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Fly larvae composting of fibrous food industry waste - Impact of pre-treatment

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Abstract

With increasing population worldwide comes higher demands of food and greater waste generation. Effective ways for safe nutrient recycling and waste treatment are thus needed to minimize the human impact on the environment. Utilizing techniques in which waste is turned into valuable products could be a driving force for making biodegradable waste treatment more applicable and attractive on a commercial scale. For biodegradable waste, one method is to use insect larvae that feed and grow on the biodegradable material. The larvae can subsequently be used as feedstuff in animal feed. An additional product generated in the process is the treatment residue, a compost-like material that can be used as soil enhancement. The black soldier fly (BSF, *Hermetia illucens* (L.) Diptera: Stratiomyidae) larvae (BSFL) can be reared on biodegradable waste, however nutrient and physical composition of the biodegradable waste affects the larvae growth. Peels from fruit, from the fruit industry, are not optimal for fly larvae treatment in terms of nutritional composition and thus methods to alter the biodegradable waste before BSFL treatment of this particular substrate is of interest. Pre-treatment of the peels is a possible method to increase BSFL composting efficiency by altering the biochemical composition and/or structure. To address this, two pre-treatment methods were used in this study, *Rhizopus oligosporus* and ammonia. In this study, the impact of pre-treatment on black soldier fly larvae treatment efficiency were evaluated based on biomass conversion of banana and orange peel into larval biomass. In the orange peel study, two larval densities (1.9 and 4.0 larvae cm^{-2}) were evaluated. The impact of pre-treatment on amino acid content and fiber content were evaluated in the banana peel study. For the banana peel study, the Biomass conversion rate (BCR) decreased in both pre-treatments. The untreated peels (*Control*) had the highest BCR with a mean 7.1±0.6 % on a total solids (TS) basis. The protein conversion rate was highest in the Control at 49.1±7.1 % and there was no significant difference in concentration of the essential amino acids lysine and methionine in the larvae in terms of pre-treatment method. The fiber components in the banana peels were to some extent degraded by the pre-treatments, but there was no significant difference in fiber decomposition by the BSFL in terms of pre-treatment method, as fiber decomposition were only observed in the Control. However, the Control was regarded as inaccurate so fiber degradation by BSFL could not be verified for any setup. For the orange peel trial, the *Control* had the highest BCR, 8.5±0.8 % on TS basis, in the 1.9 larvae cm^{-2} density setup. For the 4.0 larvae cm^{-2} density setup, the ammonia pre-treatment had the highest BCR with 9.2±0.3 % on TS basis. *Rhizopus oligosporus* pre-treatment generated poor results regarding BCR in the orange peel study (0.9±0.1 & 1.3±0.4 % TS) contrary to the banana peel study (6.4±0.2 % TS). Higher larval density increased BSFL composting efficiency for all treatments. Further studies could explore the impact of ammonia pre-treatment further, larval density and other possible pre-treatments.

Populärvetenskaplig sammanfattning

Fluglarver som ersätter sojafoder, med kompostering på köpet

Att kompostera dina matrester från köket har du säkert hört har fördelaktiga effekter på miljön. Kanske har du också hört att det går att utvinna biogas av detta för att driva bilar och att värma upp hushåll? Att få ut ett proteinrikt djurfoder av det komposterbara avfallet kanske inte låter lika bekant? Fluglarvkompostering kallas processen där fluglarver äter organiskt material, det vill säga det du slänger i komposten (gammalt bröd, äggskal, fruktskal, köttrester och dylikt), för att växa till sig och sedan användas i djurfoder till grisar, höns och fiskar. Idén bakom detta är att försöka få ut en värdefull produkt, i detta fall proteinrika larver som djurfoder, ur det komposterbara avfallet som i många avseenden ses som bara ”skräp”. I dagens djurproduktion är sojabaserat proteinfoder mycket vanligt. Dessa är generellt inte hållbara då en betydande andel odlas i regnskogsskövlade områden, vilket påverkar den biologiska mångfalden negativt, samt att dessa måste transporteras världen över. Så att kunna ersätta denna proteinrika soja med fluglarver istället är ett *win-win* scenario med fluglarvskompostering och ett bättre alternativ för miljön. Forskning om fluglarvskompostering är ett aktivt ämne och pilotanläggningar är redan i bruk. Det som saktar ner utvecklingen för denna typ av behandlingsteknik är lagar om livsmedelssäkerhet. I och med att larverna äter organiska avfallsrester är det viktigt att dessa inte är kontaminerade, eftersom detta annars skulle kunna föra vidare biologiska föroreningar i näringskedjan såsom sjukdomsalstrande mikroorganismer och prioner, reaktiva proteiner som orsakar bl.a. galna kosjukan.

Syftet med denna studie var att testa bananskal och apelsinskal som substrat till fluglarverna. Fruktodlingar är vanligt förekommande i låg- och medelinkomstländer runt om i världen. Dessa avfallsfraktioner anses inte ha något värde och hamnar ofta på deponi eller dumpas där det finns plats. Fruktskal innehåller mycket kolhydrater, dock inte speciellt mycket socker, vilket gör att skalen i dess rena form inte är optimal mat till larverna. En fördel med skalen som föda åt larverna är däremot att de inte har blandats med andra, smutsigare avfallsfraktioner.

För att göra näringsämnena i skalen mer tillgängliga för larverna, så att larverna växer sig större, utvärderades två olika metoder. En metod var att tillsätta ammoniak till skalen. Ammoniak är ett ämne som innehåller kväve, som är viktig i uppbyggandet av proteiner som är byggstenar i cellerna på alla levande organismer. Den andra metoden var att förbehandla skalen med svampen *Rhizopus oligosporpus*, för att bryta ner kolhydraterna i skalen till socker som i så fall skulle vara mer tillgänglig för larverna. Dessutom utvärderades två olika larvdensiteter. Detta test visade om det var möjligt att få större totalvikt larver per mängd fruktskal om mer skal gavs per larv eller om mer larver applicerades per skal. Larvkomposteringsbehandlingen pågick i tre

veckor och analys gjordes därefter för att ta reda på proteinmängder, kolhydratmängder och den totala vikten larver som uppnåtts.

Resultatet visade att de förbehandlingsmetoder som användes, ammoniak och svampen, inte gav någon förbättrad effekt på fluglarvskomposteringen. Dock gav en högre larvdensitet, fler larver per mängd fruktskal, en högre bioomvandlingsfaktor. Det vill säga man fick ut en högre totalvikt larver per vikt fruktskal man gav larverna med en högre larvdensitet. Resultaten gav inte heller något stöd för att larverna brutit ner kolhydraterna i skalen eller att proteinmängderna i larverna från de olika behandlingarna skilde sig. Mängden av en del aminosyror skilde sig åt i larverna beroende på vilken förbehandlingsmetod skalen fått. Dock var det ingen skillnad på mängden av aminosyrorna lysin och metionin i larverna. Lysin och metionin var av intresse då dessa aminosyror är essentiella för rovfisk, hönsdjur och gris. Studien fokuserade även på att undersöka om larverna brutit ner kolhydraterna i olika molekyler. Kolhydraterna cellulosa, hemicellulosa och lignin fanns i skalen som gavs till larverna. Resultatet visade att mängderna kvar i behandlingsresten efter avslutad behandling ej gav bevis för att den toala mängden av dessa minskat. Det vill säga resultatet kunde inte säkerställa att någon nedbrytning av dessa kolhydrater skett.

Potentialen för fluglarvkompostering är stor, dock visade denna studie att en förbehandling med ammoniak eller *Rhizopus oligosporus* inte har en önskvärd effektivisering av fluglarvskomposteringen och därav inte är ett alternativ som verkar livskraftigt i stor skala. Alternativa lösningar som exempelvis samkompostering bör utvärderas vidare för att öka effektiviteten. Omställningen till mera hållbart producerade fodertyper för djur är ett aktivt ämne och fluglarver används redan idag och kommer med all sannolikhet vara en del av den förändringsprocessen i större omfattning i framtiden.

Contents

Abbreviations

BCR	Biomass conversion rate
BSF	Black soldier fly
BSFL	Black soldier fly larvae
PrCR	Protein conversion ratio
Red	Material reduction
SR	Survival rate
TS	Total solids
VS	Volatile solids

1. Introduction

Population worldwide is increasing and estimated to reach 9.8 billion by 2050 (United Nations, 2017). Global Annual waste generation is expected to increase by 70 % from 2016 levels to 3.40 billion tonnes in 2050 (World Bank, 2019). Population growth and urbanization, increased consumption by the population, coupled with urbanization and industrialization, leads to waste-related environmental problems (Ngoc & Schnitzer, 2009). In addition, unclear regulations and division of responsibility on the government side, contribute to the inadequate or lacking waste management services in low-income countries (Ngoc & Schnitzer, 2009). In Kampala, as an example, Komakech et al (2014) demonstrated that almost 90 % of the municipal solid waste reaching landfills was biodegradable. For efficient and economically feasible waste management service to be provided to a greater number of people, it has been suggested the private sector could have beneficial effects on the waste treatment field (Lohri et al., 2014). A majority of the waste treatment in low- to middle-income countries are handled by the informal sector (Linzner & Lange 2013). However, in some cases, waste may not be handled by the informal sector, due to differing motivations and lack of agreement among stakeholders (Linzner & Lange 2013). As the cost of the treatment is higher than the revenue from the products, biodegradable waste treatment is generally not considered economically viable (Hogg et al., 2003; Lohri et al., 2017).

As global population increases and available arable land decreases, more efficient methods of animal feeding continues to gain interest. FAO forecasts a further reduction of 19 % of available arable land (hectare per capita) by 2020 compared to 2000 (FAO, 2006). Soybean (*Glycine max* (L.) Merr.) is a high-quality annual forage legume (Hintz et al., 1992) and is also the world's most important source of a high-quality vegetable protein (Pottorff, 2007). The soybean is one of the largest sources in the world for production of edible oils and animal protein feed (Sugiyama et al., 2015). Europe is a major importer of soybean produced in South America. Between 1986 and 2008, 44 % of the total soybean production in Brazil and 45 % in Argentina was exported to the EU countries (Boerema et al., 2016). However, the soy production today is in many cases associated with deforestation and generates three to four-fold higher carbon dioxide emissions than regional cereals due to long transports oversea to Europe (Garcia-Launay et al., 2014). It is thus of interest to find more environmentally friendly alternatives. One potential use of agricultural waste is to produce additional food resources such as insects as a feed supplement (Van Huis, 2013). The use of flies to recover nutrients, and especially fat, from organic waste was mentioned for the first time over a century ago (Linder, 1919). The black soldier fly, (BSF, *Hermetia illucens* (L.) Diptera: Stratiomyidae), is a fly who's larvae (BSFL) have been reported to consume and degrade various biodegradable wastes (Wu et al., 2015; Rehman., et al 2017; Lalander et al., 2015) with material degradations up to 70 % (Diener et al. 2011a). Moreover, the larvae can rapidly and efficiently convert high amounts of low-quality organic waste into high-value insect biomass that is rich in protein and fat, thus providing an alternative source for feed (Khusro et al., 2012), human food (Wang and Shelomi, 2017) and biodiesel production (Feng et al., 2018). Challenges arise regarding economic

feasibility, as in some cases, the possible products created from the waste are less worth than the economic inputs needed to produce them (Hoornweg and Bhada-Tata, 2012). It is thus of interest for BSFL composting to produce as much larval biomass possible that is conceivable for the substrates available. Concerns about substances in animal by-products, such as prions, that could potentially be accumulated in BSFL restricts the implementation of this technology. According to Regulation (EC) No 1774/2002, biodegradable waste containing animal by-products cannot be used as a substrate for the larvae in the European Union. This restriction promotes research on vegetable substrates in BSFL composting.

Banana, from the family *Musaceae* and genus *Musa*, is a crop grown in 120 countries throughout the world (Byarugaba-Bazirake 2008). Tanzania is a low-income country that contribute a large share of the production of banana in Africa, while also generating around 60 tonnes ha^{-1} of banana-related biodegradable waste per production year (Tock 2010; Emmanuel 2014). Challenges follow to handle these amounts of biodegradable waste in an economically and environmentally viable way. Single source biodegradable waste, such as homogenous peels from the fruit industry, compared to co-composting of different substrates, are less likely to carry and spread disease because potential vectors that are associated with mixed biodegradable waste are less likely to be present. However, an imbalanced amount of dietary constituents in the substrate, such as proteins, fibers and fat, makes the BSFL grow at a slower rate and obtain a lower weight compared to food waste and fecal sludge (Nyakeri et al., 2017). As banana peels contain large amounts of dietary fiber (Anjum et al., 2014), it is not favourable in that context. Homogenous substrates with high levels of lignin and hemicellulose, such as banana peels, are particularly poor for BSFL composting (Nyakeri et al., 2017; Kumar et al., 2018). A few studies have been conducted on methods to improve the biodegradability/digestibility of substrates in order to increase efficiency and biomass conversion rate by the BSFL (Yu et al. 2011; Li et al. 2015a; Rehman et al. 2017). The ability of the BSFL to degrade fiber are inconclusive (Li et al., 2015b; Rehman et al. 2017). One suggested method involves addition of microorganisms to the substrate, to improve the degradation of the substrate before the BSFL treatment, with the aim of improving conversion rates (Yu et al. 2011). The low content of protein and amino acids in certain plant substrates is also a limiting factor for larvae development. A study on rumen bacteria by Wang and Tan (2013) showed that ammonia assimilation makes microorganisms convert nitrogen sources into more easily available substances, such as amino acids, for other mammals. Adapting a pre-treatment to either have microorganisms initiate the degradation of the substrate prior to BSFL composting or adding a non-protein nitrogen source to enhance protein production in the larvae are thus of interest. Using banana peels as a substrate for BSFL composting was evaluated by Isibika et al (2019). When banana peels were used as a substrate, all pre-treatment methods tested except heating resulted in higher BSFL conversion and final larval weight. In that study, *Rhizopus oligosporus* pre-treatment and *Rhizopus oligosporus* + ammonia pre-treatment resulted in the most efficient BSFL treatment, in terms of protein produced kg^{-1} material used. That study did not investigate the fiber degradation potential of the treatments and could not verify whether the BSFL can degrade fibrous components.

1.1 Aim and object

The aim of this study was to understand the impact of pre-treatment with *Rhizopus oligosporus* and ammonia on banana peel in terms of amino acid and fiber content and composition, and to furthermore understand the impact of the pre-treatments on the efficiency of BSFL composting of banana and orange peel. Moreover the impact of larval density on the BSFL composting efficiency of pre-treated orange peel was investigated.

Specific objects were to understand:

- How pre-treatment with *Rhizopus oligosporus* and ammonia impact on biomass conversion rate
- Establish mass balance over amino acids over the entire treatment, including the pre-treatment, of banana peel
- Establish mass balance over fibers (cellulose, hemicellulose and lignin) in the entire treatment, including pre-treatment, of banana peel
- How the larval density impact on biomass conversion rate for orange peel

2. Background

2.1 The Black soldier fly (*Hermetia illucens)*

The black soldier fly is found in warmer regions of the world in tropical or subtropical climates (Dortmans et al., 2017; Rozkosny, 1983; Üstüner et al., 2003; Martínez-Sánchez et al. 2011). It is one of five species belonging to the subfamily Hermetiinae (Woodley, 2001). The BSF larvae's natural diet consists of excreta, fruit and vegetable wastes, manures and cadaver (Rozkosny, 1983; Schremmer, 1986). Flies are up to 20 mm long. The larvae develop through seven larval instars (Schremmer, 1986; Gligorescu et al., 2019) and generally grow to 18–20 mm in length (Rozkošny´, 1997). In a temperature-controlled greenhouse, under laboratory conditions, mating usually occurs two days after eclosion (hatching) and oviposition (egg laying by females) four days after eclosion (Tomberlin and Sheppard, 2002). The development of the BSF from egg to prepupae takes, in laboratory conditions set at 27 ˚C, on average 23 days, and from egg to adult on average 40–43 days (Tomberlin et al., 2002). Temperatures and relative humidity greatly influence the time of development, mating and oviposition of the species (Barry, 2004, Tomberlin et al., 2009).

2.2. BSF in waste treatment

The treatment could have potential environmental benefits to the society (Smetana et al., 2016), with biodegradable waste reduction being the prominent one, as well as lower greenhouse gas emissions and less land and water require for production of larvae biomass compared to livestock (Van Huis, 2013). BSFL consume biodegradable waste, convert it into larval biomass, and leave behind a compost-like residue (Zurbrügg et al., 2018; Xiao et al., 2018). Advantages in using the *Hermetia illucens,* compared to other studied fly species are for instance that the fly does not feed and thus is not a vector in disease transmission (Sheppard et al., 2002) and is thus a suitable fly choice for fly larvae composting (Cicková et al., 2015).

According to Dortmans et al. (2017), an exemplary BSF biodegradable waste facility would consist of waste preprocessing (e.g. mincing, dewatering, removal of inorganics such as plastics and metals), biodegradable waste treatment by BSFL, separation of BSFL from the process residue followed by refinement of the larvae and residue into marketable products (Figure 1). Feasibility depends primary on the amount of larval biomass produced from a certain amount of waste, measured as the biomass conversion rate. The BCR in BSFL composting varies depending on the type of feeding substrate and may range from as little as 3 % for biogas digestate to 23 % on a wet-weight basis for fresh human excreta (Banks et al., 2014; Lalander et al., 2015; Newton et al., 2005; Spranghers et al., 2016). For BSF biodegradable waste systems, new methodologies to quantify the sustainability of waste management and feed production in terms of hygiene and environmental aspects are under development (Chaudhary et al., 2018; Smetana et al., 2016). Today, only a few large scale facilities for BSFL composting exists, such as Agriprotein Technologies (Philippi, Cape Town, South Africa) and Enterra Feed Corporation (Langley, Canada). Local conditions such as climate (temperature, humidity), revenue from sales of derived products (protein meal, oil, whole larvae) and potential revenue from sales of the residue for further implement (biogas, soil amendment) also impact on the viability of the technology (Dortmans et al., 2017). Additionally, for it to be functionally

operational in a larger scale, the availability of adequate amounts of fresh biodegradable waste at a feasible cost is needed as well on regular basis. Moreover, as the lifecycle of BSF is controlled totally by the operator(s), they have to create an environment that best mimics the natural habitat of the fly (Dortmans et al., 2017).

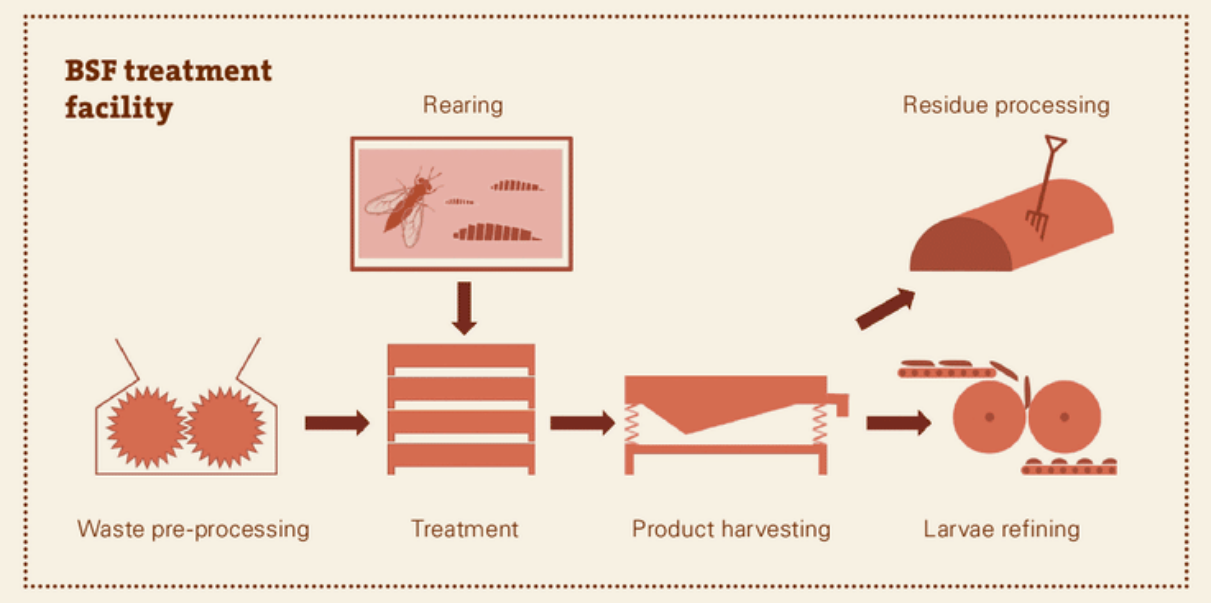

Figure 1: Steps in the BSF treatment facility. Taken from (Dortmans et al., 2017).

2.3 Products of BSFL composting

2.3.1 Larvae

The larval biomass can be used as a protein source in animal feed and the fat fraction for biodiesel production (Surendra et al., 2016). Larvae from the black soldier fly contain around 40 % protein, regardless of the substrate it has been reared in (Lalander et al., 2019). As BSF stop feeding in its larval stage, it has a higher fat concentration than other larvae (Cicková et al., 2015). According to Ewald et al (2020), fatty acid composition in the feeding substrate affects the fatty acid content and profile of the larvae. The fatty acid content was found to vary between 15 and 60 % fat on a total solids basis. In addition, it was concluded that larger larvae generally contain more saturated fatty acids compared to smaller larvae.

2.3.1.1 Animal feed

Environmental concern for deforestation, often associated with soy production (Garcia-Launay et al., 2014), prompts interest in exploring other sources for protein-rich animal feed. In poultry production for example, alternative protein sources either for total or partial replacements have to be evaluated in an effort to meet the dietary requirements and reduce feed costs (Ramos-Elordury et al., 2002; Das et al., 2009). Protein-rich insects are being considered, to reduce the cost of protein supplements in poultry feed. Insects have been used as a food source for several different species of animals (Finke et al., 1985). Research has demonstrated that several insects including silkworms, locusts and fly larvae can be safely fed to chickens without compromising

the quality and palatability of the meat (Khusro et al., 2012). In swine production, lysine is the initial limiting amino acid in the feed for pig development, and thus this amino acid is a driving force in the selection of feed for pigs (Göransson, 2009), and as a consequence, higher levels of lysine in the BSFL is desirable if they are to be used for swine production. Kroeckel et al (2012) examined different set-ups of replacing fishmeal by BSFL in fish feed for aquaculture production. They found that partial replacement with BSFL in the fish diet was a feasible alternative regarding environmental impact of the feed. As for replacement, Lock et al (2016) concluded that inclusion levels up to 50 % BSFL in the feed was possible without having a negative impact on fish growth. Also, Wang and Shelomi (2017) endorsed the use of BSFL in animal feed, but stresses the fact that regional legal restrictions in the production of larvae is a challenge for further expansion. Making the process of larvae production completely and guaranteed free of chemicals and microbial contaminations is an important step for the future of larvae production (Van Huis, 2019).

2.3.1.2 Biodiesel

Biodiesel have been produced from larvae fat in laboratory settings. In a study where the larvae were grown on biogas digestate as a substrate, Wu et al (2015) successfully yielded biodiesel when extracting the fat from the larvae. Digestate has a very low biomass conversion rate in BSFL composting compared to other substrates (Banks et al., 2014; Lalander et al., 2015; Newton et al., 2005; Spranghers et al., 2016), and in cases where biodiesel production from BSFL is prioritized, biodiesel extractions from larvae fed on other substrates could potentially be higher.

2.3.2 Residue

The compost-like treatment residue that forms from BSFL composting can be used as an organic fertilizer (Xiao et al., 2018). It has been shown that the larval manure (frass) possesses similar qualities as commercial fertilizers (Choi et al., 2009), and a study by Green and Popa (2012) suggests that the use of BSFL to process biodegradable waste could recover nitrate and thus offset expenses from nitrogen fertilization in crops when used as a fertilizer. Setti et al (2019) evaluated the residue as a potential replacement to peat for cultivation of lettuce, basil and tomato, and additions of 20 % residue showing significant positive impact on yield.

2.4 BSFL compost processing

The biochemical mechanisms of the BSFL composting process have not been extensively analysed (Gold et al., 2018). However, larvae of other well-studied close related species such as the house fly, *Musca domestica* (Diptera: Muscidae) and fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) suggests that the BSFL has a similarly evolved digestive system (Terra and Ferreira, 2012; Terra and Ferreira, 1994; Terra and Regel, 1995). As with other insects and mammals, the nutrients for the fly larvae are obtained by feeding (Cohen, 2005). The main component for the structural composition and development of the fly larvae is carbohydrate monomers and glucose. Components for protein production and tissue development, such as amino acids, are important molecules and arginine, histidine, isoleucine, leucine, lysine, methionine are considered essential. The larvae hydrolyse biomolecules into smaller molecules for absorption through the gut cells by the metabolism (Chapman, 2013). The fly larvae excrete

enzymes like amylase and maltase onto the feeding substrate. These enzymes originate from the salivary glands, however most digestive process occurs in the midgut of the larvae (Terra et al., 1988). The environment in the compost changes during the biodegradation to a more alkaline environment (Cicková et al., 2015). There is no consensus on the capability of fiber degradation by the BSFL. Li et al (2015b) BSFL composted fermented corncobs and found a 2% reduced of the lignin content but did find any reduction in cellulose or hemicellulose content. However, in a study by Rehman et al (2017), larvae fed on cow manure and soybean curd did reduce the amounts of cellulose, hemicellulose and lignin by 65-, 64- and 37 % respectively. Also, Li et al., (2011) found a 5 % and 17 % reduction in hemicellulose and cellulose, respectively, when BSFL composting cow manure (Li et al., 2011). Likely, the BSFL do not have enzymes to degrade fibres, however, possible microbes in the larval gut and in the feeding substrate does, according to some, have the ability to hydrolyse them and make the substrate nutrients available (Espinoza-Fuentes and Terra, 1987; Lemaitre and Miguel-Aliaga, 2013; Terra and Ferreira, 2012).

2.5 Pre-treatment of substrates

Food substrates with a low protein to carbon ratio and high amounts of fiber have been shown to inhibit BSFL growth rate and biomass yield because of poor digestibility and low nutrient utilization (Tomberlin et al. 2009; Tschirner 2015; Lalander et al. 2019). Thus making, in this case banana- and orange peel, not an optimum substrate as lignin content is high and fat and protein levels are low. Co-composting of substrates have been studied (Barragán-Fonseca et al. (2018) in terms of NFC (non-fibrous carbohydrates) and protein levels, that shows significantly different larvae growth between mixtures depending on nutritional composure in the feeding substrate. However, biodegradable waste that are highly nutritious, like that from the food industry, such as bread or restaurant wastes are already applied elsewhere (Mertenat et al., 2019, Smetana et al., 2019, Smetana et al., 2016), and are thus not always a viable option for BSFL composting. Pre-treatment of a substrate is thus an alternative/complement to co-composting of substrates for more efficient nutrient utilisation.

2.3.1 *Rhizopus oligosporus*

The fungi *Rhizopus oligosporus* is aerobic and produces a wide range of extracellular enzymes including carbohydrases, protease, lipases and phosphatase (Varzakas, 1998). These enzymes result in hydrolysis of macromolecules and the subsequent metabolism along the hydrolytic by-products alters the biochemical structure (de Reu et al., 1997; Handoyo and Morita, 2006) of the substrate. Gibbs et al (2004) found an improved digestibility of proteins in soybeans where the fungi *Rhizopus oligosporus* had grown. The fungi digest cellulose fibers (Collins et al., 2018), which is of interest for improved degradation of peels. The hypothesis of using *Rhizopus oligosporus* as a pre-treatment was that the fungi would process the fiber components (lignin, cellulose and hemicelluloses) of the peels to make it more accessible for ingestion by the larvae in terms of molecular composition and/or structure.

2.3.2 Ammonia

In composting, nitrogen is transformed by microorganisms by assimilation, nitrification, denitrification and ammonification (Meng et al., 2016). Ammonia assimilation have been

shown to transform nitrogen sources into proteins (Wang och Tan, 2013). Although there are more factors, nitrogen transformation is mainly driven by the ammonia assimilating bacteria (Sasaki et al., 2004). The hypothesis with the ammonia pre-treatment was that the added nitrogen source to the substrate would make the substrate more nitrogen-rich for the larvae with the help of ammonia assimilating bacteria, and thus try to increase BCR and protein conversion rate.

3. Material and methods

3.1 Material

3.1.1 Black soldier fly larvae

Black soldier fly larvae (BSFL) used for the experiment were obtained from a colony at the Swedish University of Agricultural Sciences (Uppsala, Sweden) that had been in operation since 2015. The BSFL were reared on chicken feed (Granngården Hönsfoder *Start,* metabolisable energy content of 11.2 MJ kg^{-1}, 80 % moisture) for around 5 days prior to the experiments.

3.1.2 Substrates for the study

Orange and banana peels that were used in the study were delivered by *Grönsakshallen Sorunda* (Stockholm, Sweden). The peels were mashed to a homogenous mix using a food processer (Robot Coupe Blixer 4 V.V) (Fig 2). The peels were thereafter stored at -20˚C until use.

Figure 2: A picture of the mashing of banana peels using a food processer; Robert Almqvist 2019-10-01

3.1.3 Pre-treatments

For ammonia pre-treatment, Ammonia (Nitor Ammoniak 24.5 % 1 L) were purchased at a local paint store in central Uppsala.

For *Rhizopus oligosporus* pre-treatment, spores were grown on agar plates (Malt extract agar (MEA), National Veterinary Institute, Uppsala, Sweden) at 28 ˚C for 1 week.

3.2 Methods

3.2.1 Experimental outline

The experiment was carried out in two trials, a banana peel trial and an orange peel trial (Figure 3). The banana peel trial used larger boxes (60 x 40 cm) and the orange peel trial used smaller ones (21 x 17 cm). The smaller boxes had a lid, with a meshed covered opening for aeration, to decrease the escape of larvae. The treatments were divided in two steps: the first step was the pre-treatment and the second the BSFL composting process. The banana peel study focused on the change in substrate composition, in terms of fibre and amino acid content and composition before and after pre-treatment and fly larvae composting. The orange peel study focused on possible differences in the biomass conversion rate and material reduction of the pre-treatment and composting process depending on substrate type (un-treated peels (Control), *Rhizopus* pre-treated peels and ammonia pre-treated peels) as well as larval density in the boxes. The orange peel trial was conducted in smaller boxes, but the larval density in one of the treatments was similar to that used in the banana peel trial (2.0 larvae cm^{-2} as compared to 1.7 larvae cm^{-2}). In addition, a treatment with 2x the density (4.0 larvae cm^{-2}) was included in order to verify the impact of larval density on process efficiency, in terms of biomass conversion rate and material reduction. The experiments were conducted in triplicates (n=3). (Figure 3, Table 1).

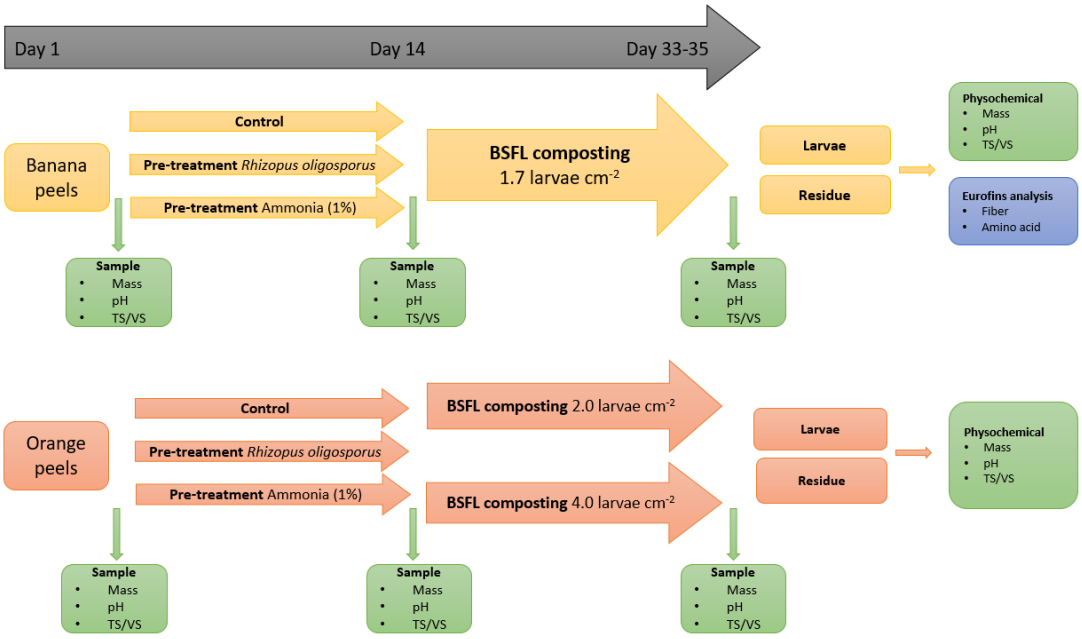

Figure 3: Procedure of the banana peel study and the orange peel study. Pre-treatments of the substrates lasted 14 days. BSFL composting lasted 19- 21 days. Physicochemical analyses were made for both the banana peel trial and orange peel trial. The banana peel trial also examined fiber and amino acid composition of the larvae and residue.

Table 1: The set-up of the orange and banana trials displaying the pre-treatment, BSFL composting area (cm^2), amount of larvae, larval density (lv cm^{-2}), larval feeding dose (g VS $larva^{-1}$) and whether or not lids were used.

	Pre-treatment	BSFL composting tray area (cm^2)	Larvae amount	Larval density (lv cm^{-2})	Larval feeding dose (g VS $larva^{-1}$)	Lid on tray
Banana peel trial						
Control	-	60 x 40	4000	1.7	0.2	No
Rhizopus oligosporus	*Rhizopus oligosporus*	60 x 40	4000	1.7	0.2	No
Ammonia	Ammonia	60 x 40	4000	1.7	0.2	No
Orange peel trial						
$Control_{2.0\ density}$	-	21 x 17	700	2.0	0.2	Yes
$Control_{3.9\ density}$	-	21 x 17	1400	4.0	0.2	Yes
Rhizopus $oligosporus_{2.0\ density}$	*Rhizopus oligosporus*	21 x 17	700	2.0	0.2	Yes
Rhizopus $oligosporus_{3.9\ density}$	*Rhizopus oligosporus*	21 x 17	1400	4.0	0.2	Yes
$Ammonia_{2.0\ density}$	Ammonia	21 x 17	700	2.0	0.2	Yes
$Ammonia_{3.9\ density}$	Ammonia	21 x 17	1400	4.0	0.2	Yes

3.2.3 Set up

The experiments were conducted in a greenhouse located at Ekologicentrum, Swedish University of Agricultural Sciences (Uppsala, Sweden). A wagon was used for stacking the trays used in the experiment and placed in a 28 ˚C cabinet. The cabinet was ventilated (Fig .4).

Figure 4: A picture of stacked trays in a wagon placed in 28 ˚C cabinet; Robert Almqvist 2019-10-16

3.2.4 Pre-treatments

Banana and orange peel were divided into three treatments: an untreated Control that was not subjected to pre-treatment; one treatment for *Rhizopus oligosporus* and one treatment for ammonia. For the *Rhizopus oligosporus* treatment, a *Rhizopus oligosporus* spore solution (4.2 x 10^8 spores mL^{-1}) were added to the peels in a dose of 100 ml kg^{-1} fresh substrate. The mixture was placed in trays in a ventilated 28 ˚C, 60 % humidity treatment chamber for 14 days. In the ammonia pre-treatment, 24.5 % ammonia solution, amounting to a dosage of 1 % N (*w/w*) were added per kilo fresh substrate. The mixture was subsequently placed in a big plastic bag, sealed and placed in a treatment chamber at 28 ˚C for 7 days.

The pre-treated substrates were kept at -20 ˚C until use.

3.2.5 Banana peel BSFL composting process

An amount of 2,300 g, approximately a third of the total amount, of each treatment (Control, *Rhizopus oligosporus* treated and ammonia treated) were placed in trays (60 x 40 cm). In each tray, 4000 BSFL (5 days old) (2.2 mg $larva^{-1}$) were added. The amount of substrate added in each tray was in total 0.2 g volatile solids (VS) per larva. Feeding occasion two and three were given in similar portions 6 and 12 days into the BSFL composting, respectively, and the BSFL composting lasted for 19 days. The BSFL composting was conducted in a treatment cabinet at 28 °C and trays were reorganized in random locations in the tray wagon each other day to assure that each replicate was exposed to the same amount of ventilation.

3.2.6 Orange peel BSFL composting process

In the orange peel trial, smaller boxes (21 x 17 cm) were used. Two larval densities were evaluated; 2.0 larvae cm^{-2} (700 larvae box^{-1}) and 3.9 larvae cm^{-2} (1400 larvae box^{-1}). The feeding procedure was the same as for the banana peel study, divided into three feeding occasions, in which the second and third was provided after 6 and 12 days, respectively. The same total amount of substrate was provided in both trials, resulting in a larval feeding dose of 0.2 and 0.1 g VS $larva^{-1}$, respectively. The boxes were randomly placed in the tray wagon during the experiment and were kept in a 28 ˚C cabinet. Trays were reorganized in random locations in the tray wagon each other day to assure that each replicate, was exposed to the same amount of ventilation.

3.2.7 Sampling

The procedure for sampling was the same in both trials. Measurements for pH, total solids (TS) and total volatile solids (VS), and larvae weight were taken at the beginning of the experiment, at each feeding, and at the end of the experiment. One sample was taken from each replicate. Three sub-samples, collected at random places in the same tray were pooled into one sample. The TS content and amount of VS gives an estimate of changes in the content of water and inorganic constituents in the sample. The pH shows how acidic or alkali the substrates are and

thus, the environment of the larvae, as well as the maturity of the BSFL compost. Larvae weight gives an estimation on larval development during the BSFL composting process. All measurements were taken once for each replicate, while pH measurements were taken twice for each replicate in the banana study. At the end of the experiment, the larvae were sieved from the substrate, counted, and samples were taken for measurements for TS and VS of the larval biomass. Average larval weight were calculated by taking a sample weight of ten larvae. At the end of the experiment, survival rate was calculated using an estimated average larval weight attained by weighing and counting three sub-samples of larvae approximately 100 larvae. The average larval weight could then be divided by the total larval harvested. For the banana peel trial, substrate and larvae samples were sent for amino acid and fiber analysis (see 3.2.9 Eurofins).

3.2.8 Physico-chemical analysis

The pH was measured by dissolving 5 g of material into 25 ml deionized water in a 50 ml centrifuge tube (Falcon 50 ml). The solution was homogenized and tempered to room temperature prior to measuring the pH. TS in the material were measured by placing 10 g of substrate into aluminum cups, weighing it, dry it at 70˚C for 48 h. Using Equation 1, the total solids content in the sample could be calculated. The VS was measured by burning the dried substrate in a furnace at 250 ˚C for 2 h and 550 ˚C for 4 h. Equation 2 was used to calculate the amount of VS on a TS basis in the material.

3.2.9 Eurofins

Samples from the banana peel trial, for amino acid and fiber content and composition analysis, were sent to an accredited lab (Eurofins Food & Feed Testing Sweden AB, Lidköping, Sweden). The analyses made were (SS-EN ISO 13903:2005) for amino acid profile, (Swedish Standards Institute) in accordance to (EU 152/2009 (F) EUDAKG) and (NF V 18-122) for fiber content.

3.2.10 Calculations

The percentage total solids (%TS) percentage volatile solids (%VS) of total solids were calculated as:

$$\%\mathrm{TS} = \frac{m_{TS}}{m_{WW}} \times 100\,, \qquad \text{(Equation 1)}$$

and,

$$\%\mathrm{VS} = \frac{m_{TS} - m_{Ash}}{m_{TS}} \times 100\,, \qquad \text{(Equation 2)}$$

where, m_{TS}, m_{WW} and m_{Ash} is the mass of the dry, wet and ash mass, respectively.

The survival rate (%SR) of the larvae in each tray/box were calculated as:

$$\%\mathrm{SR} = \frac{\mathrm{BSFL}_{out}}{\mathrm{BSFL}_{in}} \times 100\,, \qquad \text{(Equation 3)}$$

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

The percentage material reduction on TS basis (%Mat.red.$_{TS}$) was calculated as:

$$\%\text{Mat. red.}_{TS} = (1 - \frac{\text{m}_{res} \times \text{TS}\%_{res}}{\text{m}_{sub.in} \times \text{TS}\%_{sub.in}}) \times 100\ , \qquad \text{(Equation 4)}$$

where, m_{res} and $m_{sub.in}$ is the mass of the residue and substrate respectively, and $TS\%_{res}$ and $TS\%_{sub.in}$ is the TS in the residue and substrate, respectively, in %.

The percentage waste-to-biomass conversion rate on TS basis (%BCR$_{TS}$) was calculated as:

$$\%\text{BCR}_{TS} = (\frac{\text{m}_{BSFL} \times TS\%_{BSFL}}{\text{m}_{sub.in} \times \text{TS}\%_{sub.in}}) \times 100\ , \qquad \text{(Equation 5)}$$

where, m_{BSFL} and $m_{sub.in}$ is the mass of the larvae (BSFL) and substrate (sub.in), respectively, and $TS\%_{BSFL}$ and $TS\%_{sub.in}$ the TS in the larvae and the substrate, respectively, in %.

The percentage protein conversion ratio on a total solids basis (%PrCR$_{TS}$) was calculated as:

$$\%\text{PrCR}_{TS} = (\frac{m_{BSFL} \times \text{TS}\%_{BSFL} \times \text{Pr}\%_{BSFL}}{m_{sub.in} \times \text{TS}\%_{sub.in} \times \text{Pr}\%_{sub.in}}) \times 100\ , \qquad \text{(Equation 6)}$$

where, m_{BSFL} and $m_{sub.in}$ was the total fresh weight mass of the larvae and substrate respectively. $TS\%_{BSFL}$ and $TS\%_{sub.in}$ was the TS of the larvae and substrate respectively and $Pr\%_{BSFL}$ and $Pr\%_{sub.in}$ the percentage crude protein (% of TS) in the larvae (BSFL) and the substrate (sub.in), respectively.

3.2.11 Statistical analysis

Evaluation of the results from the BSFL composting process were made using a one-way ANOVA test with a 95 % confidence interval for the triplicate setup used in the experiments. For the orange peel trial, comparisons were made within and between different treatments regarding biomass conversion rate, material reduction and survival rates as well as a two-way ANOVA for the BCR in response to treatment and larval density. In the banana peel study, additional comparisons in amino acid- and fibrous composition were also made using a one-way ANOVA.

4. Results

4.1 Physico-chemical

TS decreased in both trials after pre-treatment and increased after the BSFL composting, while the VS decreased after both the pre-treatment and BSFL composting process (Table 2). The pH fluctuated between approximately pH 6 and pH 9 in the banana peel trial and reached around 9 towards the end of the experiment in all treatments. In the orange peel trial, pH remained low throughout the entire experiment in the Control and *Rhizopus oligosporus* treatment. For the ammonia treatment, pH stayed around 8-9 for the major part of the experiment.

In the banana peel trial, survival rates were highest in the Control followed by *Rhizopus oligosporus* treatment, around 95 % and 97 %, respectively. Survival rate in the ammonia treatment were noticeably lower than in the other treatments, at close to 59 %. The larvae were larger in the ammonia treatment compared to other treatments and reached an average weight of 90 mg. Larvae size in the Control and *Rhizopus oligosporus* were approximately the same, with the *Rhizopus oligosporus* larvae being slightly larger at 69±1.9 mg compared to 64±3.0 mg in the Control. In the orange peel trial, the survival rate in the Control was the highest for both larval densities at 88 % for the 2.0 larvae cm^{-2} setup and 93 % for the 3.9 larvae cm^{-2} setup. Survival rates were higher in the higher density setup in all treatments. Larvae weights were highest in the ammonia treatment for both densities. The larvae weight were noticeably lower in comparison to the other treatments for larvae in the *Rhizopus oligosporus* treatment for both densities.

Table 2: Physico-chemical results from the pre-treatment and BSFL Composting process, Differences in TS, VS and pH. Data presented as averages (± sd) are shown for the BSFL composting process (n=3)

	Inflow				**After pre-treatment**				**After BSFL Composting**			
	TS(%)	VS (% of TS)	pH high	pH low	TS (%)	VS (% of TS)	pH high	pH low	TS (%)	VS (% of TS)	pH high	pH low
Banana peel trial												
Control	13.1	85.9	6.9	6.7					35.9±8.5[a]	80.4±4.4[b]	9.7	9.5
Rhizopus oligosporus	13.1	85.9	6.9	6.7	10.7	81.4	8.3	8.1	20.1±3.9[b]	79.0±2.3[b]	9.5	9.1
Ammonia	13.1	85.9	6.9	6.7	10.8	82.9	7.9	7.6	19.4±4.1[b]	80.8±1.5[b]	9.3	9.0
Orange peel trial												
$Control_{2.0\ density}$	19.3	97.6	4.8	4.5					20.0±7.4[a]	92.4±0.1[a]	7.3	6.6
$Control_{3.9\ density}$	19.3	97.6	4.8	4.5					20.0±2.0[a]	93.7±1.1[a]	7.0	4.2
Rhizopus oligosporus$_{2.0\ density}$	19.3	97.6	4.8	4.5	14.4	93.0	3.9	3.4	25.9±2.0[a]	91.7±1.0[a]	3.9	3.7
Rhizopus oligosporus$_{3.9\ density}$	19.3	97.6	4.8	4.5	14.4	93.0	3.9	3.4	22.6±4.7[a]	90.6±1.6[a]	4.0	3.8
$Ammonia_{2.0\ density}$	19.3	97.6	4.8	4.5	17.3	96.7	9.7	9.5	19.7±4.0[c]	95.3±1.4[a]	8.8	8.5
$Ammonia_{3.9\ density}$	19.3	97.6	4.8	4.5	17.3	96.7	9.7	9.5	17.7±3.0[a]	94.0±0.2[a]	8.9	8.6

[a]*Significantly different within trial and other trial*
[b]*Significantly different other trial but not within trial*
[c]*Significantly different within trial but not for pre-treatment type in other trial*

For the banana peels, material reduction was highest on both TS and VS basis in the Control with 38 % and 42 % reduction, respectively (Table 3). In the orange peel trial, Control $_{700}$ (2 larvae cm^{-2}) had the highest material reduction on TS basis compared to any other setup, at 63 %. On TS basis, material reduction was low for *Rhizopus oligosporus* treatment in both the banana peel trial (around 11 %) and orange peel trial, with a reduction of only around 2 % in the 2.0 larvae cm^{-2} density setup. Notably, there was a very small variation in the replicates of the ammonia treatment for the orange peel 3.9 larvae cm^{-2} density setup (NH_4^+ $_{1400}$) and the Control and NH_4^+ pre-treatment had more consistent results than *Rhizopus oligosporus,* which had a higher variance in both trials.

The Control had the highest biomass conversion rate (BCR) in the banana peel trial at 7 %. The *Rhizopus oligosporus* treatment had the lowest variance among triplicates in the banana peel trial. In the orange peel trial, ammonia pre-treatment had the highest BCR for 3.9 larvae cm^{-2} density setup (NH_4^+ $_{1400}$) on TS basis at 9 %. For the 2.0 larvae cm^{-2} density setup, BCR was highest in the Control. The BCR for *Rhizopus oligosporus* treatment was exceptionally low in the orange peel study for both larval densities compared to the other treatments at just around 1 % in both densities. Higher larval density increased BCR in all treatments. Untreated peels had higher BCR for orange peels than banana peels.

Table 3: Physico-chemical results from the pre-treatment and BSFL Composting process, biomass conversion rate, material reduction, survival rates and larval size. Averages (± sd) are shown for the BSFL composting and total process (n=3)

	After pre-treatment		After FLC				Total process	
	Red TS [%]	BCR TS [%]	Red TS [%]	BCR TS [%]	Survival rate [%]	Larval size [mg larvae^{-1}]	Tot-Red$_{TS}$ [% of TS]	Tot-BCR$_{TS}$ [% of TS]
Banana peel trial								
Control	-	-	38.3±12.2^{a}	7.1±0.6^{a}	95.4±8.0^{b}	64.2±3.0^{c}	38.3±12.2^{b}	7.1±0.6^{a}
Rhizopus oligosporus	37.9^{a}	62.1^{a}	11.6±20.7^{a}	6.4±0.2^{a}	96.8±4.1^{b}	69.1±1.9^{a}	45.1±12.9^{c}	4.0±0.1^{b}
Ammonia	18.1^{a}	81.9^{a}	28.6±9.6^{a}	5.2±0.4^{c}	58.6±15.1^{a}	89.6±15^{c}	41.5±7.9^{b}	4.2±0.3^{b}
Orange peel trial								
Control$_{2.0\ density}$	-	-	63.1±3.3^{a}	8.5±0.8$^{a\,d}$	87.7±12.9^{a}	63±12$^{c\,d}$	63.1±3.3^{a}	8.5±0.8$^{a\,d}$
Control$_{3.9\ density}$	-	-	55.6±7.9^{a}	8.8±1.0$^{a\,d}$	92.6±7.4^{a}	34±4.1$^{a\,d}$	55.6±7.9^{a}	8.8±1.0$^{a\,d}$
Rhizopus oligosporus$_{2.0\ density}$	46.9^{a}	53.1^{a}	1.7±15.8^{a}	0.9±0.1^{a}	41.2±3.6^{a}	17.5±1.7$^{a\,d}$	46.0±8.4^{c}	0.5±0.0^{a}
Rhizopus oligosporus$_{3.9\ density}$	46.9^{a}	53.1^{a}	4.5±24.1^{a}	1.3±0.4^{a}	45.9±5.4^{a}	10.5±1.9$^{a\,d}$	49.3±12.8^{c}	0.7±0.2^{a}
Ammonia$_{2.0\ density}$	10.3^{a}	89.7^{a}	39.3±3.3^{a}	5.5±0.5$^{c\,d}$	40.7±1.6$^{a\,d}$	83±10.5$^{c\,d}$	45.6±3.0^{a}	4.9±0.4$^{a\,d}$
Ammonia$_{3.9\ density}$	10.3^{a}	89.7^{a}	43.5±0.3^{a}	9.2±0.3$^{a\,d}$	65.7±1.1$^{a\,d}$	47.9±2.2$^{a\,d}$	49.3±0.3^{a}	8.2±0.3$^{a\,d}$

[a]Significantly different within trial and other trial
[b]Significantly different other trial but not within trial
[c]Significantly different within trial but not for pre-treatment type in other trial
[d]Significantly different other larval density

For the banana peel trial, protein conversion ratio was highest in the Control at 49 %. There was no significant difference in crude protein content in the different treatments. Amounts of essential amino acids such as lysine and methionine were also not significantly more concentrated in any larvae, regardless of feeding substrate (Table 4).

Table 4: Crude protein content, protein conversion ratio, lysine, and methionine content for larvae in the banana peel trial. Averages (± sd) are shown (n=3).

	Control		*Rhizopus oligosporus*		Ammonia	
	Average	sd	Average	sd	Average	sd
Total solids larvae($\%_{ww}$)	25.9	± 1.3	23.5	± 1.9	24.5	± 2.3
Crude protein larvae ($\%_{TS}$)	34.8	± 7.1	36.0	± 0.3	38.5	± 4.9
Crude protein (g 100 g^{-1} substrate)$_{TS}$	2.5	± 0.5	2.3	± 0.0	2.0	± 0.3
Lysine larvae ($\%_{DM}$)	2.2	± 0.2	2.4	± 0.1	2.7	± 0.3
Lysine (mg 100 g^{-1} substrate)$_{TS}$	155	± 15	154	± 7	141	± 13
Methionine larvae ($\%_{DM}$)	0.5	± 0.1	0.5	± 0.1	0.5	± 0.0
Methionine (mg 100 g^{-1} substrate)$_{TS}$	32	± 4	30	± 3	26	± 1
Protein conversion ratio (%PrCR$_{TS}$)	49.1	± 7.7	31.5	± 6.9	32.1	± 5.4

Statistical analysis using ANOVA showed that the biomass and protein conversion ratio was significantly higher in the Control. The treatment was the factor that demonstrated significant difference for BCR in both trials and the protein conversion ratio in the banana peel trial (Table 5). The crude protein content in the larvae did not significantly vary depending on substrate type.

Table 5: One-way ANOVA values with p-value and adjusted R^2 value.

Response	Trial	Factor	*p*-value	Adjusted R^2
BCR	Banana peel	Treatment	0.005	0.794
Protein conversion ratio (%PrCR$_{TS}$)	Banana peel	Treatment	0.085	0.586
Crude protein larvae ($\%_{TS}$)	Banana peel	Treatment	0.735	0.000
Crude protein (g 100 g^{-1} substrate)$_{TS}$	Banana peel	Treatment	0.307	0.123
Survival rate	Banana peel	Treatment	0.059	0.760
TS in residue	Banana peel	Treatment	0.111	0.620
BCR	Orange peel	Treatment	0.000	0.872
BCR	Orange peel	Larval density	0.414	0.000
BCR	Orange peel	Treatment + Larval density	0.000	0.914
Survival rate	Orange peel	Treatment	0.000	0.798
TS in residue	Orange peel	Treatment	0.065	0.204

Significance level<0.05.

4.2 Amino acid profiles

For the substrates, individual amino acids were either decreased or increased during the process by either pre-treatment or BSFL composting (Table. 6. 7. 8). Methionine was not present in any substrate, but was found in the residue of the Control after BSFL composting. Other amino acids created that were not found in the feeding substrate were hydroxiproline, isoleucine and ornithine.

Total amount of amino acids in the BSFL treatment residue and larvae after the treatment was higher than that present in the inflow substrate and increased by 37 % in the Control, 5 % in the *Rhizopus oligosporus* pre-treatment and 24 % in the ammonia pre-treatment (Table 6. 7. 8).

Table 6: Amino acid amounts in the substrate, larvae and residue in the Control during pre-treatment and BSFL composting process. Averages (± sd) are shown (n=3)

	Inflow material		**After BSFL composting**			
			Amount [g]	% of inflow		
	Amount [g]	%	Larvae+ res	Larvae + Res	Larvae	Residue
Alanine	3.34	100	5.28	158.1±32.1	64.6±9.7	93.5±25.0
Arginine	2.70	100	3.37	125.0±25.3	49.6±5.5	75.4±21.8
Aspartic acid	6.19	100	6.65	107.4±31.0	31.9±4.2	75.6±30.4
Glutamic acid	4.86	100	8.05	165.7±39.9	55.0±4.8	110.8±38.1
Glycine	3.02	100	4.35	143.8±38.5	49.0±8.4	94.8±32.9
Histidine	1.87	100	1.45	77.7±10.2	33.7±4.5	44.0±8.3
Hydroxiproline	0	-	0.34	-	-	-
Isoleucine	0	-	2.84	-	-	-
Leucine	3.76	100	4.91	130.7±31.4	44.1±4.1	86.5±29.2
Lysine	2.62	100	2.88	110.1±9.2	55.1±3.3	55.1±7.7
Ornithine	0	-	0.11	-	-	-
Phenylalanine	1.73	100	2.99	173.0±49.9	49.0±4.3	124.0±49.4
Proline	2.89	100	4.39	151.9±34.7	69.5±16.1	82.4±19.1
Serine	3.33	100	3.56	106.9±31.0	35.6±5.7	71.3±28.0
Threonine	2.79	100	3.25	116.8±35.6	35.5±4.9	81.2±33.7
Tyrosine	1.88	100	2.89	153.7±26.5	70.2±14.1	83.5±17.2
Valine	3.84	100	4.21	109.7±26.6	40.5±6.8	69.2±21.9
Cysteine + Cystine	1.42	100	0.85	60.3±23.0	11.8±0.4	48.5±23.2
Methionine	0	-	0.87	-	-	-
Total	**46.2**	**100**	**63.28**	**136.8±32.4**	**49.4±6.0**	**87.4±28.7**

Table 7: Amino acid amounts in the substrate, larvae and residue in the Rhizopus oligosporus *during pre-treatment and BSFL composting process. Averages (± sd) are shown (n=3)*

	Inflow material		**After pre-treatment**		**After BSFL composting**			
					Amount [g]	% of inflow		
	Amount [g]	%	Amount [g]	% of inflow	Larvae+ res	Larvae + Res	Larvae	Residue
Alanine	5.68	100	6.72	118.4	2.05	121.1±9.3	36.1±1.4	85.0±10.1
Arginine	4.59	100	4.28	93.3	1.28	99.3±6.2	27.9±1.0	71.4±7.0
Aspartic acid	10.52	100	8.57	81.5	2.01	82.0±5.6	19.1±1.3	62.9±5.8
Glutamic acid	8.26	100	9.42	114.0	2.97	134.6±11.0	36.0±0.5	98.6±10.5
Glycine	5.14	100	5.17	100.6	1.43	113.8±9.9	27.7±0.8	86.1±10.6
Histidine	3.18	100	0.69	21.9	0.59	61.9±4.4	18.7±2.1	43.2±6.5
Hydroxiproline	0	-	0	-	0	-	-	-
Isoleucine	0	-	1.42	-	1.00	-	-	-
Leucine	6.40	100	6.06	94.7	1.64	101.8±8.4	25.7±0.5	76.1±8.4
Lysine	4.45	100	4.37	98.1	1.52	84.2±6.7	34.1±2.0	50.1±6.9
Ornithine	0	-	0	-	0.12	-	-	-
Phenylalanine	2.94	100	3.70	125.5	0.85	131.9±8.6	29.0±1.7	102.9±7.1
Proline	4.91	100	4.69	95.4	1.75	103.9±8.5	35.6±0.9	68.3±9.4
Serine	5.67	100	4.55	80.3	1.15	80.1±4.3	20.3±0.4	59.9±4.7
Threonine	4.74	100	4.37	92.3	1.00	95.7±7.4	21.2±1.2	74.5±8.0
Tyrosine	3.20	100	3.14	98.1	1.30	120.3±12.5	40.6±3.2	79.6±9.4
Valine	6.53	100	5.12	78.4	1.51	83.4±6.7	23.2±0.3	60.2±6.9
Cysteine + Cystine	2.41	100	1.54	63.7	0.18	52.9±2.9	7.3±0.4	45.6±2.6
Methionine	0	-	0	-	0.30	-	-	-
Total	**78.6**	**100**	**73.8**	**93.9**	**22.67**	**104.5±7.3**	**28.8±0.5**	**75.7±7.6**

Table 8: Amino acid amounts in the substrate, larvae and residue in the Ammonia during pre-treatment and BSFL composting process. Averages (+/- sd) are shown (n=3)

	Inflow material		**After pre-treatment**		**After BSFL composting**			
					Amount [g]	% of inflow		
	Amount [g]	%	Amount [g]	% of inflow	Larvae+ res	Larvae + Res	Larvae	Residue
Alanine	4.22	100	20.91	495.1	10.18	241.1±46.2	42.5±6.1	198.6±46.8
Arginine	3.41	100	3.23	94.6	3.80	111.3±10.2	32.4±2.6	78.9±7.6
Aspartic acid	7.83	100	6.79	86.8	7.86	100.4±7.3	22.6±2.0	77.8±5.8
Glutamic acid	6.14	100	5.99	97.5	9.49	154.6±9.0	38.1±4.4	116.5±4.8
Glycine	3.82	100	3.26	85.3	4.78	125.1±11.3	32.3±4.6	92.8±7.1
Histidine	2.36	100	0	-	1.65	69.9±7.7	22.7±1.7	47.3±6.9
Hydroxiproline	0	-	0	-	0	-	-	-
Isoleucine	0	-	0	-	3.16	-	-	-
Leucine	4.76	100	4.12	86.5	5.24	110.1±8.8	29.8±2.7	80.4±6.3
Lysine	3.31	100	0	-	2.97	89.5±43.1	41.0±3.3	48.5±44.0
Ornithine	0	-	0	-	0	-	-	-
Phenylalanine	2.19	100	0	-	3.22	147.2±11.6	33.6±1.9	113.6±10.3
Proline	3.66	100	3.17	86.7	4.08	111.5±13.7	39.2±8.8	72.3±11.8
Serine	4.22	100	3.54	83.9	3.68	87.2±9.5	23.4±2.3	63.7±8.0
Threonine	3.52	100	3.12	88.5	3.58	101.6±11.8	24.4±1.7	77.1±10.4
Tyrosine	2.38	100	0.73	30.8	3.18	133.5±10.9	48.1±5.7	85.4±5.3
Valine	4.86	100	3.99	82.1	4.37	89.9±7.5	26.2±3.2	63.7±4.6
Cysteine + Cystine	1.79	100	1.11	61.7	0.91	50.7±6.5	8.4±0.5	42.3±6.8
Methionine	0	-	0	-	0.25	-	-	-
Total	**58.5**	**100**	**60.0**	**102.5**	**72.4**	**123.8±6.6**	**32.9±3.4**	**90.9±3.4**

4.3 Fiber content

For the *Rhizopus oligosporus* treatment; hemicellulose, cellulose and lignin were degraded by 46 %, 9 % and 12 %, respectively. The ammonia pre-treatment did also degrade hemicellulose, cellulose and lignin by 1 %, 10 % and 14 %, respectively (Table 10).

In the BSFL composting process, the amount of hemicellulose that remained in the residue of the total amount that was added before the BSFL composting process were 86 % for Control, 87 % for *Rhizopus oligosporus* and 90 % for ammonia (Table 10).

For cellulose, the factual amount in the residue after larval activity were 116 % for Control, 119 % for *Rhizopus oligosporus* and 107 % for ammonia (Table 10).

The amount of lignin remaining after BSFL composting of the amount added to the larva were 70 % for Control, 98 % for *Rhizopus oligosporus* and 87 % for ammonia (Table. 10).

Statistical analysis with one-way ANOVA showed that there was no significant difference in degradation of hemicellulose, cellulose and lignin by the larvae depending on feeding substrate (Table 9).

Table 9: ANOVA values for fibrous degradation in the BSFL composting process with P-value and adjusted R^2 value

Response	Factor	*p*-value
Hemicellulose degradation	Treatment	0.970
Cellulose degradation	Treatment	0.791
Lignin degradation	Treatment	0.324

Significance level: $p<0.05$

Table 10: Fiber composition amounts in the substrate and residue after pre-treatment and BSFL. Inflow material shows the total amount added before pre-treatment. The % of inflow shows the amount left of inflow material in that process. Averages (+/- sd) are shown (n=3)

	Inflow material		**After pre-treatment**		**After BSFL composting**			
					Amount [g]	% of inflow		
	Amount [g]	%	Amount [g]	% of inflow	Larvae+ res	Larvae + Res	Larvae	Residue
CONTROL								
Hemicellulose	272.8	100			238.9±56.0	87.5±20.6	1.2±0.0	86.3±20.6
Cellulose	337.9	100			397.1±93.1	117.5±27.4	1.6±0.0	115.9±27.4
Lignin	497.1	100			350.8±73.8	70.1±14.7	>0.0	70.1±14.7
R. OLIGOSPORUS								
Hemicellulose	463.8	100	249.6	53.8	219.3±53.0	47.2±11.4	0.6±0.0	46.6±11.4
Cellulose	574.6	100	524.8	91.3	628.5±127.3	108.5±22.4	0.1±0.2	108.4±22.2
Lignin	845.3	100	742.0	87.8	732.8±200.2	86.6±23.8	0.4±0.1	86.2±23.7
AMMONIA								
Hemicellulose	345.0	100	341.6	99	312.7±50.8	90.6±15.1	1.2±0.5	89.4±14.6
Cellulose	427.4	100	386.0	90.3	417.7±68.7	97.7±16.2	1.0±0.1	96.7±16.1
Lignin	628.8	100	539.4	85.8	469.8±54.7	74.7±8.7	0.4±0.0	74.3±8.7

5. Discussion

5.1 Results

5.1.1 Biomass conversion rate & material reduction

In the banana peel trial, none of the pre-treatments increased BCR. This is not in accordance with the findings of Isibika et al. (2019) (Table 11), that found that the *Rhizopus oligosporus* pre-treatment with 14 days pre-treatment resulted in more than twice as high BCR on a VS basis. The BCR demonstrated by Isibika et al. (2019) was 15.0 % on a VS basis, compared to the BCR found of the *Rhizopus oligosporus* treatment found in this study at 6 % on a TS basis. The pre-treatment was 14 days in both cases. Additionally, the Control had a similar biomass conversion rate in that study as the one in this study at around 7 % TS. These conversion rates, however, are lower than those observed in Nyakeri et al (2017), where a BCR of around 11 % TS for untreated banana peel was observed. In the banana peel trial, the ammonia pre-treatment resulted in the lowest BCR of the treatments, at 5 % TS, whereas in Isibika et al (2019) almost twice as high BCR was observed (9.6±3.9 % on VS basis). In the orange peel trial in this study, the BCR improved for all treatments with a higher larval density, most notably in the ammonia pre-treatment, at 6 % for the lower density and 9 % for the higher density. The orange peel trial used 2.0 and 3.9 larvae cm^{-2} density setups, while the banana peel study used 1.7 larvae cm^{-2}. In Lindberg (2018), orange peels were used as a substrate as well, however the larval density in that study was 6.3 larvae cm^{-2}. Similarly to this study, an ammonia 1 % (*w/w*) pre-treatment was used and a BCR of 5.0±0.7 VS was achieved. That result is very similar to the 5 % VS BCR found in the $ammonia_{2.0\ density}$ in this study. There was a difference in larval feeding dose and BSFL composting time between the experiments, however. Higher composting time seems to increase the overall BCR in the banana peel pre-treatments, when comparing these results with those from Isibika et al. (2019), while the affect is not as notable for orange peels compared with the results obtained by Lindberg (2018). In Isibika et al. (2019), a larval density of 0.6 larvae cm^{-2} were used, which could be a contributing factor for differing results. According to Parra et al (2015), larval density and feeding rate of the larvae have a significant impact on the bioconversion process of the substrate used, and larval density is the most influential element for development. That study also suggests that an optimal larval density would be 1.2 larvae cm^{-2}. The BCR is one driving force in determining the feasibility of a feeding substrate when used in BSFL composting. In some cases, the quantity of larvae output may be prioritized before the nutritional composition (amino acids, fat, fiber) of the larvae and as such, a high BCR rate is desired. In this study, single source substrates were used, and as mentioned, larval density improved BCR for all treatments. This corresponds to the results by Karol et al (2018), were low nutrient concentration in the feeding substrate resulted in higher larval yield when using higher larval density, indicating an interaction between the larval density and the capacity to extract available resources of the feeding substrate. The nutritional value of the feeding substrate has a large impact on the BCR. As the BCR for mixed food waste was as high as 24.3±0.9 % TS in Lindberg (2018), reaching a BCR equal to or greater than that is ideal, but probably not possible while using a single type substrate with low nutritional value. Also, in Nguyen et al. (2013) and Oonincx et al. (2015), the BSFL had a

slower development time when fed vegetable substrates low in protein, whereas vegetable substrates of high protein content had faster development times. It is thus of interest whether a higher BCR would have been observed if the separation process had been postponed in this experiment. In addition, Simon et al. (2011) suggests that high protein diets does not only decrease development time but also survival rates of the larvae. Lalander et al., (2019) suggested that low protein content could make larvae development slow because of the amount of substrate required for sufficient protein intake for development.

Table 11: Values in comparison to studies conducted by Isibika et al (2019) and Lindberg (2018).

	Experiment	Pre-treatment	Pre-treatment time (d)	BSFL composting tray size (cm)	Lid on tray	Larval density (larvae cm^{-2})	Larval VS feeding (g VS $larva^{-1}$)	BSFL composting time (d)	BCR (% of VS)
Banana peel									
	This trial	*Rhizopus oligosporus*	14	60 x 40	No	1.7	0.2	19	6.7±0.6
		Ammonia 1 %	7	60 x 40	No	1.7	0.2	19	5.6±0.3
	Isibika et al (2019)	*Rhizopus oligosporus*	7	21 x 17	Yes	0.6	0.40	30	6.7±1.9
		Rhizopus oligosporus	14	21 x 17	Yes	0.6	0.38	30	15.0
		Ammonia 1 %	7	21 x 17	Yes	0.6	0.42	30	9.6±3.9
		Ammonia 0.8 %	7	21 x 17	Yes	0.6	0.42	30	7.1±0.5
		Trichoderma reesei	14	21 x 17	Yes	0.6	0.38	30	11.6
Orange peel									
	This trial	*Rhizopus oligosporus*	14	21 x 17	Yes	2.0	0.2	21	0.9±0.0
		Rhizopus oligosporus	14	21 x 17	Yes	4.0	0.1	21	1.3±0.3
		Ammonia 1 %	7	21 x 17	Yes	2.0	0.2	21	4.9±0.8
		Ammonia 1 %	7	21 x 17	Yes	4.0	0.1	21	8.2±0.6
	Lindberg (2018)	*Trichoderma reesei*	16	60 x 40	No	6.3	0.11	31	2.5±0.9
		Ammonia 1 %	16	60 x 40	No	6.3	0.12	35	5.0±0.7

Other BSFL composting scenarios favor systems where the highest amount of substrates can be degraded at a given timeframe, thus the material reduction rates are of main priority. As for material reduction, in this study, all treatments and set-ups reached around 50 % total material reduction. For the BSFL composting process only though, the *Rhizopus oligosporus* pre-treatment was significantly lower, indicting an altering of the substrate by the pre-treatment (see 5.1.3 Fiber analysis). Lalander et al (2019) suggested that a lower BCR in the BSFL composting process could be due to high substrate lignin concentration. The weight of the

increasing larvae yield with mixed food waste compared to the hygienic advantages of a single type substrate could be evaluated against each other in a larger scale of BSFL composting.

5.1.2 Amino acid analysis

When using the larvae from BSFL composting as either feed or human food, the nutritional value of the larvae are of interest. While the fat composition and concentration have been studied before (Cicková et al., 2015; Ewald et al., 2020), protein content and amino acid composition is an area worth notice. Lalander et al. (2019) evaluated the amino acid profile of the larvae reared on different substrates, but no mass balances and fate of certain amino acids from the rearing substrate were made in that study. The banana peel treatments used in the trial for amino acids, showed minor differences in composition in the larvae. The protein conversion rate was significantly higher in the Control. In Lalander et al. (2019) eight different urban organic waste fractions were evaluated and the larval protein content did not vary greatly on a TS basis. In this study, the protein conversion rate correlated with BCR, but in Lalander et al (2019) it did not. In that study, abattoir waste had the highest BCR, while poultry feed had the highest protein conversion rate. However, on essential amino acids, the amino acid methionine compromised 0.5 % of the crude protein (TS) in the larvae for all treatments in this study. That is lower than the 2.1 % measured in other studies, with other substrates (Kroeckel et al., 2012; St-Hilaire et al., 2007; Stamer, 2015; Zhang et al., 2007) and the 1.8 % measured in Lalander et al (2019). Based on the results in this study it is reasonable to assume that the pre-treatments of the banana peel does not improve the subsequent methionine neither lysine content in the larvae to those levels in larvae fed on other, more protein-rich substrates. Regarding protein content in larvae by adding a nitrogen source (in ammonia pre-treatment), it did not significantly increase protein amount on TS basis. The protein amount in the larvae were 39 % in the ammonia treatment, 36 % in the *Rhizopus oligosporus* treatment and 35 % in the Control. Adding a non-protein nitrogen source (ammonia pre-treatment) did thus not have a significant impact on the essential amino acids of the larvae. Additionally, there was a five-fold increase in alanine content in the ammonia pre-treated substrate. As the pre-treatments were done in singlets, a more secure verification of the amino acid change during pre-treatment is suggested.

5.1.3 Fiber analysis

The pre-treatments were done in singlets and the decreased amount of fiber in the pre-treatments and thus the results have no variances. However, as mentioned earlier, the value measured is a mean from three sample takes from the same batch, so an accurate measurement of the single pre-treatment is obtained. The *Rhizopus oligosporus* capability to degrade fibrous components were observed by a degradation of 46 % of the hemicellulose. As seen in Section 5.1.1 (Biomass conversion rate & material reduction), it is hypothesized that the BSFL composting time could affected the BCR positively for banana peels, as results from Isibika et al (2019) had higher conversion rates and also used longer BSFL composting time. As banana peels are more fibrous than orange peels, and considering that the *Rhizopus oligosporus* pre-treatment degraded almost half of the hemicellulose but little (approx. 13 %) of the lignin in the banana peel, the composition of the VS provided to the larvae in the *Rhizopus oligosporus* treatment likely had

a higher percentage lignin than the Control and ammonia pre-treatment. The increase in BCR by a longer BSFL composting time for banana peels and not orange peels, when compared to Lindberg (2018), could possibly be due to that the orange peels was near to completely exploited by the larvae after 21 days, the BSFL composting time in that study, while that time was not enough time for BSFL composting of banana peels. A theory is that the increased concentration of lignin in the peels after *Rhizopus oligosporus* pre-treatment (the *Rhizopus oligosporus* pre-treatment degraded more hemicellulose than lignin) seems to make the available nutrients harder and slower for the larvae to ingest. As a result, a higher BCR can be seen in Isibika et al (2019) with a BSFL composting time of 30 days even with similar amounts of TS banana peels being treated by the BSFL. In contrast to Rehman et al (2017) and Li et al (2011), degradation of fiber components by the larvae could not be confirmed in this study, as the results are too inconsistent. The lignin content decreased by the larvae in the Control to 70 % of its original amount, which can be seen as a confirmed degradation. However, when separating the larvae from the residue in the Control, the fibers were detached from the rest of the residue (Figure 5), which in turn made it difficult to pick accurate samples. The fibers are visually seen as a leftover, which is a suggestion that breakdown by the BSFL have not occurred. As mentioned in previous studies, microorganisms in the larval gut is the possible reason for the ability to degrade fiber components, rather than enzymes produced by the larvae itself (Espinoza-Fuentes and Terra (1987); Lemaitre and Miguel-Aliaga (2013); Terra and Ferreira (2012)). The reason why inconsistent results of fiber degradation by the larvae is seen between different studies could thus possibly be because of circumstantial quantities of these gut bacteria that may wary as well as the treatment set-ups and material being different in the studies.

Figure 5: Picture of a banana peel Control tray during separation. The compost-like residue can be seen to the left and strains of detached fiber on the right; Robert Almqvist 2019-10-28

5.1.4 Additional results

As mentioned in Bradley and Sheppard (1984), BSFL secrete substances in the substrate they are consuming that inhibits the presence of other fly species. Also, BSFL activity significantly decreases the presence of pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., and other Gram-negative bacteria (Erickson et al., 2004; Lalander et al., 2013; Choi and Jiang., 2014). In this study, higher larval density, and thus BSFL activity, seemed to prevent fungal colonization on the treatments to a higher degree (Figure 6). A cause for this was probably that the substrates were more mixed and less untouched in the boxes containing more larvae because of larval movement. The fungi development seems to thus be interrupted by the presence of more larvae, as well as having lower amount of substrate to grow on. By measuring pH, a change in hydrogen/hydroxyl release from the substrates by larval ingestion may be tracked. The pH reached a more alkaline environment in most treatments compared to the value at the beginning. Progression in the BSFL composting process usually turn the processed material more alkaline (Cicková et al., 2015).

a) b)

Figure 6: Pictures of treatment residue from Control boxes of the a) 3.9 larvae cm^{-2} (1400 larvae) and b) 2.0 larvae cm^{-2} (700 larvae) densities. The box in b) have been colonized by fungi. Robert Almqvist 2019-11-18

The separation process of the residue and larvae is a step that is heavily dependent on the TS and general physical structure of the treatment residue. When no adjustment in moisture content is made to the residue it may be either too wet for dry separation of the larvae from the treatment residue.

a) b)

Figure 7: Pictures of treatment residue from Rhizopus oligosporus treatment after BSFL composting in the a) banana peel and b) orange peel trial. The residue had a tar-like structure, more severe in the orange peel trial, that was unfavorable for sieving; Robert Almqvist 2019-10-29; 2019-11-18

Ignoring moisture adjustment reduces one moment in the process, but neglecting it could lead to difficulties in the subsequent separation process with the residue becoming too wet and viscous (82-86 % moisture content) for sieving (Diener et al., 2011b). The separation process in this study was easiest for the Control residue (Figure 5) and hardest for the *Rhizopus oligosporus* residue in both trials. Separation of the ammonia residue was harder than the Control, but easier than the *Rhizopus oligosporus* in the banana peel trial, while it behaved similarly to the Control in the orange peel trial. The *Rhizopus oligosporus* had a TS of 20 % compared to the Control's of 36 % in the banana peel trial. The Control residue had a fine structure, whereas the *Rhizopus oligosporus* residue rather resembled a slurry, more so in the orange peel than the banana peel trial. Differences in residue structure have been observed when using food waste by Cheng et al (2017), and additionally that study suggests a TS value of >25 % in the initial substrate when using food waste as a substrate, in order to have a feasible separation of larvae from treatment residue and they observed a smooth and orderly separation with TS values of >50 %. As the TS value was in fact around 25 % in the *Rhizopus oligosporus* treatment residue for orange peels, it should be mentioned that the effects of the fungi breaks down the cell walls (fibers) of the peels. This in turn, although it results in a higher TS value, leads to a slurry structure because of the cell walls in the peel residue being broken. The separation process in this study was thus, questionably, performed either too early for water to evaporate, or the composition of the residue was not favorable in the context of sieving. As can be seen in Figure 7, the residue of the *Rhizopus oligosporus* treatment for both banana and orange peel had a tar-like composition.

5.2 Implementation of results

For BSF technology in colder climates, a setback for implementation is that production of larvae has to be conducted in a heated environment, which depending on the source of energy, may have negative environmental impacts (Halloran et al., 2016). In places around the world where

banana and orange are cultivated in large scales however, and thus generating fruit waste such as peels, the climate is already favorable for the BSF, so environmental impact caused by heating of BSFL treatment facility in those areas are negligible. In this study, feeding of the larvae was divided onto three occasions to minimize the crust formation and the anaerobic degradation of the substrate before it would be digested by the larvae. According to Liu et al (2017) however, number of feedings should be kept at minimum to reduce operational costs in a commercial scale scenario, and selection of harvest time being prioritized to ensure maximum larvae production. This should be put in context against the optimum feeding dose recommended by Parra et al (2015) at 163 mg $larva^{-1}$ day^{-1} on dry basis. The BSFL composting time could be more evaluated as well. With the different results with Lindberg (2018) and Isibika et al (2019) as seen in Section 5.1.1 (Biomass conversion rate & material reduction) and 5.1.3 (Fiber analysis), BSFL composting time appear to have a substantial impact. The economic, practical and environmental consequences of treating more material under shorter time with less larvae yield versus less material under longer time with more larvae yield is a subject that requires more in depth investigations, and could also be impacted by local context.

Whereas the material reduction of some substrates is high (Lindberg (2018) reached more than 80 % for ammonia treated orange peels), heavy metal accumulation is a concern (Gold et al., 2018) when the residue is to be applied as a soil conditioner. As for larvae, concentration of microorganisms in and onto it is a potential health risk when used as feeding for animals (Lalander et al., 2013). A lack of profound knowledge about the fate of certain chemicals, microorganisms and biomolecules in BSFL composting limits the use of mixed food waste, out of safety reasons, as a substrate for feeding larvae (EFSA., 2015; Makkar et al., 2014). Also, as of 03/2020, it is still illegal to use animal by-products, which could be present in mixed food waste, for BSFL composting according to EU law. The use of homogenous vegetable biodegradable waste for BSFL composting thus remains of great interest for the future of this technology. In this study, except ammonia pre-treatment combined with higher larval density, the use of pre-treatments did not significantly increase the total biomass conversion rate for banana peels or orange peels. According to a study by Lindberg (2018), mixed food waste was superior as substrate compared to pre-treated cauliflower and orange peel. In addition to the results in this study, the biomass conversion rate of peels with pre-treatment in BSFL composting are of mixed and inconsistent results (Isibika et al., 2019; Lindberg., 2018). However, the indirect value of having a clean homogenous substrate for BSFL composting in terms of hygiene should not go unnoticed. As a result of above mentioned studies, homogenous substrate mixtures could be a preferable method for research to increase BCR and potential effectiveness, revenue and feasibility for BSFL composting.

6. Conclusion

For banana peels, neither ammonia pre-treatment and *Rhizopus oligosporus* pre-treatment improved the biomass conversion or material reduction rates compared to the Control. The pre-treatments used in the banana study were not favorable to enhance the total biomass conversion rate on banana peels.

For orange peels, *Rhizopus oligosporus* pre-treatment significantly decreased biomass conversion rate and material reduction. Ammonia pre-treatment increased biomass conversion rate in the higher larval density setup.

Higher larval density had higher biomass conversion rate for the same amount of substrate in all treatments. Whether it is more economically and environmentally viable to have higher larval density were not analyzed in this paper.

There was no significant difference in crude protein- and amino acid content on TS basis in the larvae depending on treatment.

There was no significant difference in fiber reduction by the larvae depending on feeding substrate. Values are inconclusive to determine whether degradation of hemicellulose, cellulose and lignin by BSFL have occurred.

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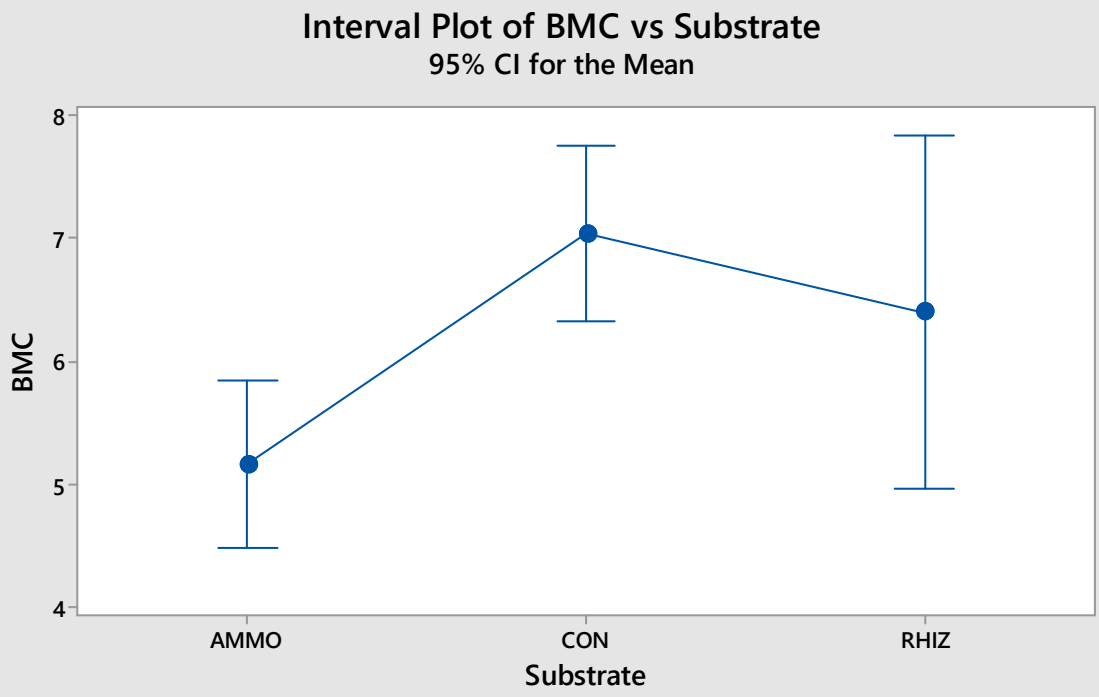
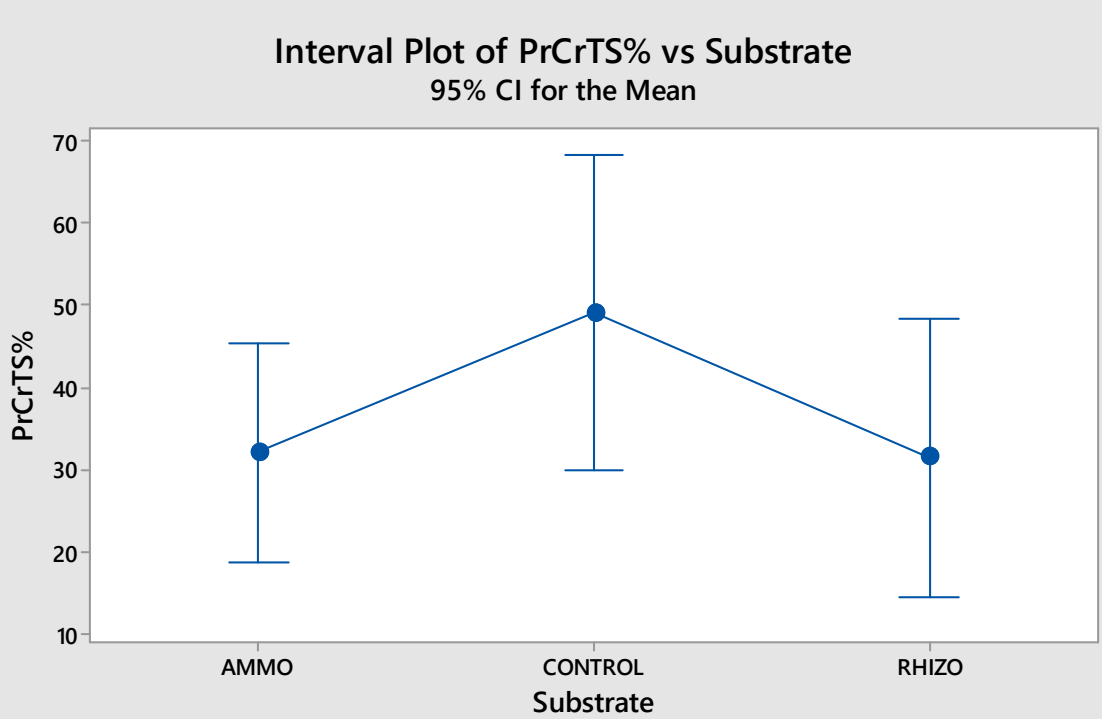
Appendix

Figure S1: One-way ANOVA of biomass conversion rate depending on substrate type for banana peels



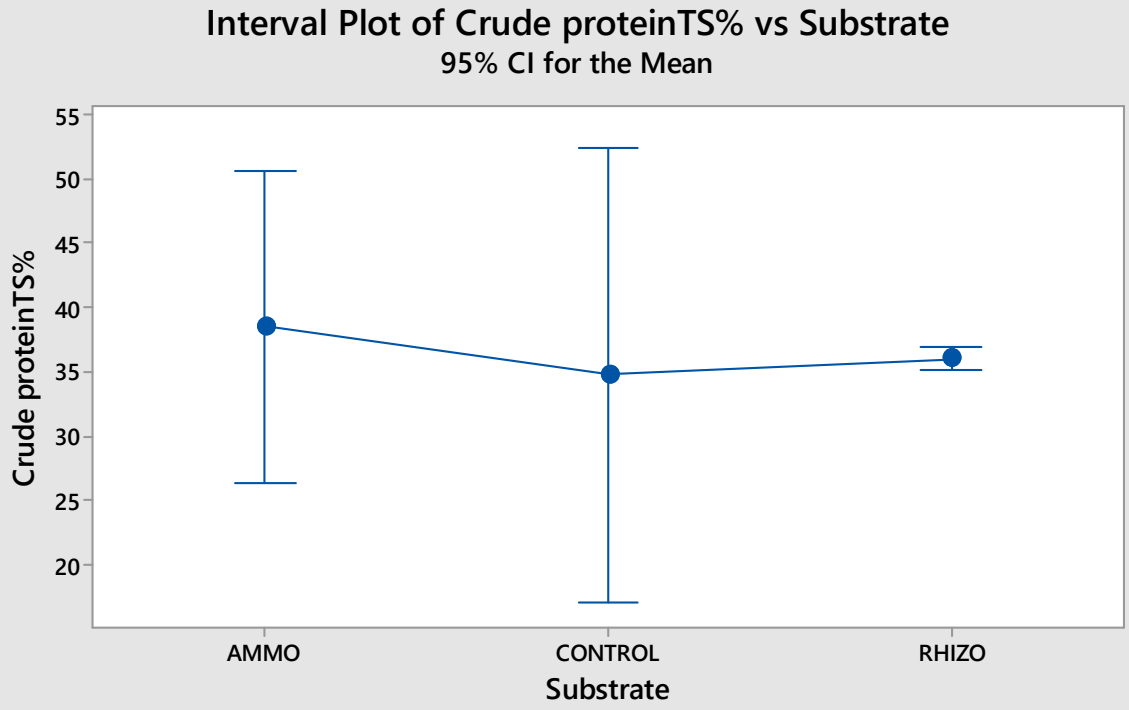
Individual standard deviations are used to calculate the intervals.

Figure S2: One-way ANOVA of protein conversion rate on TS basis depending on substrate type for banana peels



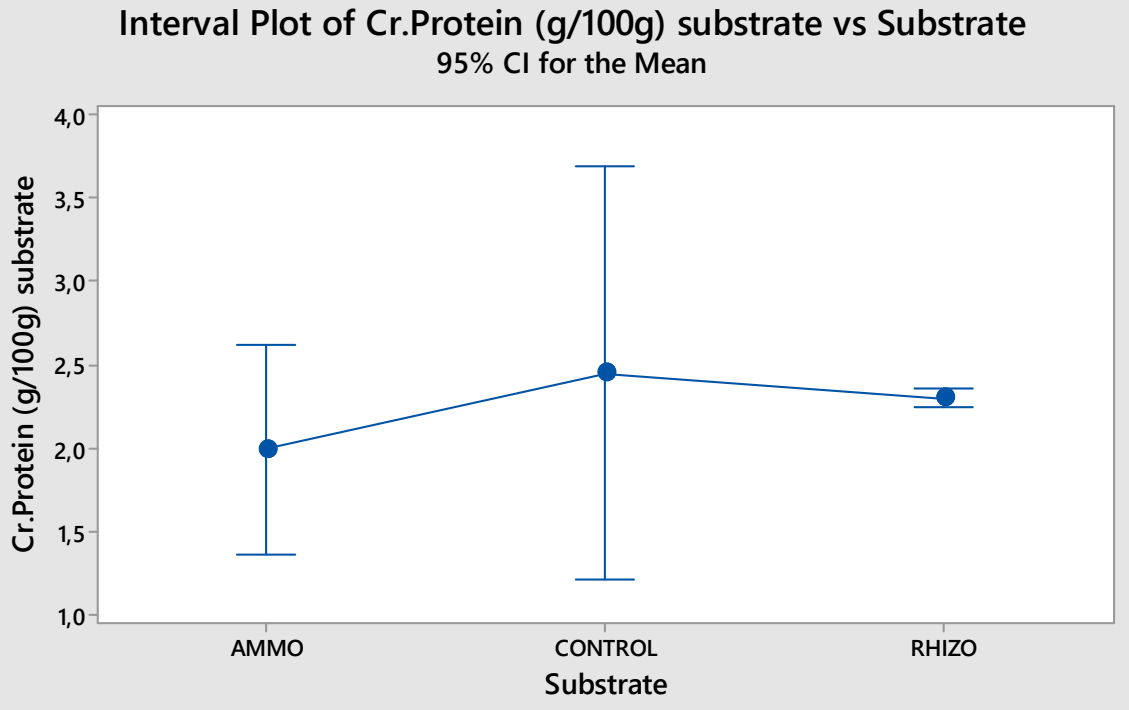
Individual standard deviations are used to calculate the intervals.

Figure S3: One-way ANOVA of crude protein amount in the larvae on TS basis depending on substrate type for banana peels



Individual standard deviations are used to calculate the intervals.

Figure S4: One-way ANOVA of total crude protein amount harvested in the larvae on TS basis depending on substrate type for banana peels



Individual standard deviations are used to calculate the intervals.

Figure S5: One-way ANOVA of biomass conversion rate depending on substrate type for orange peels

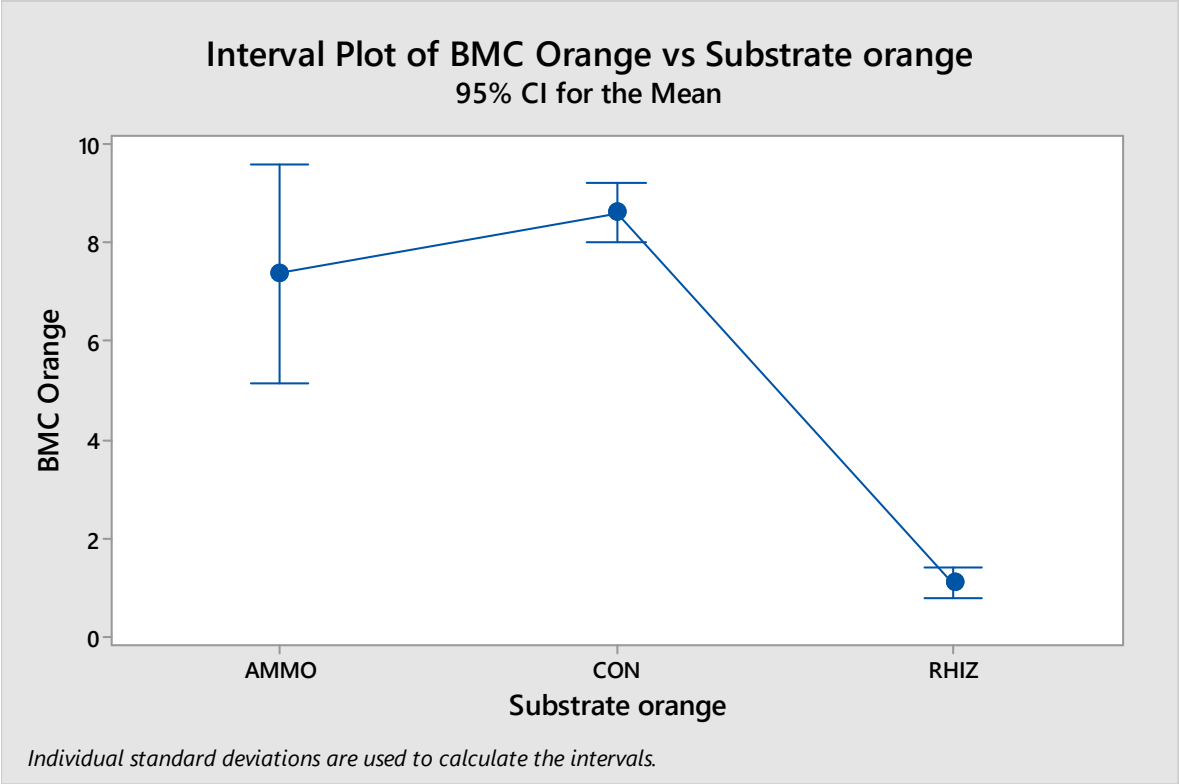


Figure S6: One-way ANOVA of biomass conversion rate depending on larval density for orange peels

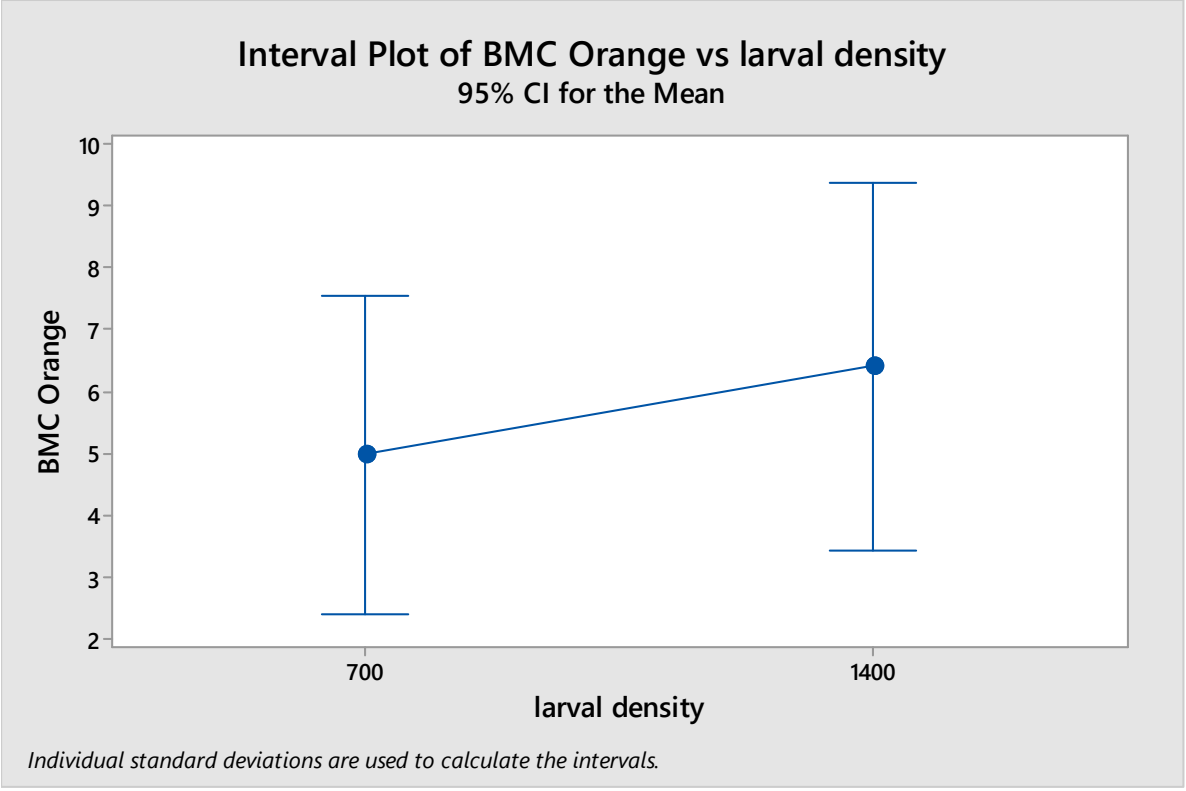


Figure S7: One-way ANOVA of hemicellulose degradation by larvae depending on substrate type

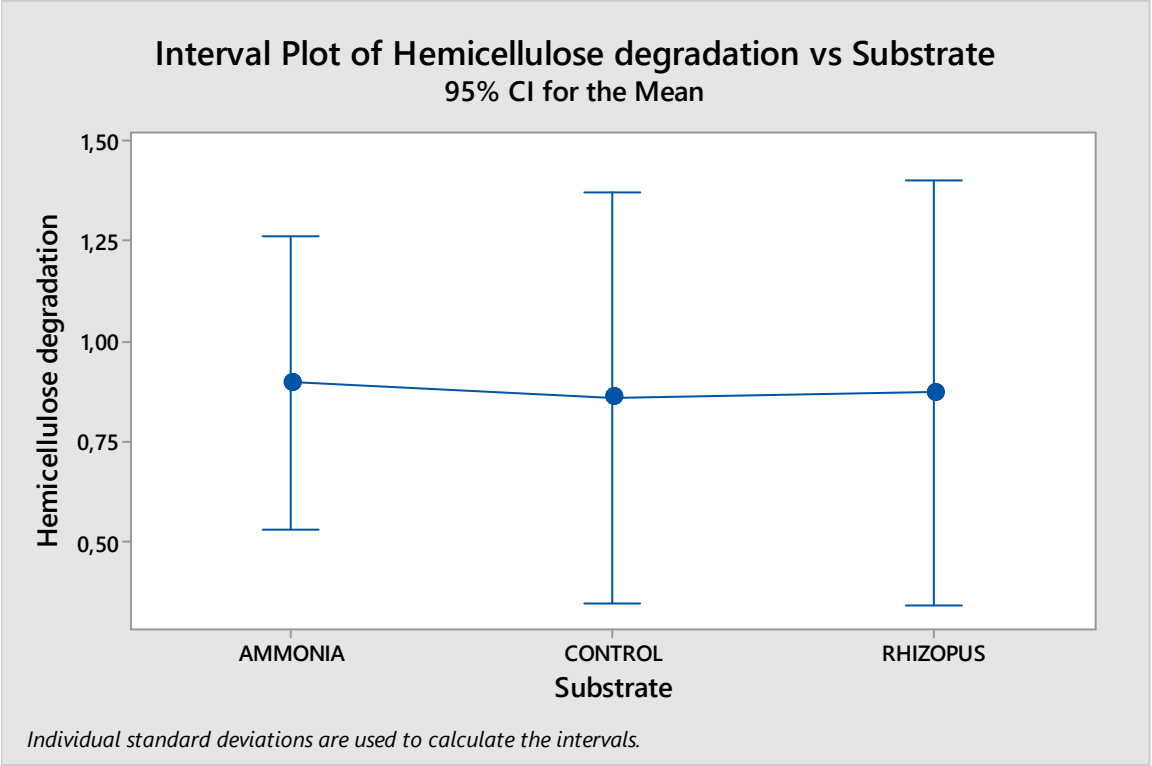


Figure S8: One-way ANOVA of cellulose degradation by larvae depending on substrate type

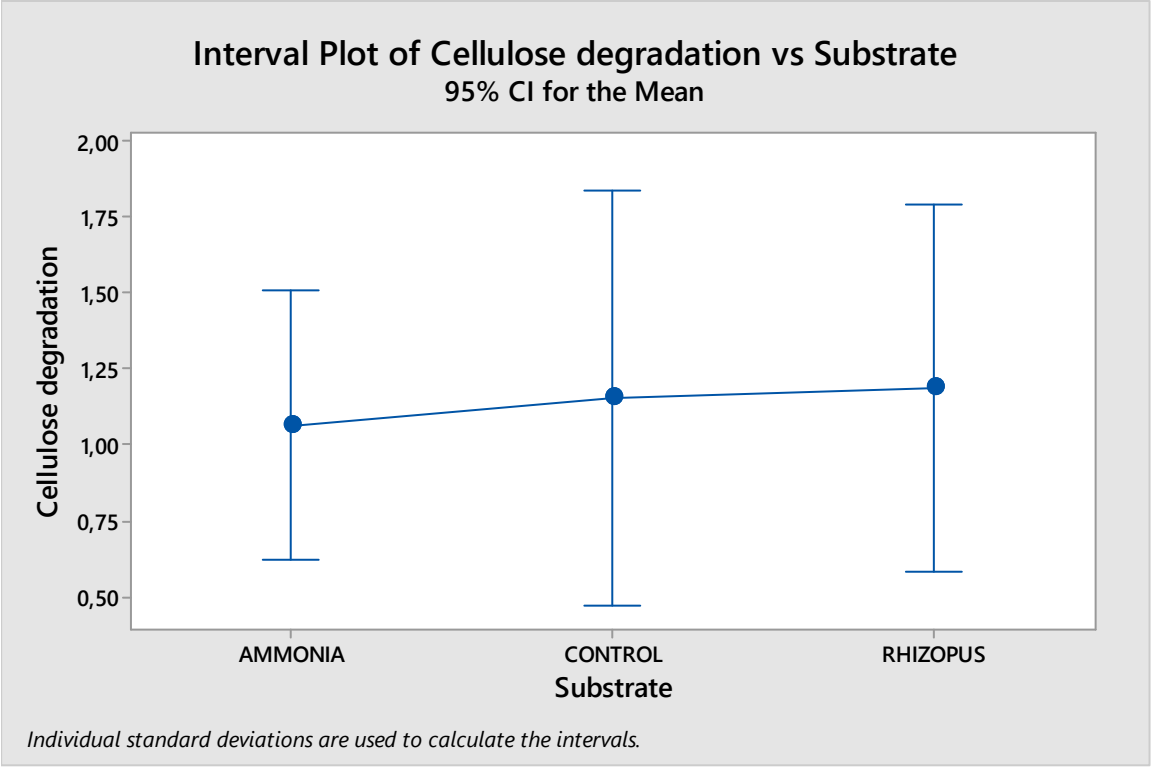


Figure S9: One-way ANOVA of lignin degradation by larvae depending on substrate type

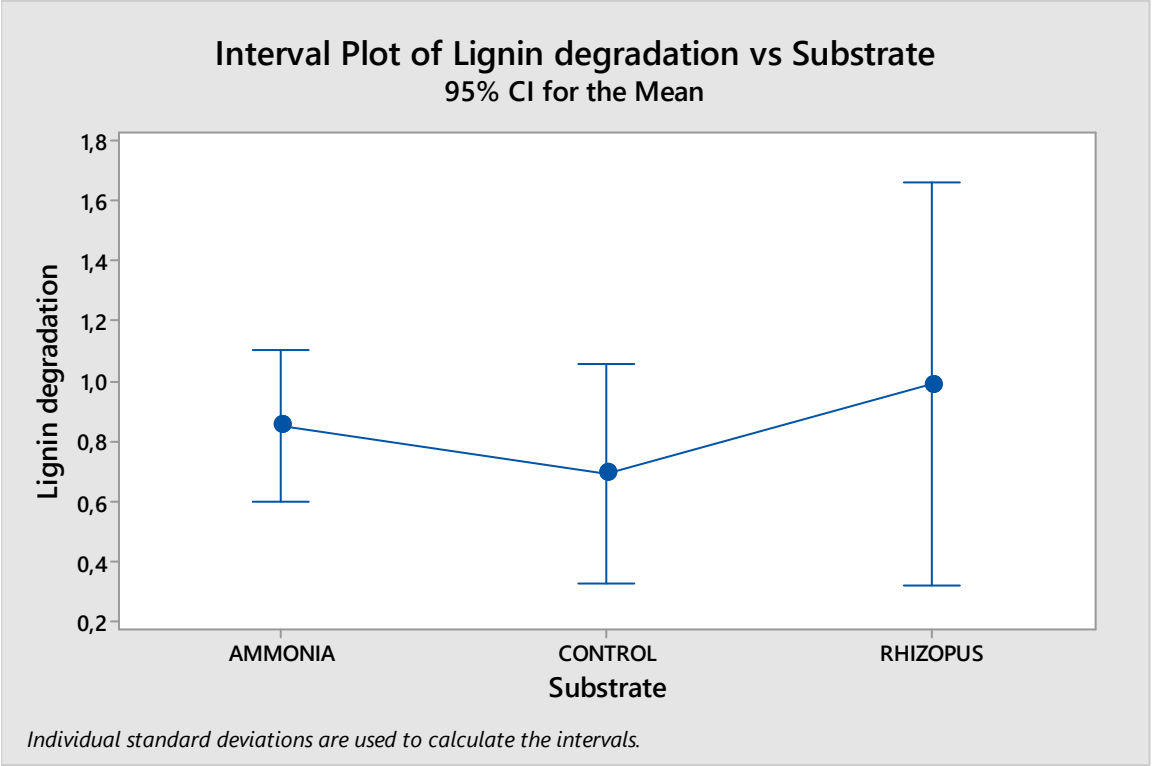


Figure S10: One-way ANOVA of survival rate depending on substrate type

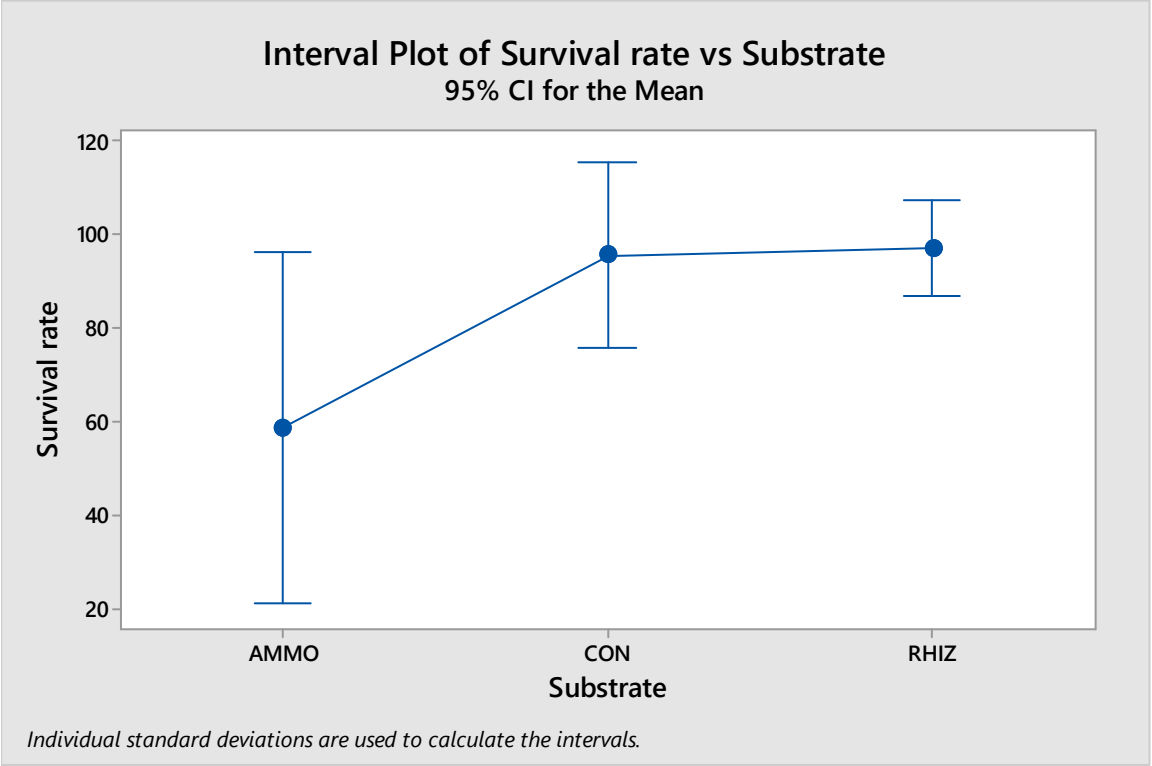
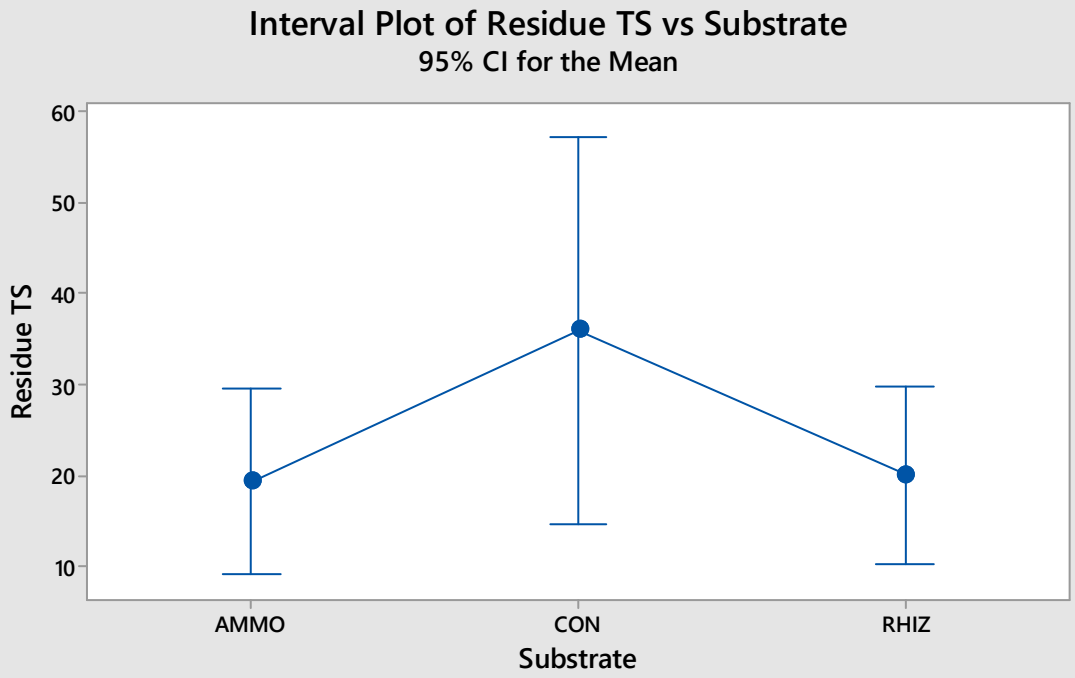
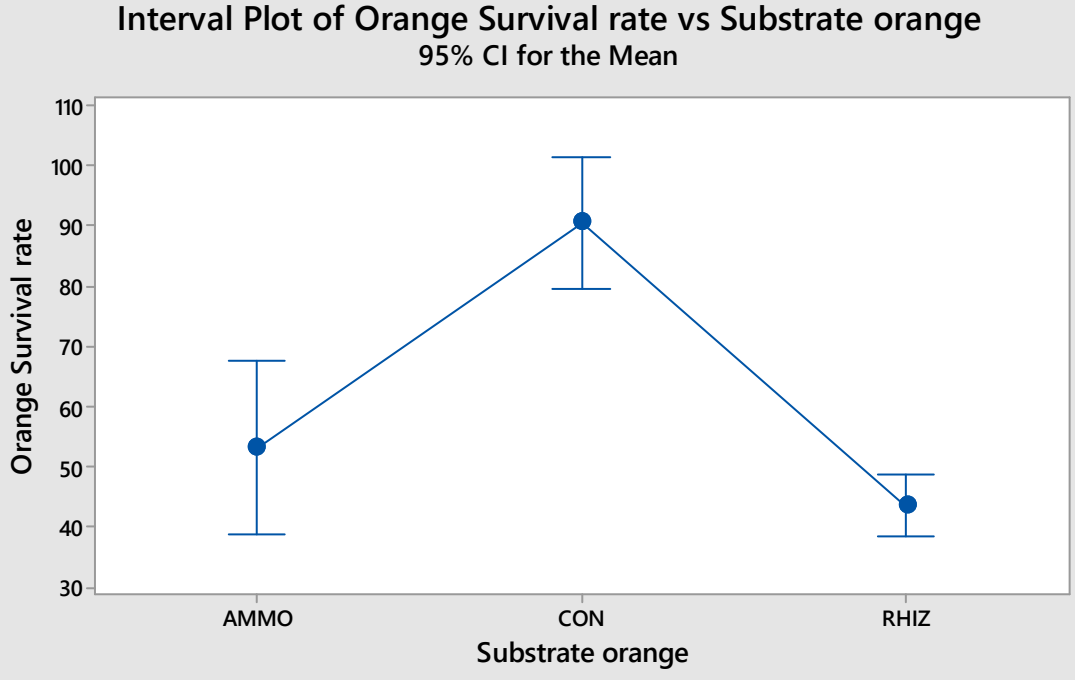


Figure S11: One-way ANOVA of residue TS depending on substrate type



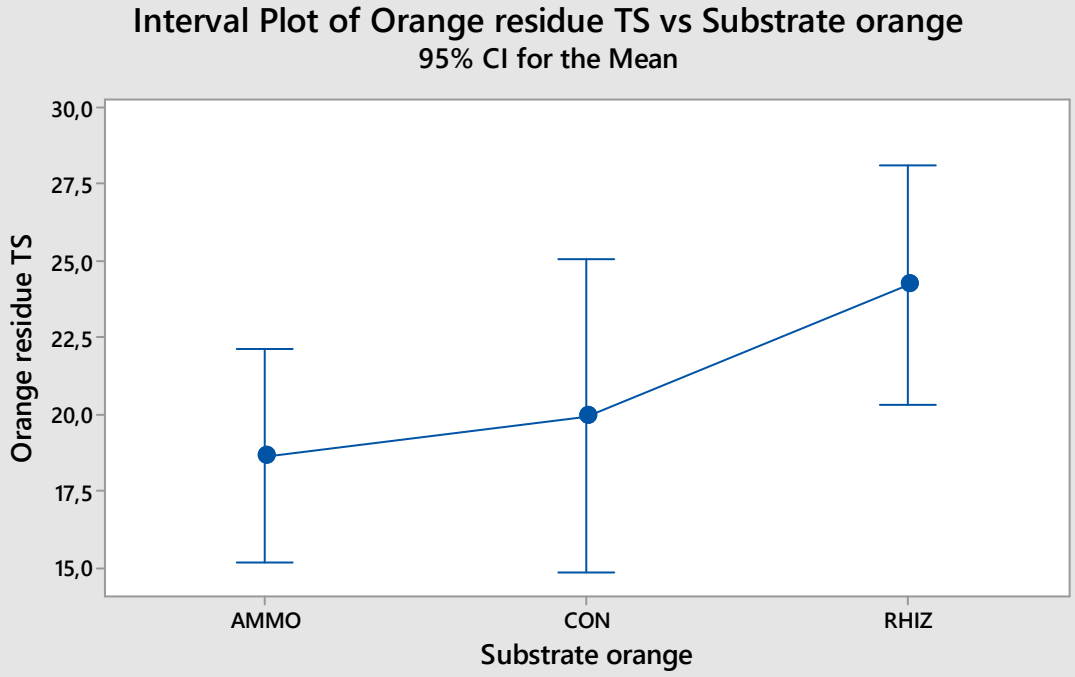
Individual standard deviations are used to calculate the intervals.

Figure S12: One-way ANOVA of survival rate depending on substrate type in orange peels



Individual standard deviations are used to calculate the intervals.

Figure S13: One-way ANOVA of residue TS depending on substrate type in orange peels



Individual standard deviations are used to calculate the intervals.

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