not observe an impact on final larval or pupal weight when they set initial substrate pH to 4, 6.1, 7.5 and 9.5. Meneguz et al. (2018) did, however, find that feeding regime impacted the development time and larvae weight. Larvae that were fed daily as compared to once got bigger, digested more substrate but took longer to develop. In addition to the abovementioned factors it has been observed that BSF from different colonies, i.e. different strains, differ in the development time, growth and ability to reduce organic material (Zhou et al., 2013).

2.2.4 Larva Anatomy and Metabolism

The gut of BSFL consists of three parts: the foregut, midgut and hindgut, which all can have different bacterial communities (Engel and Moran, 2013). In most insects, the main digestion occurs in the midgut, while the foregut may act a temporary food storage and the hindgut may act as a storage for faeces (Engel and Moran, 2013). Both the hindgut and the foregut are shred several times as the larvae develops and the lining of the midgut is also shred repeatedly (Engel and Moran, 2013). This makes the larvae gut in general a very instable microbial habitat, but Engel and Moran (2013) pointed out that many insects have special “crypts” that help microbes survive.

Kim et al. (2011) studied digestive enzyme activities in the salivary gland and gut of BSF and suggested that since most of the activity was found in the gut of the larvae it should be considered the primary organ for digesting food in BSFL. The salivary glands did have some enzymatic activity, but much lower compared to the activity in the gut. Furthermore, the study confirmed that the black soldier fly is a polyphagous insect (it eats many different kinds of substrate) based on the types of enzymes found in the larvae. This has, for instance, been seen by Nguyen et al. (2015), who found that BSFL were able to degrade and reduce the amount of pig liver, pig manure, kitchen waste, fruits and vegetables and rendered fish. Other studies have also used BSFL to degrade chicken manure (Sheppard et al., 1994), dairy manure (Li et al., 2011b) and human faeces (Lalander et al., 2013).

However, even if the material is degraded the larvae do not necessarily grow well in all of them. Lalander et al. (2019) studied the growth and development of BSFL in 11 different substrates (for instance food waste, sludge and abattoir waste) and found that in order to grow the larvae needed a substrate (or substrate mix) that is both high in protein and have a large percentage of easily available carbon. They observed that if the protein content was high the development time was shorter compared to low protein diets, but without energy in the form of easily degradable carbon they did not grow large. Similar results were obtained in a study by Jucker et al. (2017) who observed a shorter development time on a diet with more protein (vegetable mix compared to fruit mix). They also observed that the fat content in larvae increased on a diet with less protein and more carbohydrates.

The metabolic rate in BSFL has been shown to increase with temperature (Gligorescu et al. 2018). As already mentioned in 2.2.3, Gligorescu et al. (2018) studied BSFL metabolism and development with a protein diet, a carbohydrate diet and a balanced diet at two different temperatures. At 20°C, the larvae had a lower food intake compared to at 27°C. At the lower temperature the food intake and metabolic rate was not significantly different between diets, while a difference was observed for the higher temperature. At 27°C the balanced diet had the highest food intake followed by the carbohydrate diet.

2.2.5 Impact of Bacteria on Black Soldier Flies

According to Engel and Moran (2013), bacteria can be a purely nutritional source for insects, but there are several other functions of the gut microbiota, including:

- Nutritional symbioses, were bacteria in the gut help the insect digest certain compounds;
- Detoxification of food;
- Stimulation of the immune system.

Some bacteria might even be necessary for the larva to develop at all, which was seen to be the case for stable fly larvae in an experiment by Lysyk et al. (1999). Six different bacteria isolated from stable fly eggs were inoculated on plates with egg yolk as growth medium, both individually and as mixed communities. Lysyk et al. (1999) found that the larvae developed to pupa if either *Acinetobacter* spp., *E. coli*, *E. breve* or *F. odoratum* were present, while the larvae failed to develop in the presence of only *Aeromonas* spp. or *Serrti marcescens* or on an uninoculated plate. In a similar study it was found that only 4% of house flies survived to adulthood on egg yolk medium that was not inoculated with bacteria (*E.coli* migula), while emergence ranged between 33% and 63% on plates with bacteria depending on the growth medium (Watson et al., 1993).

However, some bacteria might not be essential for development but could potentially be beneficial to the larvae in other ways. For instance, some bacteria have been observed to metabolize lignin (Brown and Chang, 2014), which is a compound that BSFL are believed not to degrade on their own, as seen in a study by Li et al. (2011b) in which the relative lignin content increased after BSFL composting. Furthermore, Jeon et al. (2011) found aerobic bacteria in the gut of BSFL that are able to degrade cellulose and thus highlighting the importance of the gut bacteria in fly larvae composting. According to Jeon et al. (2011), the bacterial diversity in the gut was influenced by the nutritional complexity of the substrate, as higher bacterial diversity was observed when larvae were reared on food waste rather than calf forage or cooked rice. Similarly, Bruno et al. (2018) observed a significant difference in the anterior midgut microbiota depending on substrate, when BSFL were reared on a standard diet, a vegetable mix and a fish meal diet. The microbial community in the substrate, however, was not seen to be significantly affected by the presence of BSFL (Bruno et al., 2018). In a more comprehensive study by Wynants et al. (2018) seven different substrates were used at

four different locations and at different scales (lab scale as well as commercial production facilities). No significant relationship between the microbial communities in the substrate compared to the communities in the larvae was observed. However, their observations also generally showed that the microbial community of the larvae and the residue were more alike, compared to the larvae and the initial substrate. Wynants et al. (2018) therefore suggested that other factors besides the substrate type is important in deciding the microbial community in BSFL, for instance the bacteria in the surroundings as well as local (and time dependent) environmental conditions that might benefit certain bacteria. They also suggested that some bacteria might thrive in the larval gut and thus are common in excreta from larvae, which then influence the residue. Hence, the observations by Wynants et al. (2018) differs from what Jeon et al. (2011) and Bruno et al. (2018) saw in that Wynants et al. (2018) did not see an influence of the substrate on the bacterial community while the other two studies indicated otherwise. This could be because Wynants et al. (2018) analysed the bacterial community of the entire larvae while Jeon et al. (2011) and Bruno et al. (2018) only analysed the gut microbiota.

Even if the relationship between substrate and gut bacteria is still unclear, there are some studies that suggest that seeding a substrate with bacteria can have an effect on the weight and development of larvae. In 2011, Yu et al. used four different strains of *Bacillus* (three *B. subtilis* isolated from the larvae gut and one *B. natto* from a commercial substrate for BSFL) as additive to chicken manure when composting with BSFL. Only one bacteria was added to each treatment. The survival was not affected significantly but the larvae grew faster and larger when accompanied with any of the studied *Bacillus* spp. The weight increase with *Bacillus* spp. ranged from 9-22% better than the control, with *Bascillus subtilis* strain S15 giving the highest increase. A more recent study also added *B. subtilis* (BSF-CL) to chicken manure and got a weight increase of 15.9% (Xiao et al., 2018). The bioconversion increased to 11.5±0.2% with bacteria compared to 10.2±0.1% without, corresponding to an increase of 12.7% (Xiao et al., 2018). The reduction ration was increased with 13.3% but was not significantly ($p<0.05$) different from the control treatment. Xiao et al., (2018) also observed that seeding with bacteria caused a faster increase in substrate temperature and left less nutrients in the residue.

Similar results were obtained by Zheng et al. (2012) who studied how BSFL fat accumulation was impacted when a mixture of bacteria was added to the substrate (which was a mix of restaurant waste and rice straw). A mixture called Rid-X, which contains millions of non-specified bacteria and enzymes, was added. They found that both biomass and fat accumulation increased when seeding with bacteria. For instance, on a diet with 20% rice straw and 80% restaurant waste the total larvae biomass yield from 1000 g feed was increased with 17% and the fat proportion increased with 10% when 0.45% (wet weight basis, WW) of Rid-X was included. The hypothesis was that the Rid-X mixture would help in making the substrate more accessible to the larvae by enzymatic degradation. The authors did, indeed, see a significant increase in degradation for cellulose, hemicellulose, lignin and protein (not lipids) when 0.35% Rid-X (WW) was inoculated, compared to BSF composting without the Rid-X addition. The degradation (WW) of cellulose was nearly 66% with 0.35% Rid-X compared to nearly 28% without. The increase in hemicellulose degradation (WW) was approximately 73% while the increase in degradation for lignin and protein were 1% and 23% respectively.

The presence of bacteria has also been shown to influence oviposition rate in BSF (Zheng et al., 2013). Female BSF laid fewer eggs in environments with bacteria that were isolated from species that are competitors of the BSF and more eggs where BSF eggs had already been laid, especially if the eggs were not sterilized. If the environment contained a more complex bacterial community (i.e. were inoculated with several bacteria strains) the females preferred it to an environment with only single bacteria. However, Zheng et al., (2013) suggested that *Gordonia* spp seemed more important than the other bacteria. *Gordonia* spp was the only bacteria isolated from BSF eggs that gave a significant positive response on oviposition even without other bacteria present. Zheng et al. (2013) discussed that since *Gordonia* spp. are known for being able to degrade environmental toxins and polymers that could be favourable for the larvae, it may be a possible explanation for the increased oviposition rate in its presence. Furthermore, Zheng et al. (2013) noted that the concentration of a bacteria sometimes mattered. For instance, *Acinetobacter* spp. isolated from the lesser mealworm first impacted oviposition rate negatively at 10^8 cfu/ml but not at lower concentrations (10^4 or 10^6 cfu/ml).

It is also possible that bacteria might affect BSFL negatively. For instance, larvae of house flies have been seen to be sensitive to infections from *Brevibacillus laterosporus* and *B. thuringiensis israelensis* (Zimmer et al., 2013). However, in a survey done by Eilenberg et al. (2015) concerning awareness of insect diseases among commercial producers, none of the responding producers reported any known diseases for BSF. Eilenberg and colleagues could neither find any mention of typical diseases for BSF in literature when writing their review article *Diseases in insects produced for food and feed* in 2015. Furthermore, BSFL contain antibacterial substances, and the fly larvae composting process have seen to have negative effects on some gram-negative bacteria as well as some pathogens and viruses (as already mentioned in 2.2.1 and 2.2.2) (Choi et al. 2012; Lalander et al., 2015; Liu et al., 2008; Erickson et al., 2004). Observations by Vogel et al. (2018) even suggest that BSFL generate different antibacterial proteins depending on the substrate.

3 MATERIALS AND METHOD

3.1 MATERIALS

In all the treatments, homogenised household food waste from Eskilstuna municipality was used as substrate. It arrived before the start of the experiments and was frozen at -20°C in smaller bags until use. The bags were either thawed in a fridge over a weekend or in room temperature overnight (approximately 15 to 20 hours). BSFL for the experiments were acquired from the BSF colony maintained by the Environmental Engineering group at The Swedish University of Agricultural Sciences, Uppsala.

3.2 EXPERIMENTAL SET-UP

In this project there were two experimental phases (Figure 2). In phase I a wide range of groupings of bacteria were studied while phase II focused on the most interesting groups and bacteria from phase I. Therefore, only single replicates were done in phase I while triplicates were done in phase II.

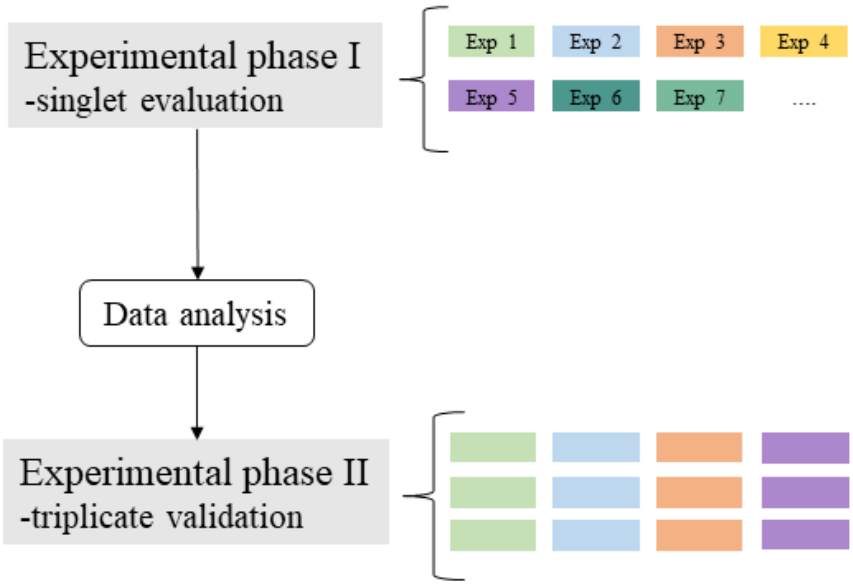

Figure 2. The experimental set-up. The study was done in two experimental phases, in which the first evaluated a wide range of bacterial groups conducted with single replicates in and the second phase validated the results by setting up triplicate experiments of the most interesting bacteria and bacteria groupings (determined by a data analysis).

3.3 EXPERIMENTAL EXECUTION

3.3.1 Phase I

In the first experimental phase, 56 treatments plus three controls were conducted (Table 8 in Appendix). Eight different bacteria were studied in groups of three and only single replicates were made (Table 1). Every possible combination was studied in order to try to understand if some bacteria behaved differently together with different companion bacteria. The treatments were named with a letter followed by the bacteria added, for instance A:123.

Table 1. List of some of the treatments done in phase I. The X marks which bacteria were included in which treatment.

Treatment	1	2	3	4	5	6	7	8
A:123	X	X	X					
B:124	X	X		X				
C:125	X	X			X			
D:126	X	X				X		
E:127	X	X					X	
F:128	X	X						X
G:134	X		X	X				
… etc.								

In phase I, the fly larvae composting was done in small lidded boxes with dimensions 13x16x10 cm (Figure 3a). The lids were used to prevent larvae from escaping or flies from laying eggs in the substrate and had a netted rectangle for air inflow. Before the start of a treatment, boxes and lids were cleaned with alcohol to remove pre-existing bacteria. During the treatment, four to six boxes were placed in a crate, which was placed in a metal rack inside a ventilation cabinet. A week into the experimental phase the racks were moved to another ventilation cabinet for logistical reasons. The treatments were done in temperatures around 25 to 28°C.

Figure 3. Pictures of the treatment set-up used in: a) phase I, with treatment box covered with netted lids, b) phase 2, where the treatment box was covered with a net, c) phase 2, in which a single treatment box was placed within a larger crate to catch larvae leaving the treatment.

3.3.2 Phase II

In phase II, the results from phase I were taken into account in order to decide which combinations of bacteria that should be further studied. Each treatment was done in triplicate, so a total of 27 treatments were done, including three controls (Table 2). Again the treatments were named with a letter followed by the numbers of the included bacteria. Note that lower case letters are different from capitals letters. D:126, S:167 and ÅÅ:678 were treatments that were used in phase I, while all treatments with lower-case names were new for phase II (Table 2).

Table 2. Complete list of treatments, with the different bacterial consortia evaluated in phase II. Each treatment was done in triplicate.

Treatment	1	2	3	4	5	6	7	8
a:16	X					X		
b:1	X							
c:6						X		
D:126	X	X				X		
e:7							X	
f:2		X						
S:167	X					X	X	
ÅÅ:678						X	X	X
K (control)								

The same treatment boxes as in phase I were used, but this time no lids were used but instead nets were placed over and secured with elastic bands (Figure 3b). This was done to increase aeration in order to make the residue drier and thus separation of larvae from the residue easier, while still preventing eggs from being laid into the boxes (by escaped BSF or fruit flies). Each small box was placed within a larger crate to collect, and later return, escaped larvae (Figure 3c). Nine treatments, one of each triplicate plus control, were started at the same time and were placed in the same metal rack in a treatment room (Figure 4). Three different stacks were used. To mitigate any effect of possible temperature difference in the stack, the order of the boxes were changed every fourth or third day. Within 24 hours after a feeding event escaped larvae were put back in the treatment box from the crate to which they had fled. The treatment boxes were also checked for BSF eggs several times during the course of the experiment, as eggs had been laid on boxes during phase I.

Figure 4. Picture of the stacked crates used in phase II. Placement of temperature and humidity sensors are indicated in the picture.

3.3.3 Fly larvae composting

The fly larvae composting was done over the course of two weeks (Figure 5). At the start of each treatment, approximately 1% (WW) per bacterial inoculate to substrate were added. Thereafter the substrate was mixed with the bacteria solution by adding a little bit of food waste in three steps to a total of 260 g and mixing thoroughly before adding more. Finally, 700 larvae were added to each box on top of the food waste.

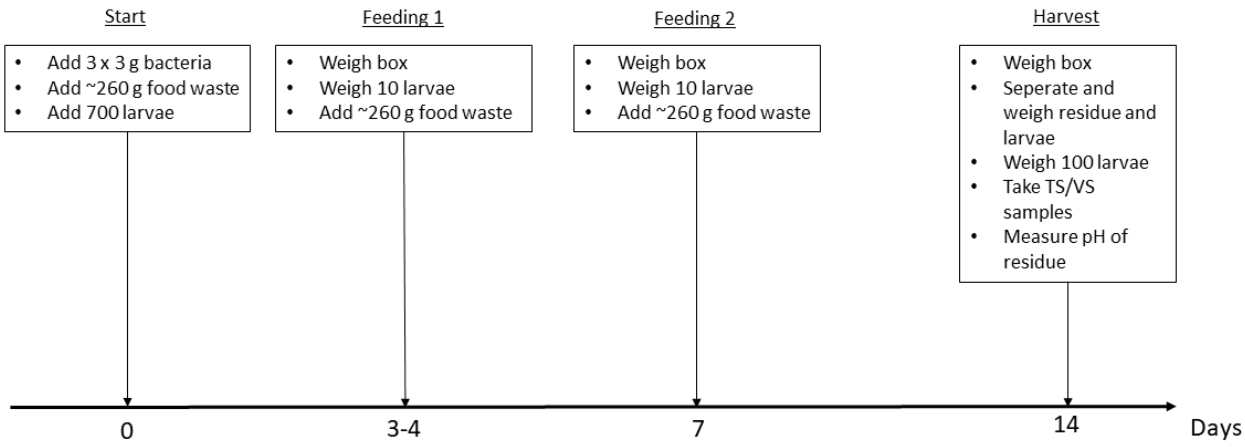

Figure 5. Schematic representation of the experimental time line. Each treatment was divided into a start, two feedings and a finishing harvest.

The larvae were fed two more times with 260 g each time. The new food waste was not mixed with the already existing material, but was instead placed in the middle of the box. The first feeding occurred after three to four days and the second feeding one week after the start (Figure 5). The new food waste was added on top of the old material without mixing. In total the larvae were fed approximately 780 g of food waste, based on a selected feeding rate of 0.2 VS/larvae and assuming a dry matter (TS) of 20% and 90% volatile solids (VS) of the food waste. The feeding was selected due to it being observed to be the optimal feeding rate (unpublished data). Before feeding, the box weight and the weight of 10 larvae were recorded. After two weeks, the boxes were harvested, which included separating and weighing the larvae and the residue (Figure 6). The larvae were separated from the residue with the help of a spoon and forceps (Figure 6). As much as possible of the residue was removed from the larvae. The total mass of larvae as well as the mass of 100 larvae was recorded. From the mass of 100 larvae the number of larvae in each treatment was estimated for calculation of the survival ratio. Thereafter the larvae were put in plastic bags and killed by being frozen at -20°C. The total mass of the residue was recorded and samples for pH and TS/VS was taken. After at least 24 h the larvae were thawed and samples for TS/VS were taken.

Figure 6. Picture of the harvest-setup. The treatment residue and larvae were separated from the box into the two smaller containers to the left.

The young larvae (mini-larvae) were fed chicken feed for about 6-8 days prior to being added to the treatments. The mini-larvae were separated by sieves, with the smallest mesh size of 2 mm, to get approximately the same size of mini-larvae. Three randomly picked sub-samples of mini-larvae were weighed and counted in order to estimate the weight of one larva and thereby approximate the total weight of the 700 mini-larvae added to each treatment.

3.3.4 Isolating and Growing Bacteria

Spore forming bacteria were isolated for the study from BSF eggs, which are lain in the form of "egg packages" containing several hundred eggs. The isolation and growing of bacteria was done by Björn Vinnerås from the Environmental Engineering group at SLU. First, 10 egg packages from BSF were washed in deionized water and then disinfection solution (70% EtOH). Spore forming bacteria were then isolated by first dispersing the egg packages in 10 mL unselective bacterial growth medium (National Veterinary Institute, Sweden) at 70°C for 10 min. After allowing the solution to cool to room temperature one mL was spread on taurocholate gelatine agar (TGA, Miclev, Sweden) plates. The plates were then incubated at 37°C for 24 h. By visual observation several different colonies were identified and clean spread on TGA plates and incubated for 24 h in 37°C. The single colonies were again put in unselective growth medium and heated to 70°C followed by clean spreading once the solution reached room temperature. After incubation in 37°C for 24 h on TGA the clean spread colonies were kept at 4°C until use.

The clean spread colonies (a total of 14) were sent for MALDI-TOF for species identification. MALDI-TOF uses mass spectrometry to get a peptide mass fingerprint, which may identify the bacteria species by comparing with fingerprints in different databases (Singhal et al., 2015). Several were found to be the same species and two did not grow properly, hence only eight were chosen for the evaluation in phase I. Four of them were identified as: *Bacillus subtilis*, *Corynebacterium* spp., *Bacillus lichniformis* and *Lycinibacillus fusiformis*, while the remaining four could not be identified (Table 3). For the experiments, one colony from each clean spread plate was added into 10 mL pre heated nonselective bacterial nutrient broth (National Veterinary Institute, Sweden) and incubated at 37°C for 24 h. Thereafter one ml of bacteria solution were incubated into 100 ml of pre-heated nonselective bacterial nutrient broth and incubated on a shaking table 100 rpm at 37 °C for 24 h. The grown bacteria solution were then used during one week, and kept at 4°C until use. To each box three ml of approximately 10^8 bacteria solution was added (three ml per bacteria added). Each bacteria species were given a code name, as not all were identified (Table 3).

Table 3. List of the code of each bacteria used in the project.

Code	Bacteria species
1	*Lycinibacillus fusiformis*
2	*Bacillus subtilis*
3	*Bacillus licheniformis*
4	N.I
5	N.I
6	N.I
7	N.I
8	*Corynebacterium* spp.

*N.I = Not identified in the MALDI TOF.

3.3.5 Sampling Procedure

The residue was mixed for a few seconds with a spoon to ensure an even sample before any samples for pH or TS/VS were taken. Roughly a tablespoon of residue was used for TS/VS determination. The larvae were thawed (from -20°C) before samples of approximately 5-8 g were taken for TS/VS. Because the food waste arrived in large buckets (approximately 30 kg), the food waste was mixed thoroughly for approximately 5 min before sampling and preparing feeding portions. After the initial stirring the food waste was stirred with a shovel for a few seconds approximately after preparing 10 feeding portions. Three samples from the top, middle and bottom of each bucket were taken for TS/VS and pH (i.e. a total of nine samples were taken per bucket). Only one sample was taken from the residue and larvae of each treatment.

3.3.6 Physiochemical Analysis

During phase I, only one temperature and humidity sensor was placed inside the ventilation cabinet, while three sensors on different levels in the stack were used in phase II (Figure 4). In phase II, the entire stack was removed from the treatment room for feeding and therefore the sensors were then moved to a different stack so that the sensors never left the treatment room.

The TS content of the substrate, residue and larvae was calculated by recording the weight of a sample before and after drying in 70°C for at least 48 h. The VS content was thereafter determined by combusting the samples at first 250°C for 2 h and then at 550°C for 5 h. The pH of the residue and food waste was measured by first mixing

residue or food waste with deionized water 1:10 and then leaving the solution at room temperature for 1 h.

3.3.7 Calculations

The survival ratio (SR) is the percentage of larvae that survived the treatment. It was calculated as:

$$SR = \frac{n_{L,end}}{n_{L,start}} \times 100 \quad (1)$$

where, $n_{L,end}$ is the number of alive larvae at the end of the treatment and $n_{L,start}$ is the number of larvae at the start of the treatment. Both $n_{L,end}$ and $n_{L,start}$ were estimated from the weight of a known number of larvae and thus SR was only considered to be a rough estimate of the survival.

The reduction ratio (RR) is the percentage of the substrate that has been digested. It is calculated as:

$$RR = \left(1 - \frac{m_r \times TS_r \times VS_r}{m_{fw} \times TS_{fw} \times VS_{fw}}\right) \times 100 \quad (2)$$

where, m_r is the WW of the residue [g], m_{fw} is the WW of the food waste [g], TS_r is the TS fraction of the residue and VS_r is its VS fraction, TS_{fw} is the TS fraction of the food waste and VS_{fw} is its VS fraction. Reduction ratio on a TS basis is obtained by setting VS to 1.

The bioconversion ratio (BCR) is the percentage of substrate that has become biomass. It is calculated by:

$$BCR = \frac{(m_{l,end} - m_{l,start}) \times TS_l \times VS_l}{m_{fw} \times TS_{fw} \times VS_{fw}} \times 100 \quad (3)$$

where $m_{l,end}$ is the WW of larvae at the end, $m_{l,start}$ is the WW of larvae at the start, m_{fw} is the WW of the food waste, TS_l and TS_{fw} are the respective TS fractions and VS_l

and VS_{fw} are the respective VS fractions. The bioconversion ratio on a WW, TS or VS basis is obtained in the same way as described for equation (2).

The respiration (RESP) and the bioconversion ratio together explain the reduction ratio. The respiration includes both the microbial degradation and larvae respiration. The respiration is thus calculated as:

$$RESP = RR - BCR \tag{4}$$

The total mean temperature for each treatment in phase II was calculated as:

$$T_{tot} = \sum_{i=1}^{9} T_i \times \frac{t_i}{t_{tot}} \tag{5}$$

where T_{tot} is the total mean temperature, T_i is the mean temperature in position i in the stack, t_i is the time in days that the treatment spent in position i and t_{tot} is the total treatment time (14 days in this study). The temperature was assumed to vary linearly from the top to the bottom of the stack.

3.3.8 Data Assessment and Statistical Analysis

Phase I Data

In order to determine whether or not any bacterial consortium performed better than others in terms of bioconversion ratio, reduction ratio or survival ratio, an exploratory data analysis was performed for phase I treatments. Since the larval biomass has a greater economic value than the residue (Lalander et al., 2018), the main focus was identifying bacteria that had a positive effect on bioconversion ratio. The analysis was divided into two steps. Limits for "high" and "low" bioconversion ratio and reduction ratio were first set. A low bioconversion ratio was set to <31.4% and a high to >36.8%, which corresponded to the control with the lowest and highest bioconversion ratio. A low reduction ratio was set to <50% while a high was set to >60%, based on the mean reduction ratio, which was 55%.

In the first step of the analysis the treatments were classified as according to the limits and then bacteria that often were included in treatments with high or low reduction ratio

or bioconversion ratio were identified. The bacteria that mainly were present in the group with high bioconversion ratio (called “upper limit group”) were selected for the next analysis step. In the second step common bacteria pairs in the upper or lower limit groups were identified. The occurrence of a bacteria pair in the two groups were calculated as the percent of the treatments in each group that contained that specific bacteria pair. This was done since the number of treatments in the upper and lower limit groups were different.

Phase II Data

The data from phase II was analysed by performing an ANOVA-test, in which the variance of bioconversion ratio, reduction ratio and survival ratio of the treatments were compared to identify any significant differences between the treatments. The confidence interval was set to 95%.

Data from both Phases

A regression analysis was also done for all treatments (from both experimental phases), in order to find which parameters that might explain differences in bioconversion ratio, reduction ratio and respiration between treatments. Both the significance (using the p-value) and the model strength (using the r^2 value) were used to determine the impact of a certain parameter on the response variables (bioconversion ratio, reduction ratio and respiration). The parameters that were used in the regression analysis were: temperature, VS/larva, VS content in food waste. Both regression models with one parameter and two parameters were evaluated. The relationship between the response variables was also investigated using regression models.

All data analysis and graphical representations were performed in R (R Core Team, 2017).

4 RESULTS

4.1 PHASE I RESULTS

All the values for bioconversion ratio and reduction ratio in the results are given on a TS-basis unless otherwise stated. The name of each treatment refers to the bacteria that are added, e.g. A:123 contains bacteria 1, 2 and 3 (Table 1).

4.1.1 Survival ratio, Bioconversion ratio and Reduction ratio

The mean survival ratio for all treatments in phase I was 91% but ranged from just over 60% for L:145 to around 100% for B:124 (Figure 7). Most of the treatments resulted in higher or similar survival ratio as compared to the controls (Figure 7).

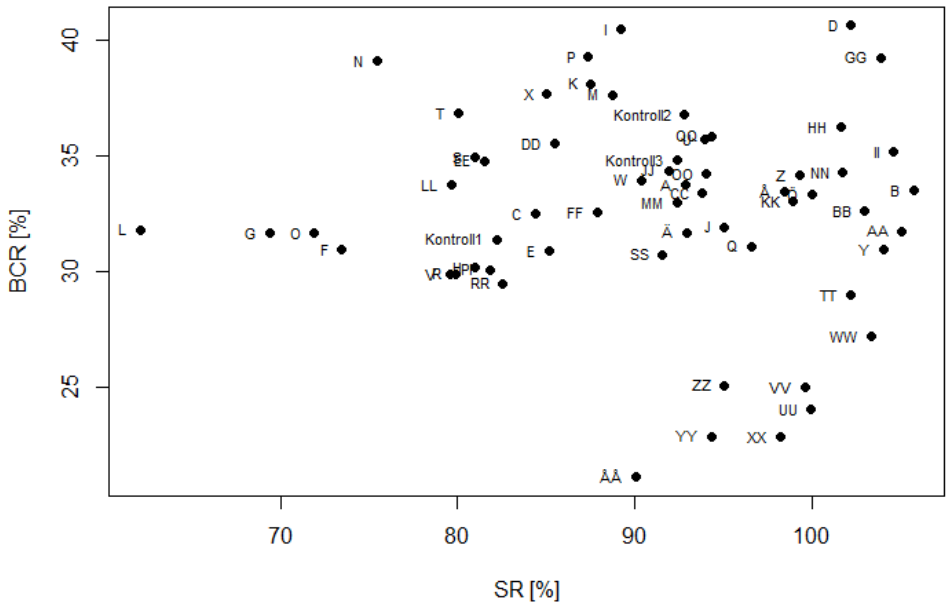

Figure 7. Scatterplot of the bioconversion ratio (BCR), on a TS-basis, against the survival ratio (SR). The controls are called “Kontroll1”, “Kontroll2” and “Kontroll3”.

The reduction ratio ranged from under 45% for F:128 to approximately 70% for W:235, while the bioconversion ratio ranged from 21% for ÅÅ:678 to 41% for D:126 (Figure 8). The mean reduction ratio and bioconversion ratio was 62% and 34% respectively, for the controls. Most treatments resulted in a reduction ratio similar or lower than the controls. In terms of bioconversion ratio, 18 of all the treatments resulted in a bioconversion ratio below 31.4% (lower limit), nine were above 36.8% (upper limit) while the majority resulted in a bioconversion ratio in between those limits (Figure 8). The treatments that generated both high bioconversion ratio and reduction ratio were D:126, I:136, GG:278, K:138 and X:236. The treatments that generated both low bioconversion ratio and reduction ratio were ÅÅ:678, XX:567, YY:568, UU:467, VV:468 and ZZ:578.

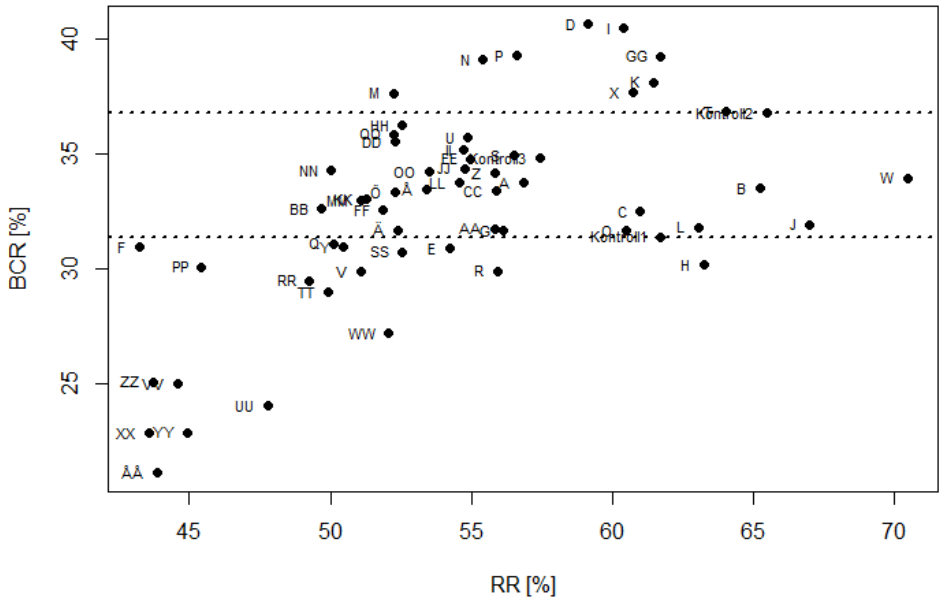

Figure 8. Scatterplot of bioconversion ratio (BCR) against reduction ratio (RR) (TS-basis). The highest dotted line is for the control with the highest bioconversion ratio (36.8%) and is defined as the upper limit for bioconversion ratio, the lowest line is for control with the lowest bioconversion ratio (31.4%) and is defined as the lower limit for bioconversion ratio. Treatments over the upper line are classified as having a "high" bioconversion ratio while treatments under the lower limit was classified as having a "low" bioconversion ratio.

4.1.2 Relationship Between Bacteria and Bioconversion ratio

Treatments containing bacteria 1, 2, 3 or 6 more often resulted in a high bioconversion ratio (over 36.8%) rather than a low bioconversion ratio (under 31.4%) while treatments containing any of the other bacteria mainly resulted in low bioconversion ratio (Figure 9). Bacteria 1 was present in 78% of treatments in the upper limit group, while 67% of the upper limit treatments contained bacteria 6. Bacteria 6 was also common in the treatments in the lower limit group (Figure 9).

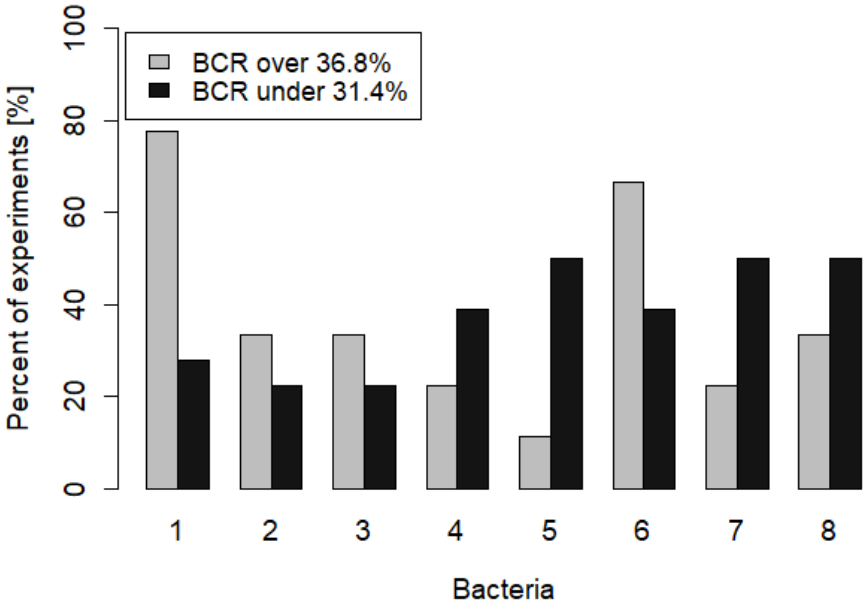

Figure 9. Bar plot of the prevalence of bacteria in treatments over the upper limit (grey) and under the lower limit (black) bioconversion ratio.

Nearly 60% of the treatments in the upper limit group for bioconversion ratio included both bacteria 1 and 6 (Figure 10a). The grouping bacteria 1 and 4 or bacteria 1 and 6 never resulted in a bioconversion ratio under the lower limit, while other groupings with bacteria 1 resulted either in a high or a low bioconversion ratio (Figure 10a). Unlike groupings with bacteria 1 or 6, some of the groups with bacteria 2 or 3 resulted in only low bioconversion ratio. Furthermore, groupings with bacteria 2 or 3 generally resulted in fewer extreme results (not prevalent in either the upper or lower limit group) than groupings with bacteria 1 or 6 (Figure 10bc compared to Figure 10ad).

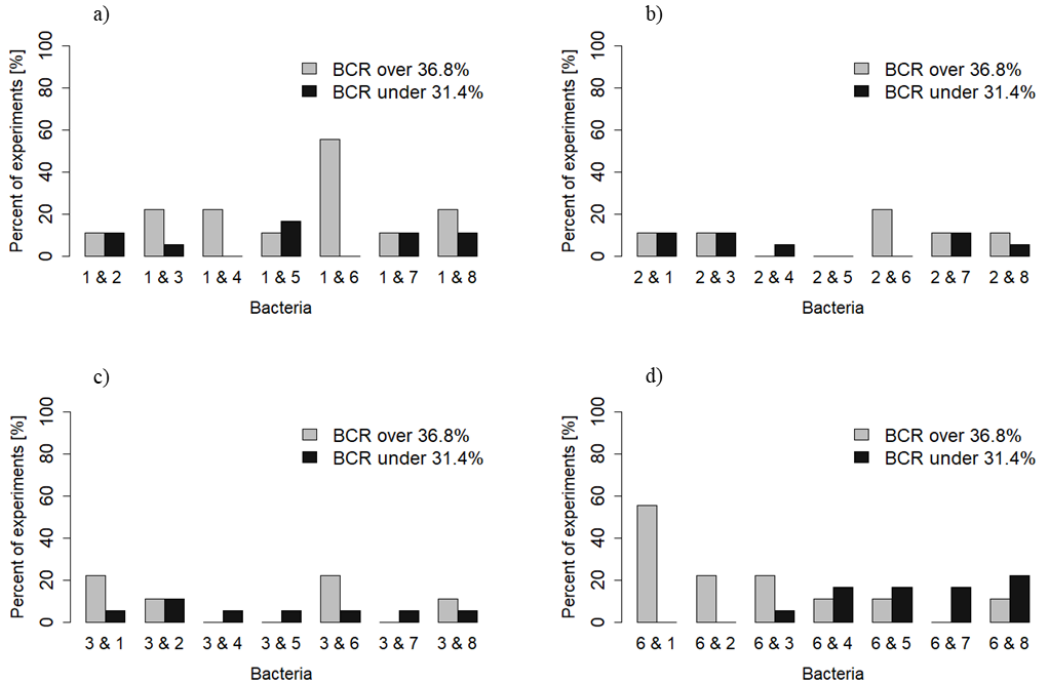

Figure 10. Bar plots of the prevalence of bacterial groupings of two in treatments over the upper limit (grey) and under the lower limit (black) bioconversion ratio (BCR) involving a) bacteria 1, b) bacteria 2, c) bacteria 3 and d) bacteria 6.

4.1.3 Relationship Between Bacteria and Reduction ratio

Treatments with bacteria 1, 2 or 3 more often resulted in high reduction ratio (over 60%) than low reduction ratio (under 50%), while treatments with the other studied bacteria generally resulted in low reduction ratio (Figure 11). Groupings including bacteria 1 more often resulted in a reduction ratio over the upper limit compared to groupings involving with bacteria 2 or 3. Treatments including bacteria 1 and 3 demonstrated the highest reduction ratio (Figure 19 in Appendix).

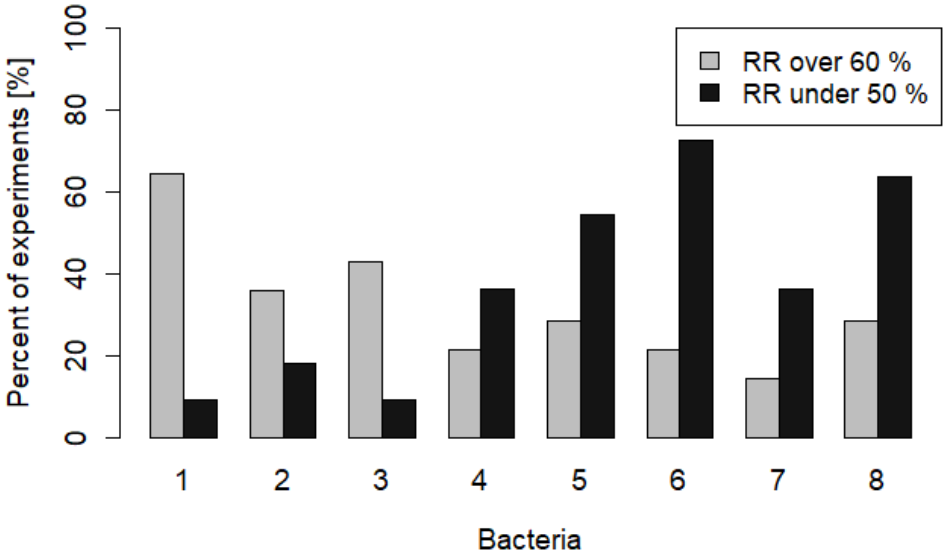

Figure 11. Bar plot of the prevalence of bacteria in treatments over the upper limit (grey) and under the lower limit (black) reduction ratio.

4.1.4 Physiochemical Parameters

The temperature varied during the experiment. The mean temperature was 25°C in the first ventilation cabinet and close to 29°C in the second cabinet (Table 4). The variation in temperature over the day was greater in the first cabinet (as high as ±5°C from the mean) than in the second cabinet. The mean relative humidity was approximately 51% throughout phase I (Table 4).

Table 4. The environmental conditions as well as substrate properties during phase I and II.

Variable	Phase I	Phase II
Temperature [°C]	Cabinet 1: 25.3±2.2	Top: 27.3±0.7
	Cabinet 2: 28.7±1.6	Middle: 26.2±0.6
		Bottom: 25.0±0.7
Humidity [%]	Cabinet 1: 50.9±8.8	Top: 37.9±6.5
	Cabinet 2: 50.9±8.5	Middle: 44.4±7.1
		Bottom: 45.9±7.4
Food waste		
- TS [%]	17.91±1.21	16.81±1.03
- VS [%]	90.81±5.17	84.6±1.28
- pH [-]	4.34±0.02	4.23±0.01

The pH of the food waste was 4.3 which was increased in all treatments after two weeks of fly larvae composting. The final pH differed between treatments from around 5 to over 8 (Figure 12). The moisture content of the treatment residue was roughly the same

as the ingoing food waste (about 82%). In some cases the moisture content had gone down after two weeks of fly larvae composting (73% at the lowest), but in most treatments the residue moisture content was roughly the same or higher compared to the ingoing food waste (Figure 12).

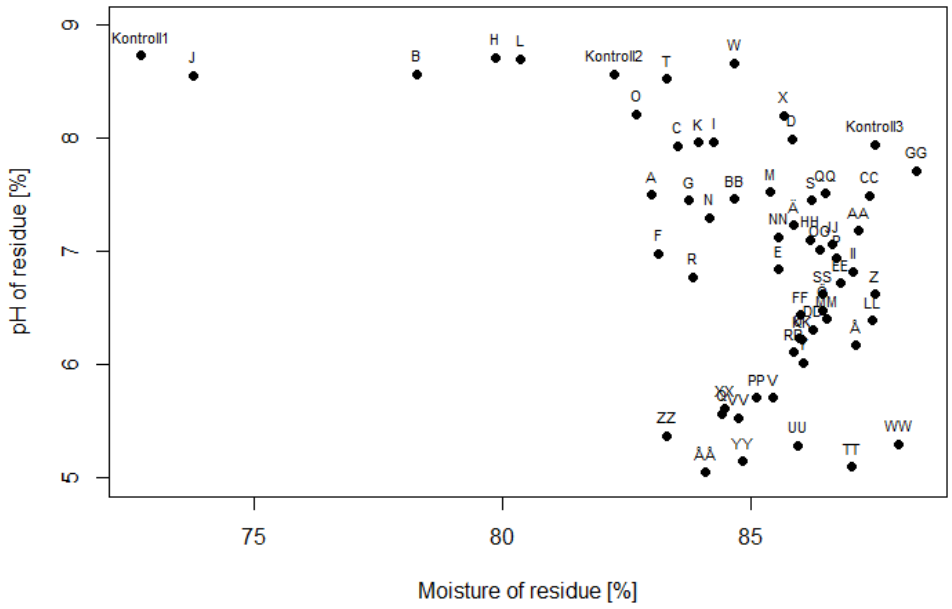

Figure 12. The pH of the residue (after harvest) plotted against the moisture content of the residue (after harvest) of the treatments in phase I.

4.2 PHASE II RESULTS

To differentiate between triplicates of the same treatment, a number (either 1, 2, or 3) will be added after the letters in the name, e.g. a1:16 is the first of the three replicates of a:16. The numbers following the colon are still the bacteria added in that treatment.

4.2.1 Survival ratio, Bioconversion ratio and Reduction ratio

The treatments in phase II resulted in a survival ratio ranging from just under 80% for ÅÅ1:678 to around 100% for a3:16 (Figure 13). The bioconversion ratio ranged from nearly 30% for control 1 to 36% for control 3 and the reduction ratio had an interval between 58% for control 1 to almost 70% for a1:16. All of the treatments thus resulted in a bioconversion ratio within the range of the controls, while most had a higher reduction ratio than the controls (Figure 13). The control had the lowest mean reduction ratio and the third lowest mean bioconversion ratio (a:16 and b:1 had lower mean bioconversion ratio mean than the control). However, none of the bacterial groupings were significantly different ($p<0.05$) from each other or the control in regards to the

three process efficiency response variables (bioconversion, reduction and survival ratio) (Figure 14).

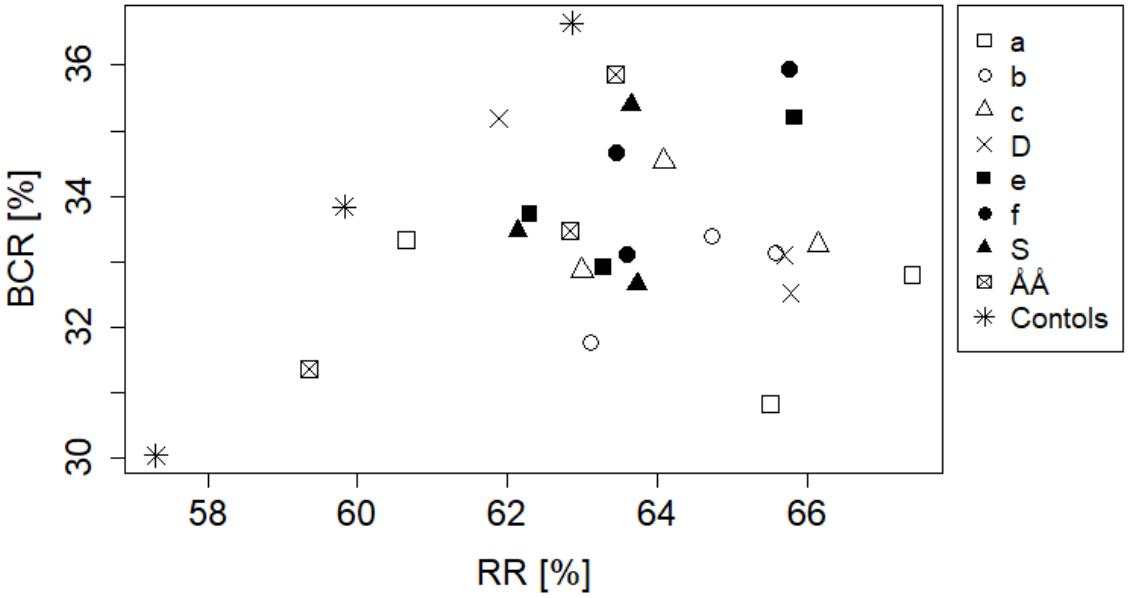

Figure 13. Scatterplot of the bioconversion ratio (BCR) against reduction ratio (RR) of the treatments in phase II (on a TS basis).

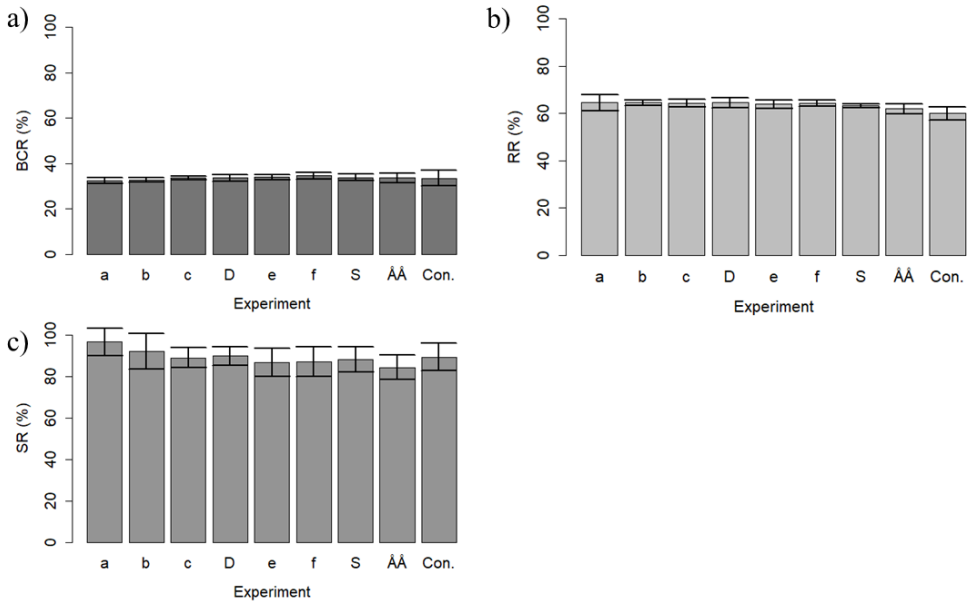

Figure 14. Bar plots of a) mean bioconversion ratio, b) mean reduction ratio and c) mean survival ratio of the treatments in phase II (on a TS-basis). Each bar is shown with an interval of ± the standard deviation.

4.2.2 Physiochemical Parameters

The moisture content of the food waste was roughly 84% in phase II, which after two weeks of fly larvae composting had been lowered for all treatments (Figure 15). The pH of the food waste increased from just over 4 to over 8 in the residues for all treatments in phase II (Table 4; Figure 15).

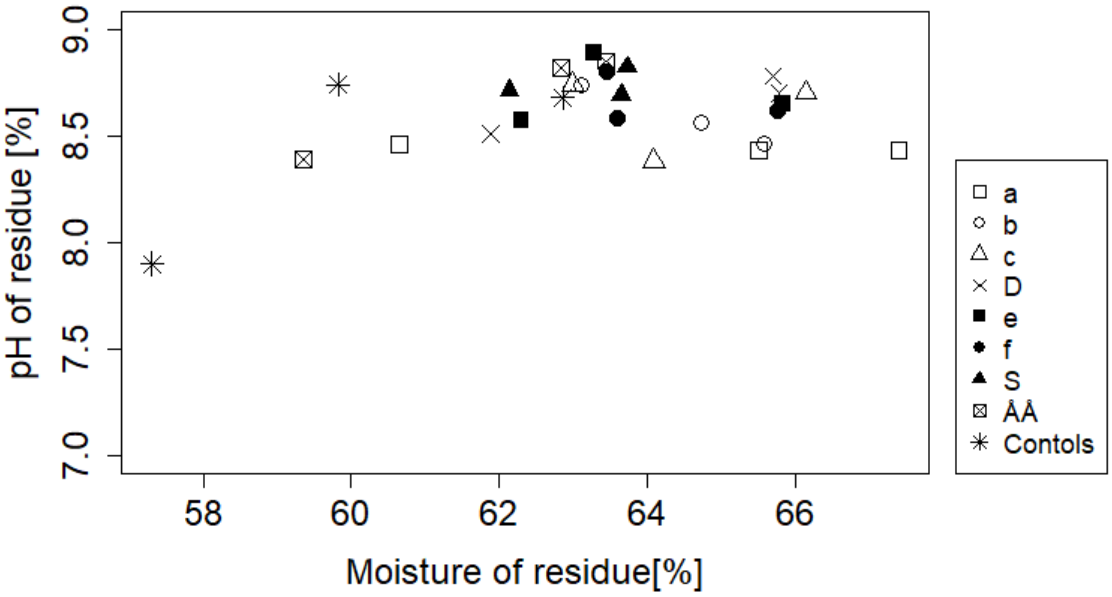

Figure 15. The pH of the residue (after harvest) plotted against the moisture content of the residue (after harvest) of the treatments in phase II.

The relative humidity (RH) was between 38% and 46% with higher RH in the bottom of the stack (Table 4). In phase II the mean temperature was 27°C at the top of the stack and then decreased to 25°C in the bottom of the stack (Table 4). The temperature at the different levels were, however, not significantly different ($p<0.05$, assumed normal distribution). The total mean room temperature over the two weeks differed slightly for the treatments (Table 5; equation (5). The highest total mean temperature was calculated for treatment a and the lowest for the control, but the difference was less than one degree (Table 5).

Table 5. The calculated total mean temperature of all treatments in phase II.

Treatment /Bacteria	Total mean temperature
a:16	26.6
b:1	26.4
c:6	26.1
D:126	26.5
e:7	26.2
f:2	26.0
S:167	26.2
ÅÅ:678	25.9
K (control)	25.7

5 DISCUSSION

5.1 PHASE I

The results of phase I indicated that seeding with bacteria could have an effect on both bioconversion ratio and reduction ratio, while the survival ratio did not appear to be affected to the same degree (Figure 7; Figure 8). This is in accordance with the results of Zheng et al. (2012), who saw an increase in weight of the larvae when fly larvae composting rice straw and restaurant waste with a mix of bacteria. Also Yu et al. (2011) demonstrated an increase in larval weight, while the survival ratio did not change significantly, when they added *B.subtilis* together with BSFL in chicken manure. However, the results of this study differed from Xiao et al. (2018) who observed an increase in both weight, bioconversion ratio and reduction ratio. In this study, the reduction ratio of the treatments containing bacteria was generally lower than the controls. However, the increase observed by Xiao et al. (2018) was not large enough to be significant ($p<0.05$).

While many of the bacteria groupings resulted in a bioconversion ratio within the control range, a few had a higher or lower bioconversion ratio than the controls. This suggested that while some bacteria combinations were neutral, others might compete with the larvae for the nutrients while others might actually improve the nutrient uptake for the larvae. After further data analysis, bacteria 1 and the grouping bacteria 1 and 6 were found to occur more frequently than other bacteria in the treatments in the upper limit group for bioconversion ratio (Figure 9; Figure 10). The only exception being treatment S:167. Hence, the hypothesis was that bacteria 1 and 6 would either together or alone be able to increase bioconversion ratio. To test this hypothesis, treatments with

the pair of bacteria (1 and 6) as well as with single bacteria were defined for phase II (Table 2). Treatment S:167 was also included to validate the results from phase I. Furthermore, the treatments with the highest (treatment D:126) and lowest (treatment ÅÅ:678) bioconversion ratio was also picked for phase II in order to validate the results from phase I. Since Treatment D:126 contained bacteria 1, 2 and 6, a treatment with only bacteria 2 was included to verify if it could affect bioconversion ratio on its own or not. Bacteria 2 occurred nearly as often in the lower limit group as in the upper limit group for bioconversion ratio and thus appeared to be "neutral". However Yu et al. (2011) and Xiao et al. (2018) used this species (*B. subtilis*) in their studies with chicken manure and found it to have positive effects on the weight of the larvae and the bioconversion ratio. Hence, bacteria 2 was also picked to see if the impact of *B. subtilis* is substrate dependant. The treatment with the lowest bioconversion ratio was ÅÅ:678, which was interesting since bacteria 6 often were included in the treatments with bioconversion ratio over the upper limit as well. As both bacteria 7 and 8 were included in treatments resulting in low bioconversion ratio (Figure 9), they were less interesting to analyse further since they seemed less likely to improve the BSFL-composting process. However, in order to validate the results in phase I bacteria 7 was also included in phase II to see if it alone could have a negative impact on the bioconversion ratio. Bacteria 7 was picked over bacteria 8 as it was also present in treatment S:167, which was the only treatment containing bacteria 1 and 6 that generated a bioconversion ratio in the control range rather than over the upper limit.

During phase I, the mean temperature changed from around 25°C to nearly 29°C as the stacks of treatments were moved to a different location. The temperatures were not significantly different ($p<0.05$) due to high variations over the day, but it is known that temperature affect BSFL-composting and it is thus possible that the differences affected the results. According to Tomberlin et al. (2009) BSFL grew larger at 27 °C compared to at 30°C, while the development time was shortened at 30°C. Additionally, Gligorescu et al. (2018) saw in increase in larvae weight at 27 °C as compared to at 20°C. Hence, the treatments done at 25°C might have resulted in smaller larvae than the ones done at 29°C. However, it could also be the other way around, if 29°C is too hot for the larvae. The treatments that were kept at 25°C at some point (none of them were done completely at this temperature) was A:123 to KK:348 (Table 8 in Appendix). In

general, treatments that partly were done at 25°C resulted in higher bioconversion ratio and reduction ratio than treatments done at 29°C (Figure 16). However, it is also possible that the differences in bioconversion ratio and reduction ratio are due to the different bacteria that were included. Especially since the bacteria groupings for the treatments were done systematically, thus starting the experimental phase with treatments always containing bacteria 1 and finishing with treatments that always contained bacteria 6 (see the order in Table 8 in Appendix).

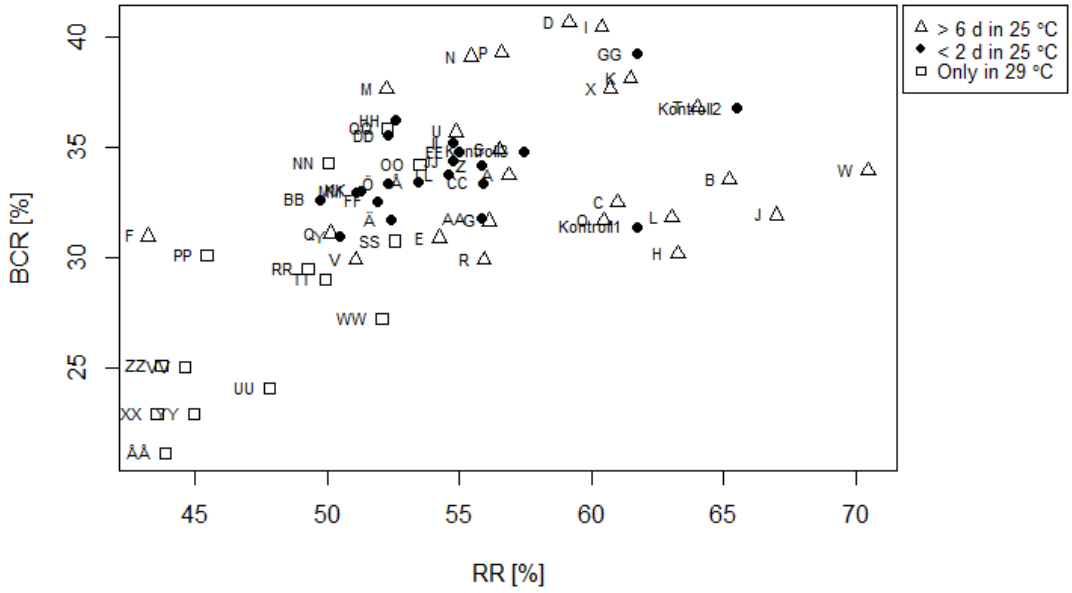

Figure 16. Plot of bioconversion ratio (BCR) against reduction ratio (RR) (TS basis) indicating temperature profile in the treatments in phase I.

5.2 PHASE II

5.2.1 Bacterial Impact on Bioconversion, Reduction and Survival ratio

No significant difference ($p<0.05$) in bioconversion ratio, reduction ratio or survival ratio were found between the treatments in phase II (Figure 14). Hence, the results indicate that seeding of food waste with the studied bacteria does not have an impact on any of the studied response variables. It is, however, also possible that the effect of the bacteria in this study is overshadowed by the effect of difference in temperature and ingoing substrate properties (e.g. VS content). Nonetheless, that would suggest that the effect of the studied bacteria is very small and thus not as interesting for process efficiency as, for instance, the temperature.

Hence, this study does not suggest a positive response in any response variable as previous studies have done (Xiao et al., 2018; Yu et al., 2011; Zheng et al., 2012). This could be due to several factors. One factor, that was not taken into account in this study but that could potentially impact the result, is the amount of bacteria added to the substrate. Yu et al. (2011) added 1% (wet weight, WW), Xiao et al., (2018) 0.1% (WW) while Zheng et al. (2012) added between 0.05 to 0.5% on a wet weight (WW) basis (with 0.35% giving the highest larval biomass and fat yield) (Table 6). In this study 1% (WW) of each bacteria was added to the substrate (Table 6). One very distinct difference between this study and previous ones, is the substrate used, since Yu et al. (2011) and Xiao et al., (2018) used chicken manure, Zheng et al. (2012) used a mix of rice straw and restaurant waste, while food waste was used in this study (Table 6). Chicken manure and rice straw contains more lignin and cellulose than food waste and is therefore less available for the larvae (Rehman et al., 2017; Zheng et al., 2012), which could be a reason behind why bacteria have an effect in such substrates. The bacteria mix (Rid-X) in the study by Zheng et al. (2012) was observed to increase the degradation of cellulose, hemicellulose, lignin as well as protein, so if the substrate is not rich in these molecules the advantage of adding bacteria might be lost. However, the positive impact of *B. subtilis* and the Rid-X mixture in the mentioned studies might be due to other reasons that have not been evaluated in this study.

Table 6. Summary of some of the important parameters for this study compared to previous studies that have added bacteria in fly larvae composting.

Study parameters	Xiao et al. (2018)	Yu et al. (2011)	Zheng et al. (2012)	This Study
Conc. of bacteria solution [cfu/ml]	10^9	10^8	N.S	10^8
Conc. of bacteria in substrate [%, WW]	0.1	1	0.05 to 0.5	1.1
Number of bacteria species added	1	1	N.S	1 to 3
Number of studied bacteria species	1	5	N.S	8
Substrate type	Chicken manure	Chicken manure	Rice straw & Restaurant waste	Food waste
Amount of treated substrate [kg]	1000	0.2	1	0.81

*N.S = not specified.

5.2.2 Bacterial Impact on Variation in Response variables

Even if no significant difference in any of the response variables was seen, the treatments with bacteria got a lower coefficient of variation in resulting bioconversion ratio (on a VS-basis) than the controls (Figure 17). The variation in bioconversion ratio was more than double for the control compared to most of the treatments with bacteria (Figure 17). The variation in reduction ratio (VS) was also lower in treatments with bacteria. Most bacterial treatments resulted in a variation more than half of the control, although the variation in treatment a:16 was almost as high as in the control (Figure 17). The only other parameter, besides bacteria, that differed between treatments in phase II was temperature, but the difference was less than one degree and not significant ($p<0.05$) (Table 5). This suggests that the bacteria potentially could stabilize the process, which in itself would be positive for large scale production even if the biomass or reduction of waste would not increase.

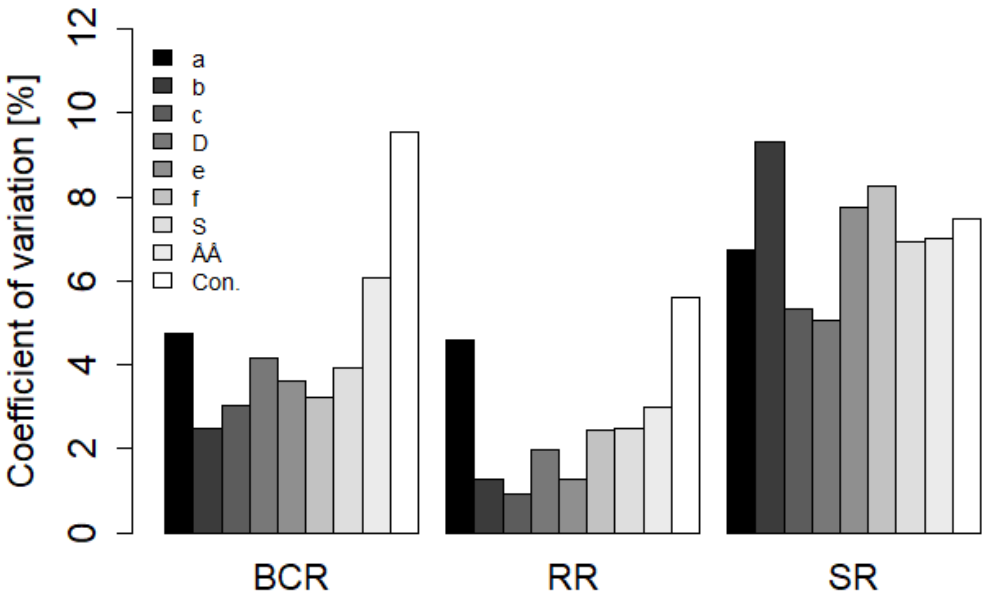

Figure 17. The coefficient of variation (standard deviation/mean) of each treatment in phase II in bioconversion ratio (BCR), reduction ratio (RR) (on a VS basis) and survival ratio (SR).

5.3 COMPARISON

Three of the studied treatments, using the same bacteria groups, plus controls were evaluated in both phase I and II. Interestingly, the same treatments gave slightly different results in phase I compared to phase II. In phase II the reduction ratio was significantly higher ($p<0.05$) compared to the results in phase I (with the exception of

the control) (Figure 18). The TS of the residue was also significantly higher in all four treatments in phase II as compared to in phase I (Figure 18). This is likely due to the increased air-flow over the treatment boxes in phase II when nets were used instead of lids.

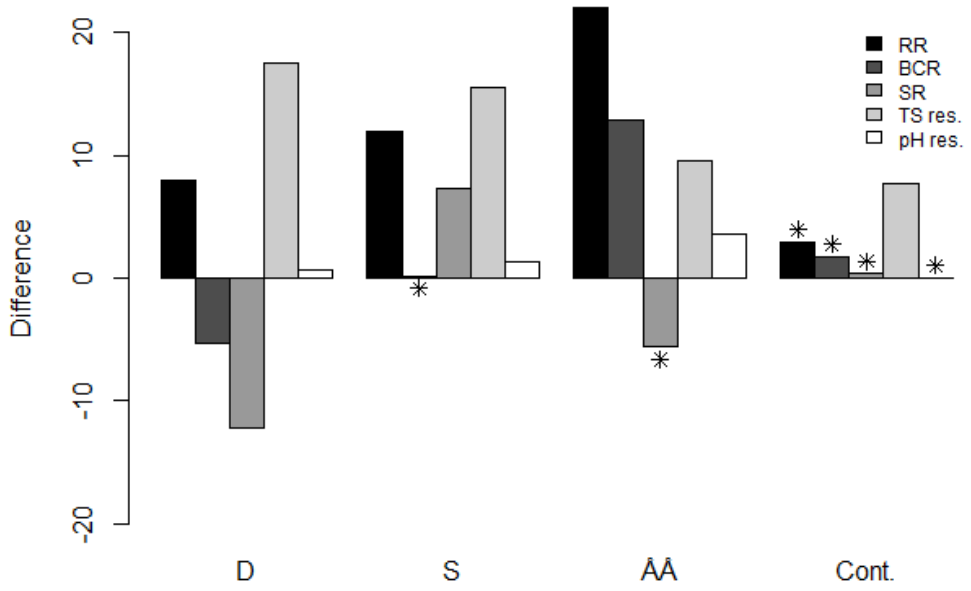

Figure 18. Comparison between results from phase I to phase II. The difference is calculated as phase II minus phase I results. Reduction ratio (RR) and bioconversion ratio (BCR) are on a VS-basis since the VS-content of the ingoing substrate differed in the two experimental phases. The stars indicate that the difference is not significant.

The difference in reduction ratio can partly be explained by the difference in temperature. When taking all the treatments from both phases into account, the difference in surrounding temperature was found to explain roughly 60% of the variation (Table 7). In general lower temperatures (around 25°C) resulted in higher reduction ratio than higher temperatures (round 29°C). Nearly 68% of the variation in reduction ratio could be explained by the effect of temperature and amount of VS in ingoing substrate combined (Table 7; Figure 20 in Appendix). However, only 15% of the variation in bioconversion ratio could be explained by temperature differences, while the VS content did not affect it significantly (Table 7). Yet, there is a relationship between bioconversion ratio and reduction ratio and thus there can be an indirect effect of the VS content on bioconversion ratio (Table 7). The difference in VS content of the food waste was not large; it was nearly 91% in phase I while it was 85% in phase II (Table 4). It may be possible that the impact of the VS content becomes more prominent

when the larvae are not provided with sufficient amounts to support their development. Furthermore, it has been suggested that the availability of carbon and nitrogen influence the bioconversion ratio rather than the sheer amount of organic matter (Lalander et al. 2019). The temperature also explained 52% of the variation in respiration (equation (**4**) and VS content of the substrate together with the temperature could explain 61% of the variation in respiration (Table 7). The respiration includes both larval and bacterial respiration but since the respiration does not have a significant ($p<0.05$) relationship with bioconversion ratio, it is likely that in this case the respiration is mainly bacterial respiration.

Table 7. Model strengths and significance of regression models with temperature, VS/larvae and VS in the FW (food waste). With one variable the model is in the form of $y = k \times x$ and with two variables it is $y= k \times x + m\times x$. The relationship between the response variables is also included. Included response variables are bioconversion ration (BCR), reduction ratio (RR) and respiration (RESP).

	Model strength (R^2) for response variables (y)		
Process parameter (x)	BCR (VS)	RR (VS)	RESP (VS)
Temperature	0.15***	0.61***	0.52***
VS/ larvae	0.02	0.06*	0.05*
VS in FW	0.09**	0.55***	0.52***
Temperature and VS in FW	0.16***	0.68***	0.61***
Temperature and VS/larvae	0.17***	0.66***	0.56***
Response variables			
BCR		0.33***	0.2
RR	0.33***		0.80***
RESP	0.2	0.80***	

Significance levels of model coefficients: $p < 0.001$ = ***, $p < 0.01$ = **, $p < 0.05$ = *

5.4 FUTURE STUDIES

Even though the bioconversion ratio, reduction ratio or survival ratio did not differ significantly when seeding food waste with bacteria, past studies with chicken manure and rice straw and restaurant waste has seen effects of adding bacteria, which suggest that the impact might be substrate dependent (Yu et al., 2011; Xiao et al., 2018; Zheng et al. 2012). Hence, future studies could investigate this further. For instance, there might have been a larger effect and importance of seeding with bacteria if the substrate had considerable lower bacteria concentrations to begin with, which is the case in the EU since the substrate needs to be pasteurised according to Regulation (EC) 1069/2009.

Adding bacteria that have been isolated from the larvae or eggs could then possibly be beneficial as previous studies with house flies and stable flies have seen that some bacteria were necessary for the larvae to develop (Watson et al., 1993; Lysyk et al. 1999).

Finally, it is quite interesting that the results of this study indicate that the variation in bioconversion ratio and reduction ratio is reduced when bacteria are added. It would be interesting to see if this is a general trend for several substrates and if the effect is dependent on when and at what concentration bacteria is inoculated.

6 CONCLUSIONS

The results in phase I suggested that bacteria could affect the reduction ratio as well as the bioconversion ratio of BSFL-composting of food waste. However, after verification with triplicates it was concluded that no significant difference compared to the control could be seen in either bioconversion ratio, reduction ratio or survival ratio. The difference in surrounding temperature between treatments was found to significantly affect mainly reduction ratio, but also the bioconversion ratio. Thus, controlling the temperature was more important for the process.

The variation in bioconversion ratio and reduction ratio was lower in treatments with bacteria, which indicate that the studied bacteria might be able to provide stability to the process. The variation in bioconversion ratio was roughly halved when any of the bacteria or bacteria groupings in phase II were inoculated in the food waste.

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8 APPENDIX

Table 8 Complete list of treatments in phase I and which bacteria were included. The list is in the order the treatments were performed. With eight to nine being started on the same day.

Treatment	Added bacteria	Added bacteria	Added bacteria
A	1	2	3
B	1	2	4
C	1	2	5
J	1	3	7
E	1	2	7
F	1	2	8
G	1	3	4
H	1	3	5
Kontroll1	0	0	0
K	1	3	8
L	1	4	5
N	1	4	7
O	1	4	8
Q	1	5	7
R	1	5	8
U	1	7	8
V	2	3	4
D	1	2	6
I	1	3	6
M	1	4	6
P	1	5	6
S	1	6	7
T	1	6	8
W	2	3	5
X	2	3	6
Kontroll2	0	0	0
Y	2	3	7
Z	2	3	8
Å	2	4	5
Ä	2	4	6
Ö	2	4	7
AA	2	4	8
BB	2	5	6
CC	2	5	7
DD	2	5	8
EE	2	6	7
FF	2	6	8
GG	2	7	8
HH	3	4	5
II	3	4	6
JJ	3	4	7
KK	3	4	8
LL	3	5	6

MM	3	5	7
NN	3	5	8
OO	3	6	7
PP	3	6	8
QQ	3	7	8
RR	4	5	6
SS	4	5	7
Kontroll3	0	0	0
TT	4	5	8
UU	4	6	7
VV	4	6	8
WW	4	7	8
XX	5	6	7
YY	5	6	8
ZZ	5	7	8
ÅÅ	6	7	8

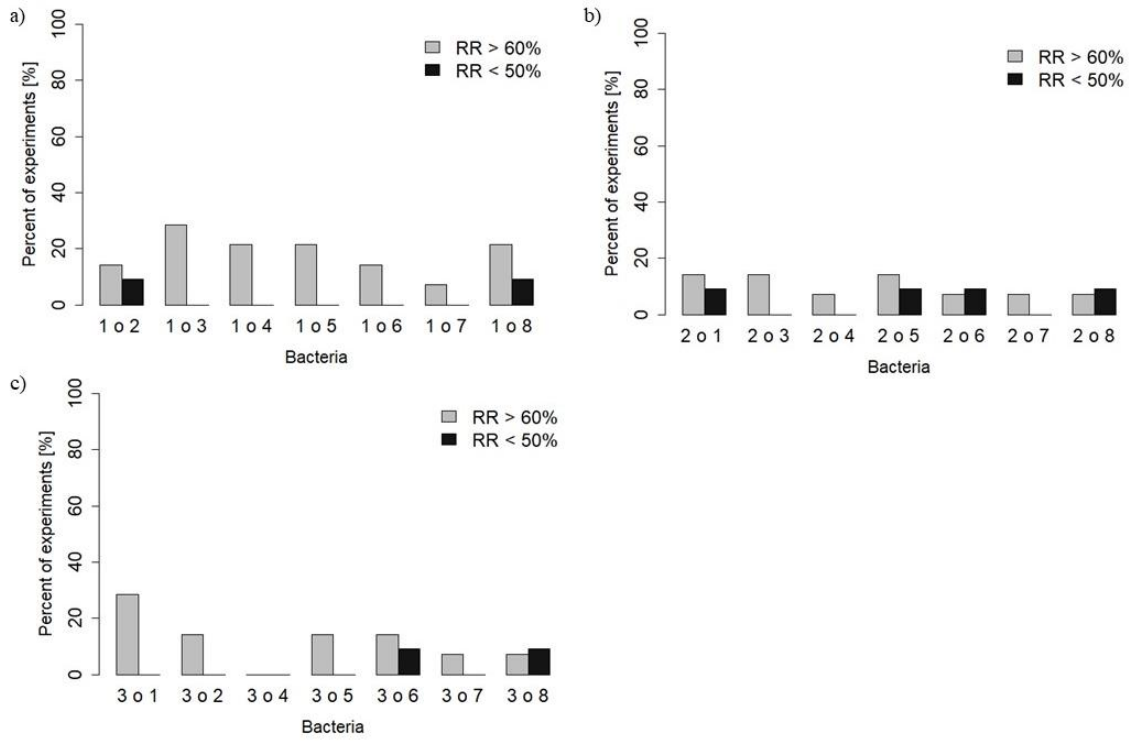

Figure 19 Bar plots of the prevalence of bacterial pairs in treatments over the upper limit (grey) and under the lower limit (black) reduction ratio (RR) involving a) bacteria 1, b) bacteria 2 and c) bacteria 3.

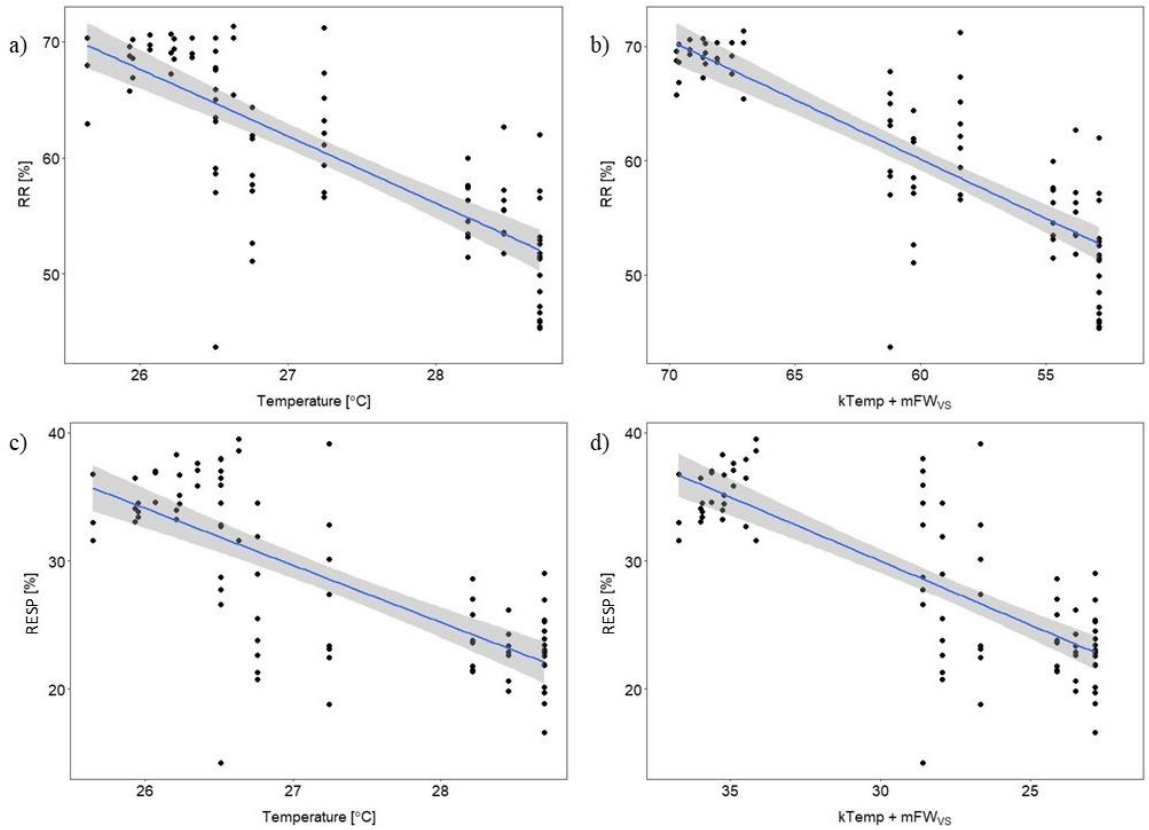

Figure 20. Plots of the measured values in both phase I and II against the modelled values for reduction ratio (RR) and respiration (RESP), on VS basis (%). a) reduction ratio against temperature (°C), b) reduction ratio against temperature and the VS content of the food waste, FW_{VS}, (%). c) and d) are the equivalent plots for respiration. The shaded area is the 95% confidence interval of the regression line (blue).