Growth of BSF (black soldier fly, *Hermetia illucens)* larvae on organic waste streams of potato processing and malting industries in the Netherlands

Experiment March 2021

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WAGENINGEN UNIVERSITY & RESEARCH

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Abstract: BSF larvae are highly efficient in converting all kinds of waste streams into larval protein-rich biomass and organic matter. These properties make the BSF larvae perfect candidates for upgrading local waste streams into organic fertilizer in the pilot project 'Kringlooplandbouw Veenkoloniën' (circular agriculture Veenkoloniën). In this report different regional substrates, such as germinated barley, barley dust, secondary food industry sludge, primary sludge, pig manure solids, or combinations of these, were used to grow BSF larvae. The growth of the larvae was closely monitored. At the end of the 7 day experiment, the dry matter of the larvae, the growth rate per day, the conversion rate of the substrate and the ease of separating the frass from the larvae were determined. The BSF larvae grew on each substrate, but the highest growth rate (fresh weight) was observed when the larvae were grown on the substrate of germinated barley + barley dust. The observed growth rate was 10.0 mg/day of fresh weight respectively. The highest substrate conversion index (WRI) based on dry matter of 11.93 g/d was observed in primary sludge + germinated barley. Overall, these regional substrates show potential as a feed stream for the cultivation of BSF larvae.

Keywords: black soldier fly larvae, BSF larvae, *Hermetia illucens*, bioconversion, waste streams, potato processing industries, malting industries, barley, sludge, pig manure, protein, fat, N, P, K.

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Photo cover: Klaas van Rozen

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Preface

ACRRES is involved in the pilot project 'Kringlooplandbouw Veenkoloniën' (circular agriculture in the Veenkoloniën) which is aimed at closing agricultural cycles on regional level. The project ran from June 2020 till February 2022. By closing nutrient cycles, emission levels are lowered and secondly, soil quality can be maintained or improved by applying local organic waste streams to the soil. Organic waste streams can be converted/processed by insect larvae and earthworms, resulting in a protein rich stream (insect larvae and worm biomass) but also in frass and vermicompost (i.e. insect larvae and worm faeces) respectively. Both streams are already used in agricultural practices on small scale. With the help of Avebe and Holland Malt, suitable organic waste streams were identified for the cultivation of insect larvae and worms. Within this project it is researched if those waste streams are beneficial for the biodiversity and profit of the agricultural practices in the region 'Veenkoloniën'. This project is a part of the project 'Innovatie biodiversiteit Veenkoloniën' (Innovation biodiversity peatlands) and the Public Private Partnership AF-17052 Biobased valorization of manure and digestate.
More information:

- https://www.nmi-agro.nl/2020/12/08/pilot-kringlooplandbouw-veenkolonien/
- https://anog.nl/innovatie-biodiversiteit-veenkolonien
- https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/Biobased-opwaarderen-mest-en-digestaat.htm
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Summary

Larvae of the black soldier fly (BSF; *Hermetia illucens)* are voracious eaters and can grow on a wide range of substrates (Newton et al., 2005). They partly break down the organic fraction of all sorts of waste streams, making them highly interesting for waste reduction projects. Conversion of waste streams by BSF larvae results in two streams, namely their own biomass (BSF larvae are rich in protein and lipids making them highly nutritious as animal feed) and fertilizer in the form of the residual substrate and frass (insect larvae faeces). A pilot for circular agricultural practices in the Veenkoloniën area of the Netherlands is interested in closing the nutrient cycles locally, and preferably combining this with improving soil quality. In this report, seven local waste streams were explored as a growth substrate for BSF larvae obtained from the potato processing industry, Avebe and the malting industry, Holland Malt. The seven substrates are the following; 1) Germinated Barley (GB); (2) Germinated Barley and Barley Dust (GB +BD); (3) Secondary food industry sludge (SFIS); (4) Primary food industry Sludge and Barley Dust (PS +BD); (5) Primary Sludge and Germinated Barley (PS +GB); (6) Pig Manure Solids (PMS) and (7) Chick Starter (CS).

The experiments were performed in triplicate. Each container contained around 10 kg of substrate (fresh weight) and the amounts were adjusted for similar substrate height in the different containers (4-5 cm). 1850 Larvae/kg substrate were added to each container. Temperature in the climate chamber was 30.1 ± 0.5 °C, relative humidity (RH) was 67.5 ± 1.8 %. The larvae were harvested after seven days and their weight and dry matter content were determined together with the amount of frass. In addition, the ease of separating the larvae from the residual substrate/frass was evaluated.

A couple of parameters were calculated from the data collected in this report, namely the growth rate (GR) in mg/d (fresh weight), the waste reduction index (WRI) (g dry matter /d) and the efficiency of conversion of the ingested food per substrate (ECI) (g dry matter larval growth/g dry matter of ingested food). Larval fresh end weight was biggest in Germinated Barley and Barley Dust (76.9 mg per larvae). Larval fresh weight decreased in the following order; Germinated Barley + Barley Dust > Chicken Starter > Secondary Food Industry Sludge > Primary Sludge + Germinated Barley > Germinated Barley > Pig Manure Solids > Primary Sludge + Barley Dust. Larval growth rates decreased in the same order as the larval fresh weights. The growth rate of Germinated Barley + Barley Dust was 10.0 mg/d. The Waste Reduction Index decreased in the following order, Primary Sludge + Germinated Barley > Chicken Starter > Germinated Barley > Primary Sludge + Barley Dust > Pig Manure Solids, where Primary Sludge + Germinated Barley had a waste reduction index of 11.93 g/d. The Efficiency of Conversion of Ingested food declined in the following order; Germinated Barley > Pig Manure Solids > Primary Sludge + Germinated Barley > Primary Sludge + Barley Dust > Chicken Starter, whereas Germinated Barley had an ECI of 0.14 g/g.

The frass obtained during the experiments was analysed for both nutritional value and heavy metal content. Germinated Barley frass and Germinated Barley + Barley Dust frass have C/N ratios comparable to compost, which makes the frass possibly suitable as a soil improver or fertilizer. Secondary Food Industry Sludge frass of Holland Malt has a high N content, but a low C/N ratio, indicating the frass could be a fast working fertilizer. The frass product of Primary Sludge mixed with either Germinated Barley or Barley Dust had a high C/N ratio, indicating that the frass is a good soil improver with a low mineralization rate. The concentrations of heavy metals are below the limits for all the frass samples. Accumulation of heavy metals in the larvae is not investigated within this research and needs further attention. BSF frass composition is dependent on the larval diet, but it seems possible to obtain frass with characteristics of an organic fertilizer as well as a soil improver.

The substrates that have potential for bioconversion by BSF larvae are Germinated Barley + Barley Dust, Secondary Food Industry Sludge of Holland Malt and Primary Sludge of Avebe + Germinated Barley, providing that residue separation can be improved. These substrates are of interest for continuous experiments and research regarding BSF larvae cultivation.

1 Introduction

Black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (BSF) larvae production is on the rise. BSF larvae can grow on a wide range of residual waste streams (vegetables, fruit, food (industry) waste, manure, slaughter waste etc) and are able to convert large quantities of the organic matter into organic fertilizer and larval protein rich biomass. The biomass can subsequently by used as feed for a variety of animals, including pigs, poultry, and fish (Veldkamp et al., 2021). The end use of the larvae is dependent on their nutritional values, which is related to the substrates they have fed on (Barragán-Fonseca, 2018; Danieli et al., 2019; Gold et al., 2018; Laganaro et al., 2021; Meneguz et al., 2018; Scala et al., 2020). Laganaro et al (2021) postulate that the quality of the substrate alters the metabolism (growth efficiency, respiration etc) of BSF larvae and as a result their overall performance. They degrade a large part of the organic fraction of their substrates. Scala et al (2020) for example found substrate reduction percentages of 59-74 % (fresh weight) for fruit and grain substrates (measured at the moment that BSF larvae growth halted). As an example of biomass growth potential, in these experiments larvae with a start weight of ~ 8 mg grew in 10-13 days up to end individual weights of ~ 140-180 mg (fresh weight). Gold et al (2018) mention a larval individual weight range of 50-299 mg (fresh weight) on a variety of substrates. They wrote an extensive review on literature data for BSF and bioconversion of different substrates/waste streams and concluded that quantity, quality and ratio of protein and digestible carbohydrate seem important for process performance of BSF larvae.

BSF larvae can contain up to 40-45 % of protein, 30-35 % of lipids on dry matter base (Nyakeri et al., 2017). Gold et al (2018) mention 32–58% proteins and 15–39% lipids on dry matter base. Lipid percentage is known to vary more than protein percentage (Scala et al, 2020) and is dependent on protein content of the substate (Barragan-Fonseca, 2018). In Barragan-Fonseca her PhD thesis, she details the influences of protein and carbohydrate content of the substrates on BSF larvae performance and composition. Danieli et al (2019) found that the amount of unsaturated fatty acids in BSF larval biomass is linked to that in the substrates.

The frass produced during the bioconversion process is a mixture of larval faeces, substrate residue and chitin-containing larval skins (exoskeletons) that can be used as soil improver/ fertilizer (Watson et al, 2021). The composition of the frass, similar to that of the larval biomass, is dependent on the feed substrate of the larvae, but is generally high in organic matter and available NPK. According to Gärttling and Schultz (2019) the average CN ratio of different frass types is 13.2 (±24 %) (Gärttling et al., 2020). Chavez and Uchanski (2021) wrote a review on different insect frasses and concluded that they have comparable or better results for plant growth than inorganic fertilizers, especially when combined with them. Chitin from the frass seems to have beneficial properties for plant growth and resistance. Of course the potential transfer of unwanted compounds from substrate to larval biomass and frass should be carefully evaluated.

The above qualities make BSF larvae ideal candidates as a waste management tool for upgrading low value waste streams into protein-rich larval biomass and organic fertilizer. BSF larvae can be easily implemented in a circular design for food systems. In the pilot 'Kringlooplandbouw Veenkoloniën', it is investigated how to close nutrient cycles and improve soil quality by organic fertilizers. BSF larvae could be very well implemented here, by upgrading locally produced low value waste streams into larval biomass and organic fertilizer. In this study BSF larvae were grown on 7 different waste streams obtained from the potato processing industry, Avebe and the malting industry, Holland Malt; (1) Germinated Barley (GB); (2) Germinated Barley and Barley Dust (GB +BD); (3) secondary food industry sludge (SFIS); (4) Primary Sludge and Barley Dust (PS +BD); (5) Primary Sludge and Germinated Barley (PS +GB); (6) Chick Starter (CS) and (7) Pig Manure Solids (PMS). The aim of this study was to evaluate total larval biomass growth, yield and macronutrient content of the BSF larvae after feeding for 7 days on the different waste streams. Breakdown of the substrates and ease of sieving the final product were also evaluated. In addition, analyses were done on the start and end products.

2 Materials & methods

2.1 Larvae and substrates

Newly hatched larvae of the Texas strain of BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae; 100 generations; 38 days egg to egg cycle) were fed with a substrate consisting of 30 % wheat bran and flour, and 70 % water during 7 days at the facilities of Bestico B.V. (Berkel en Rodenrijs, the Netherlands). Once larvae were 7 days old (starter larvae), they were sieved, packaged at 10-15 °C and shipped to the Lelystad test facility of Wageningen University & Research.

The first substrate was germinated barley (GB), obtained from Holland Malt, maltings (Eemshaven, the Netherlands). The germinated barley is a residual stream after germination, a soft and stringy substrate. The second stream obtained from the same location of Holland Malt was barley dust (BD); this substrate is mixed with other substrates, and is not tested on its own. The barley dust consists of small particles, leftovers from the barley. This waste stream is available after cleaning and sieving of the barley kernels. The third substrate from Holland Malt is secondary food industry sludge (SFIS). This substrate can be best described as doughy, sticky material. When it's dried, it becomes granular. The SFIS is obtained from the water cleaning station of the maltings at Eemshaven, the Netherlands. The fourth stream was obtained from Avebe, potato starch and protein producer, and was a primary food industry sludge (PS). This substrate is a waste stream from the factory in Gasselternijveen, the Netherlands. Wastewater from the factory is pumped into a basin where the particles (such as protein or starch) in the wastewater are allowed to settle. This settled material is collected twice a year and constitutes the PS. The primary food industry sludge can be best described as doughy and heavy. The fifth and last substrate is pig manure solids (PMS), which were obtained from Van Beek SPF Varkens B.V. (Lelystad, the Netherlands). The pig manure solids were obtained fresh and can be best described as big and lumpy turds. As a reference chick feed starter (CS) was used, obtained from the Welkoop in Ede, the Netherlands.

A couple of the above mentioned substrates were mixed, resulting in new substrates. These included a mixture of germinated barley and barley dust (GB +BD), primary sludge and barley dust (PS + BD) and primary sludge and germinated barley (PS +GB). The first can be described as sticky and moist material, whereas the second can be described as moist, but the addition of the dust makes the substrates a bit dryer and crumblier. The third is soft and moist, with the stringy material of the germinated barley.

The composition of the starting substrates, as obtained from the manufacturers, is shown in Table 1 below.

Table 1 Composition of the substrates obtained from the starch and malting industries.

Component	Germinated Barley Holland Malt	Barley Dust Holland Malt	Secondary food industry sludge Holland Malt	Primary food industry sludge Avebe
DM g/kg product	49	865	115	255
Ash g/kg DM	98	85	103	13
Protein g/kg DM	409	101	?	34
Fibres g/kg DM	214	211	?	10
Starch g/kg DM				823
N g/kg DM			96 g TKN/kg *MS	
P g/kg DM			21	

K g/kg DM	
As mg/kg DM	<1.0
Cd mg/kg DM	0.3
Ca mg/kg DM	6900
Cr mg/kg DM	7.4
Cu mg/kg DM	41
Pb mg/kg DM	1.7
Hg mg/kg DM	0.07
Ni mg/kg DM	10
Zn mg/kg DM	310

*MS = the measurement is executed with an original sludge substrate. The result is subsequently adjusted for the dry matter content of the substrate.

In Annex 1, pictures of the substrates are shown at the start of the experiment. In Annex 3, the compositions of the substrates GB, GB + BD, SFIS, PS + BD, PS + GB and CS as a reference are shown, together with the dry matter content, the organic matter content, the nutrient content and the presence of heavy metals.

2.2 Experimental set-up

The unground CS was mixed with water. The substrate PS contained a great deal of free water, this was drained before the start. All the other substrates were used as obtained from the factories. All the substrates were placed in containers, the substrate layer was about 4-5 cm's thick. The amounts of substrate were adjusted to even the substrate height in the different containers. The experiment was conducted in triplicates for each substrate, except for SFIS (single experiment) and PMS (duplicate). On top of each substrate, 1850 BSF starter larvae per kilogram (wet) substrate were incubated in industrial plastic containers (75 x 47 x 15 cm) in a climate chamber with a photoperiod of 0:24 hours (L/D). The containers were placed in three larger boxes (120 x 100 x 60 cm) mostly at random, but not completely, as the containers with the highest moisture content were placed at the bottom to avoid escaping larvae falling down in containers below them. Temperature in the climate chamber was 30.1 ± 0.5 °C, relative humidity (RH) was 67.5 ± 1.8 %. The test lasted for 7 days. Pig manure solids dried out quickly in one of the containers, therefore the top layer was sprayed with 700mL water (30 °C) on day four during the experiment in container 7B. Container 2B, containing germinated barley + barley dust was sprayed on the same day with 350 mL water (30 °C). Container 6B, containing the reference chick starter was sprayed on day six of the experiment with 3000mL water (30 °C). After watering, the substrate, larvae and water were mixed by hand.

Table 2 Amounts of substrates and larvae added. The total amount of larvae per kg substrate was 1850.

Substrates (mixing ratio wet weight based)	Qty of wet substrate (kg)	Total # of BSF larvae	Substrate DM %
GB (100 %)	8.00	14800	16
GB+BD (86.5 %+ 13.5 %)	7.00	12950	26
SFIS (100 %)	10.00	18500	15
PS+BD (90.9 % + 9.1 %)	10.00	18500	47
PS+GB (75.7 % + 18.9 % + 5.4 % BD)	10.00	18500	40
CS (64.7 % + 35.3 % water)	10.00	18500	57
PMS (100 %)	9.00	16650	31

Figure 1　Placement of the containers with substrates/larvae in the larger boxes

2.3 Sampling and analysis

Mean individual start wet weight of the larvae (7.28 mg fresh weight) was determined from three samples of ca. 450 larvae.
At the end of the test, the contents of the containers had to be separated into larvae, frass and residual substrate. Some substrates had not been eaten evenly, i.e. crusts on top of the substrates had formed, particularly primary sludge with additives and pig manure solids. This top-layer of the substrate was removed (together with chaff = substrate residue), subsequently frass and larvae were segregated manually and/or by taking samples at the end of the experiment. Triplicate samples of 2 to 20 g were obtained with a spoon after homogenization of the frass (or frass with residual substrate) with larvae and this material was weighed and manually separated and counted. The number of larvae collected in these samples varied between 27 and 230 and mean larval wet weight was calculated from these samples. Most of the introduced larvae were retrieved, survival of the larvae was reasonable to good at any tested substrate, and varied from 83 to 110 %.
Dry matter contents of the substrates at the start of the experimental period and of the left-over substrate/frass and BSF larvae at the end of the experiment were determined by oven-drying for 48 hours at 105 °C. The samples were frozen and sent to Eurofins. The samples for Agrolab group were sent in a cool box with cooling elements. Several analyses were performed on the different fractions by Agrolab group in Kiel, Germany and Eurofins Agro, Wageningen, the Netherlands. Table 3 shows an overview of which analyses were done for each fraction.

Table 3　Analyses of the different subsamples

Analysis	Larvae	Frass	Residual substrate	Laboratory
Heavy metals		X	X	Eurofins Agro, Wageningen, the Netherlands
pH		X	X	Eurofins Agro, Wageningen, the Netherlands
Weende	X			AgroLab group, Kiel, Germany
Ca+P		X	X	Eurofins Agro, Wageningen, the Netherlands
Moisture content	X	X	X	Eurofins Agro, Wageningen, the Netherlands
Fertilizer value (package)		X	X	Eurofins Agro, Wageningen, the Netherlands

Weende = analyses including the moisture content, crude ash, crude fibre, fat, crude protein and fatty acids content.

2.4 Calculations

The following parameters were calculated according to (Diener et al., 2009).

- Larval growth rate in mg/d (GR) = (final larval average weight − initial larval average weight)/number of days of the trial.

- Waste reduction index (WRI frass) in g/day = ((W − R)/W)/days of trial (d) × 100 where W= total amount of substrate provided; R = residual of the substrate (frass)
- Waste reduction index (WRI frass+ substrate) in g/day = ((W − R)/W)/days of trial (d) × 100 where W= total amount of substrate provided; R = residual of the substrate (frass+ substrate)
- Efficiency of conversion of the ingested food (ECI) in g/g = B/(W−R) where B = total larval + pupal biomass at the end (g); W = total amount of substrate provided; R = residual of the substrate.
- Decrease of dry matter in %: (total residual substrate per container (g) - total amount of substrate at the start per container (g)) / total amount of substrate at the start per container * 100

Parameters related to waste reduction efficiency (WRI and ECI) were calculated on a dry matter basis. SR was calculated on a fresh weight basis.

3 Results and discussion

The amounts of frass and residual substrate were weighed where possible. Although normally the residual substrate and frass combined is named frass, the distinction between the two is made in this report, wherever it was possible. Sometimes this distinction was difficult to make, which made it impossible to determine these numbers for all replicates of each substrate. An overview of the results is given in Table 4 below and Figures 2, 3, 4 and 5.

Table 4 Parameters at the end of the test with germinated barley (GB) with barley dust (GB+BD, secondary food industry sludge (SFIS), primary food industry sludge with barley dust (PS+BD) or germinated barley (PS+GB) , pig manure solids (PMS) and chick starter feed (CS) as a reference. All results are triplicates unless stated different. The standard deviations are indicated where necessary.

	Larval average fresh weight end (mg)	# of larvae	Total average DM of larvae (g)	Average frass g DM
GB	59.6 ± 1.7	14131 ± 565	138.0 ± 7.1	**541 ± 69
GB+BD	76.9 ± 4.3	12104 ± 681	190.0 ± 12.9	-
SFIS*	72.7	17614	277.4	867
PS+BD	42.7 ± 5.9	19071 ± 937	227.6 ± 43.1	2333 ± 605
PS+GB	62.6 ± 1.6	17305 ± 1433	316.5 ± 22.6	2305 ± 113
PMS**	45.0 ± 3.2	15802 ± 27	158.3 ± 10.7	1412 ± 132
CS	74.0 ± 24.3	17925 ± 785	434.9 ± 155.7	-

*It was not possible to determine all parameters for each substrate, those are indicated with an '-'. The results shown for SFIS are all from a single determination. The results from PMS are duplicates. Furthermore, the star signs indicate if the indexes are calculated from *single or **duplicate experiments.*

The ease of separation of frass and larvae is also a parameter that is of significance, especially when larvae are grown in an industrial environment. Easy separation makes scaling up of the process easier. Substrates that were easy to separate after larval rearing (by sieving) were Primary Sludge (in combination with Germinated Barley or Barley Dust), Pig Manure Solids and Chick Starter. The Secondary Sludge of Holland Malt was very wet and therefore difficult to sieve, this sample was therefore rinsed. The Germinated Barley led to difficulties with separation as the material was moist and sticky.

The following figures show the growth rate of the BSF larvae, based on wet weight (Figure 2), the decrease of the dry matter content at the end of the experiment (Figure 3), the WRI (Figure 4) and the ECI (Figure 5). The standard deviations are indicated where necessary.

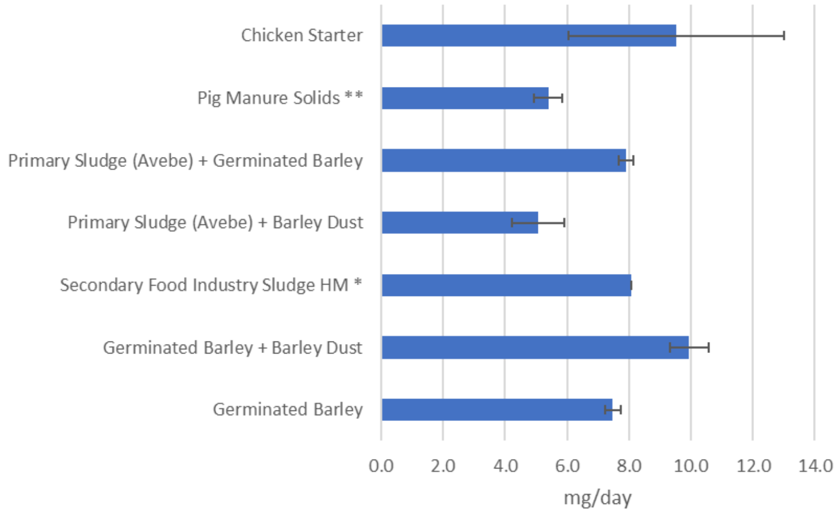

*Figure 2 Growth rate of the BSF larvae on different substrates (** duplicate; * single, no additional signs means triplicates).*

The average wet weight of the larvae at the end of the experiment was highest when the larvae where grown on GB+BD, resulting in larvae with an average weight of 76.9 mg. The larvae in the reference chicken starter reached a weight of 74.0 mg per larva. The corresponding growth rates (mg fresh weight/d) are respectively 10.0 and 9.5 mg/d. The lowest growth rate and mean individual weight were found in larvae grown on PS+BD and PMS where the growth rates were 5.1 mg/d and 5.4 mg/d respectively. The larvae grown on the other substrates had a growth rate just below the reference of the CS, namely 7.5 mg/d for GB, 8.1 mg/d for SFIS and 7.9 mg/d for PS+GB. A literature reference mentioned that BSF larvae reached a wet weight mass of 167-308 mg in 10-12 days after the start of the experiment. Those larvae were fed on a mixture of chicken feed and degassed sludge (Laganaro et al., 2021). Our growth rate on the reference substrate is a slightly higher than in the research of Veldkamp et al. (2021), where they obtained a growth rate of 7.2 mg/d for larvae reared on CS. The experiment lasted for 8 days, other parameters are similar to those described in this report. Larvae were also reared on pig manure solids, where they reached a growth rate of 3.2 mg/d. The differences in growth rates of the BSF larvae in this report when compared to other research data can be attributed to the differences in dry matter, the chemical compositions of the substrates or the duration of the experiment. Gold et al. (2018), reviews several BSF bioconversion experiments (see table 2 in their paper) where the duration of these experiments ranged between 15 till 52 days. For the experiments described in this report a test duration of 7 days was chosen, as this fits industrial production cycles.

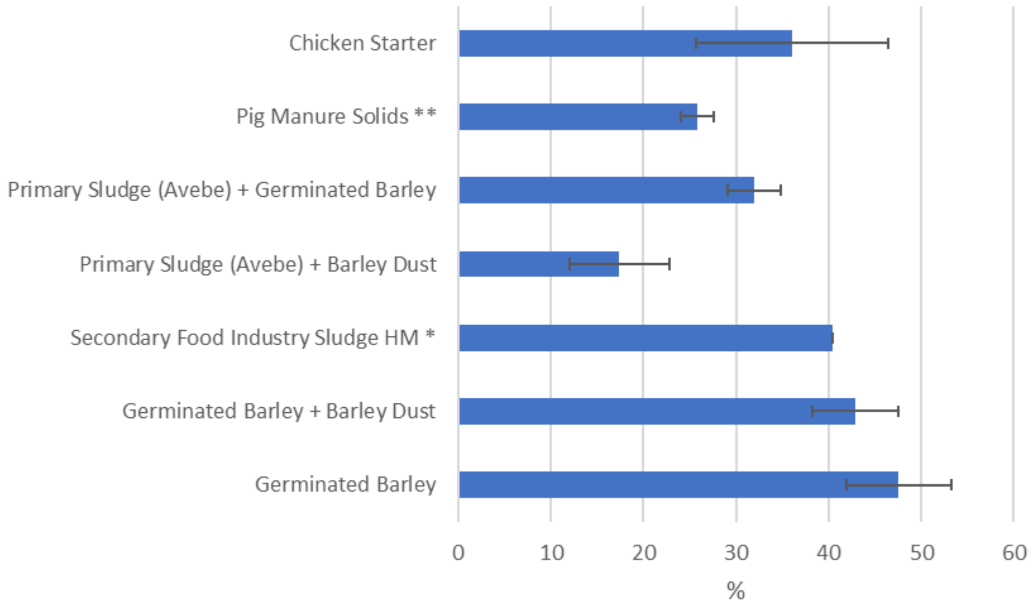

Figure 3 The decrease of the dry matter (%) of the substrates at the end of the experiment. Chicken starter is used as a reference. Standard deviations are shown where necessary. The results shown for Secondary food industry sludge are all from a single experiment. The results from Pig Manure Solids are duplicates.

The decrease of dry matter (%) was highest in the substrates where the larvae had the highest average larval fresh weight as discussed before. Dry matter decreases in GB+BD, SFIS and CS (reference) were 42.9, 40.5 and 36.1 % respectively. Interestingly, the decrease of DM was relatively high in GB, where the larvae did not reach a weight of above 60 mg. The WRI (11.30g/d) in this substrate was similar to the WRI of the reference CS (11.33 g/d). It is hypothesized that the BSF larvae where not able to convert germinated barley to biomass as effective as other substrates. This could be due to the substrate composition, but this is not further investigated in this experiment. In line with this remark, Veldkamp et al., (2021) suggests that substrates such as olive pulp or silage grass, which have a high proportion of directly available carbon but are low in nitrogen content, do not support larval development (Veldkamp et al., 2021).

The waste reduction index (WRI) and the efficiency of the conversion of ingested food (ECI) were previously calculated in this report, see section 2.4 for the formulas. The term R in the formula refers to the residual substrate, whereas it was decided to only take into account the frass (not frass+ residual substrate). Unfortunately it was not possible to separate the frass from the residual substrate in every tray, e.g. those with germinated barley + barley dust and secondary food industry sludge. Therefore it was decided to do two analyses, one where both the frass and the substrate are taken into account, and secondly where only the frass is considered.

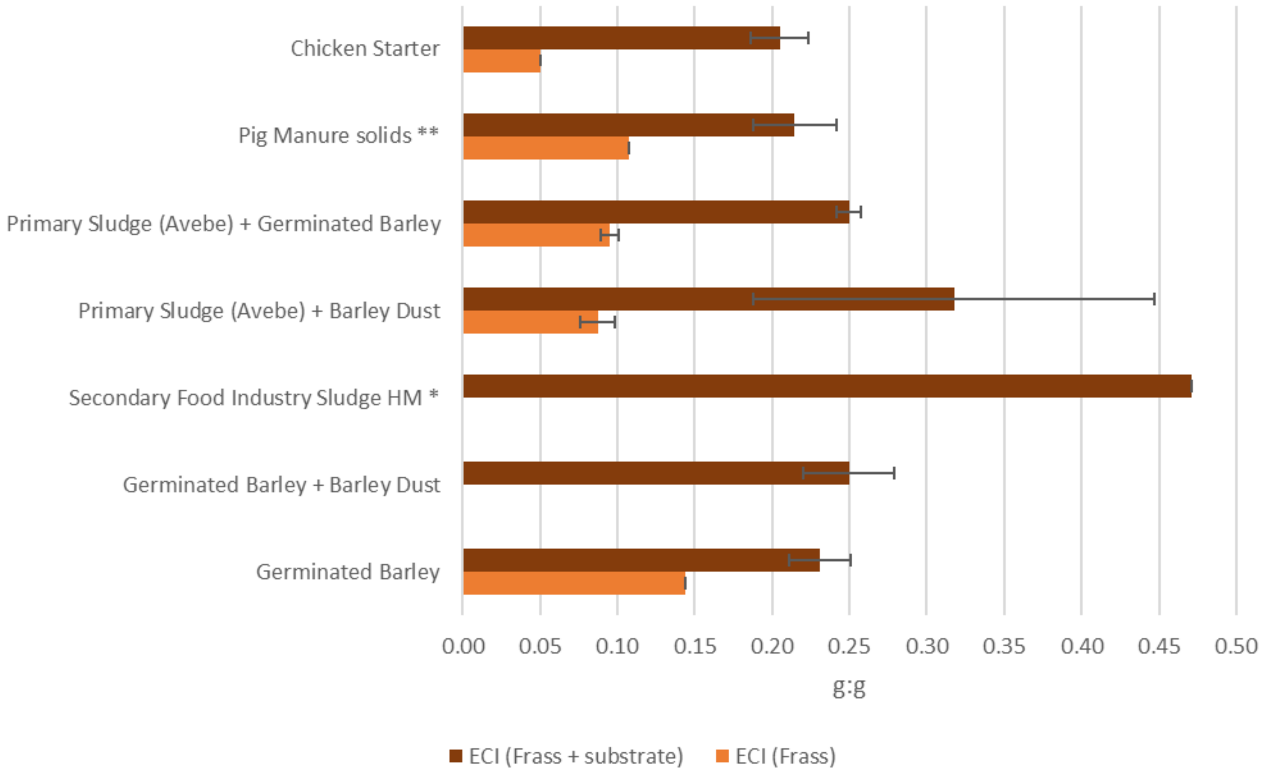

Figure 4 Efficiency of conversion of ingested food is calculated in two different ways. Dark orange bars show Frass + substrate, whereas orange bars only show frass. Results shown for Secondary food industry sludge are all from a single experiment. Results for Pig Manure Solids are duplicates. Standard deviations are shown.

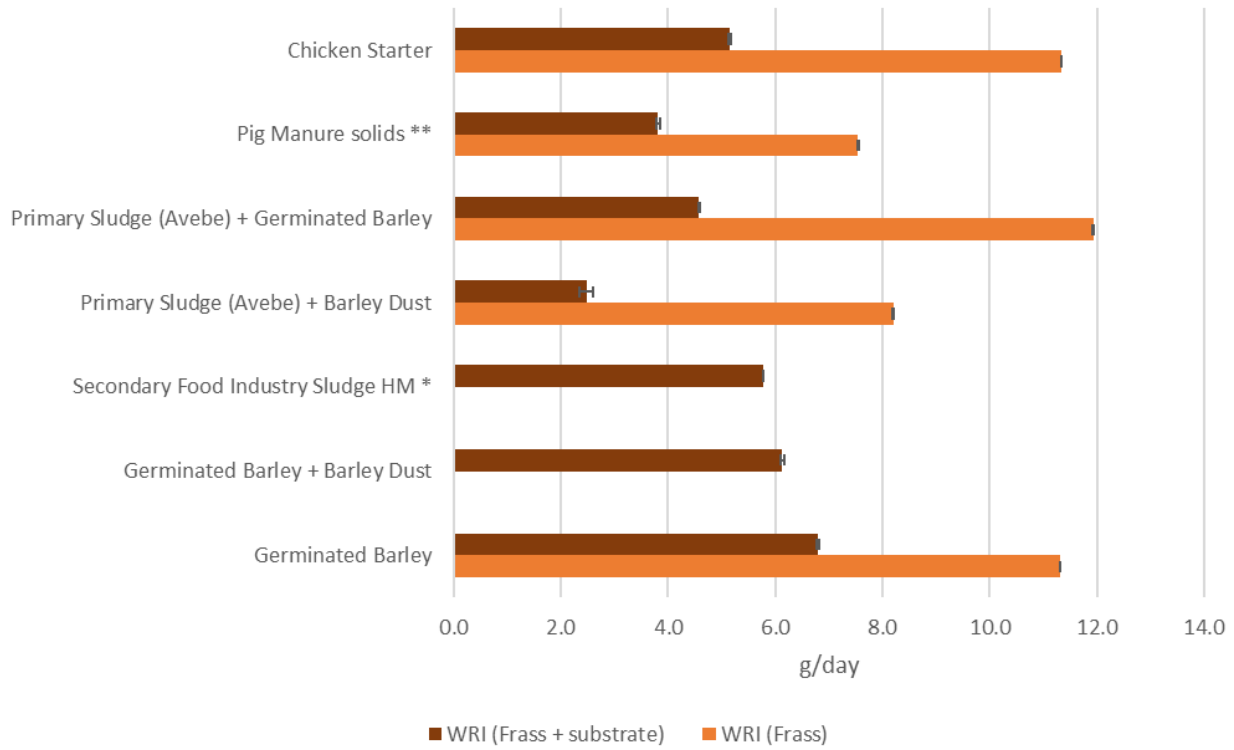

Figure 5 Waste Reduction Index (g/day) is calculated in two different ways. Dark orange bars show frass + substrate, whereas orange bars only consider frass. Results shown for Secondary food industry sludge are all from a single experiment. Results for Pig Manure Solids are duplicates. Standard deviations are shown. Differences between the orange and dark orange bar show that the waste reduction based on only frass is lower than the waste reduction based on frass + residual substrate. The frass + residual substrate means more material, resulting in a lower WRI.

When the calculation was done with the frass fraction only, it was observed that the WRI in the CS reached 11.33 g/d, which is, as mentioned before, comparable to the WRI of GB (11.30 g/d) and PS + GB (11.93 g/d).
The WRI scored lower (compared to the reference) for PS +BD (8.2 g/d) and PMS (7.5 g/d). The efficiency of the conversion of the ingested food was relatively low in the CS reference, 0.05 g/g DM. The highest ECI was found in larvae grown on GB (0.14 g/g), but it must be noted that this result was from a single event. All the other substrates scored better than the CS reference on ECI, but this does not necessarily mean a high growth rate.

Similar outcomes for the ECI were found by Liu et al, (2018). BSF larvae were grown on pig manure with an ECI of 13.81 ± 1.56 (Liu et al., 2018). Bava et al. (2019), performed an experiment where BSF larvae were reared on different organic by-products, such as okara, maize distiller, brewer's grains and a hen diet as control. The larvae were grown at 25 °C with a relative humidity of 60%, the substrate was provided ad libitum. The experiment was stopped when 40% of the larvae reached prepupal stage. The WRI ranged between 4.46 for the hen diet and 3.01 for the brewer's grain, which is relatively low. The growth rate of the larvae was also lower than ours, ranging from 5.1 mg/d for the hen diet till 1.4 mg/d for the brewer's grain. The ECI was higher when compared to our results, ranging from 0.27 for the hen diet till 0.25 in the brewer's grain (Bava et al., 2019). The differences in WRI can be explained by the experimental setup, since the larvae of Bava et al., (2019) were fed ad libitum, leading to more residual substrate + frass.

A lower WRI was also observed in this report when WRI results were compared between frass and frass + residual substrate (Figure 5). More residual substrate + frass will also increase the ECI (Figure 4), which explains why the ECI observed by Bava et al., (2019) is higher when compared to the ECI observed in this report. The growth rate of the BSF larvae depends on the amount of days the larvae were permitted to grow. A variation between the two experiment durations (between this report and Bava et al., (2019)) possibly clarifies the growth rate differences. GB+BD and SFIS showed a good larval growth rate. The newly calculated WRI and ECI values support those results. For the reference CS a WRI of 5.16 g/d was found and an ECI of 0.20 g/g. Both the GB+BD and SFIS have a WRI (6.13 g/d and 5.78 g/d) and an ECI (0.25 and 0.47 g/g) above the reference.

From the above mentioned results on larvae growth and substrate conversion, plus the ease of separation, the substrates for further research were chosen. The substrates that look most promising (for a possible business model) were Germinated Barley + Barley Dust (GB + BD) from Avebe, Secondary Food Industry Sludge (SFIS) from Holland Malt and Primary Sludge + Germinated Barley (PS + GB) from Avebe. Good larvae growth and conversion of the substrates was observed in GB + BD and SFIS. Ease of separation was more difficult, but can be improved by optimizing the substrate mixtures. PS+GB showed good larval growth and the larvae were easy to separate from the frass. The conversion of the food however could be optimized, the larvae did not eat through the whole substrate since the top of the substrate became a dry crust. The mixtures can however be optimized in future research to obtain the best results possible in terms of larval growth, substrate conversion and ease of separation.

During the test we noted that only a limited amount of larvae escaped. A few larvae escaped from substrate 6 (PMS), when water was added, but no other larvae escaped from the other substrates. It was noted that in two of the triplicates of substrate 2 (GB+BD) the larvae clustered and became inactive. Larvae also became inactive in one of the triplicates of substrate 6 (PMS), the other larvae in this sample clustered together. The inactiveness could be due to lack of food (J. van Schelt., 2021). All the other substrates supported active and lively larvae. For substrate 4 (PS + BD) it was found that more larvae were present at the end of the experiment, than before. This can be explained by the relatively small sampling size, which can lead to bigger measurement errors.

The larvae were sent for analysis to the Agrolab laboratory (Kiel, Germany). The nutritional value of the larvae, such as moisture content, crude protein content and the composition of fatty acids were analyzed (Table 5).

Table 5, *Analysis of larvae grown on the different substrates. Moisture content is displayed as percentage. All other values are expressed as a percentage of the dry matter of the larvae.*

	Germinated Barley	Germinated barley+ barley dust	Secondary food industry sludge	Primary sludge +barley dust	Primary sludge + germinated barley	Chick starter feed (reference)
Moisture content (%)	83.5	78.3	78.8	72	71.8	64.9
Crude ash (% DM)	14.5	11.1	12.7	6.8	6.4	7.1
Crude protein (% DM)	65.5	57.6	65.1	36.1	37.2	47.9
Crude fibre (% DM)	10.9	10.1	6.6	6.4	6.0	7.1
Fat (% DM)	6.7	8.3	8.5	16.1	9.2	17.9
Saturated fatty acids (% DM)	3.42	7.65	3.83	14.25	12.09	16.72
Mono unsaturated fatty acids (% DM)	1.87	2.10	2.95	3.04	3.04	4.36
Poly unsaturated fatty acids (% DM)	1.84	1.62	0.62	0.81	0.93	3.68

The nutritional composition of BSF larvae is related to the substrate they have fed on (Barragán-Fonseca, 2018). The moisture content of the larvae is relatively high (between 83.5 % and 71.8 %), especially when compared with those on the chicken starter feed, where the larvae contained only 64.9 % moisture. This means that the larvae have lower dry matter percentages, resulting in a smaller nutritional value on fresh weight basis and possibly additional processing costs (to obtain dry larvae meal). It is stated in previous research that protein content is much less variable than fat content in BSF larvae (Barragan-Fonseca et al., 2017), however, this was not observed in the current experiment, where a high variability between protein and fat content was visible between the larvae grown on different substrates. Protein, fat and all other parameters are expressed as dry matter percentages. The highest protein content was found in larvae grown on GB and SFIS with respectively 65.5% and 65.1%, whereas the reference contained 47.9% of crude protein. The lowest protein content was observed in larvae grown on PS+BD. These larvae however did contain a high amount of fat, 16.1 % (DM). Only reference substrate CS contained more fat, 17.9 % (DM). All other substrates contained almost half the amount of fat (between GB, 6.7 % DM, and PS+GB, 9.2 % DM). The high variation between the amount of crude protein and fats is in line with research of Chia et al. (2020). They too found that body fat and protein content of BSF larvae were heavily influenced by the type of substrate they grew on. The crude protein content (DM %) ranged between 30 and 46 %, when larvae were grown in four brewers′ spent grains. The fat content of these larvae ranged between 33.2% and 21.1%, for larvae reared on barley and malted barley, both supplemented with water (Chia et al., 2020). In this research, relatively low percentages of fat (6.7%) were found in larvae reared on germinated barley. In our study, the protein content of the larvae grown on all substrates resulted in a higher protein content than 40.8 ± 3.8% (DM based), which is a normal content for BSF larvae (Wang & Shelomi, 2017).

Substrates, frass and residual substrate were analyzed for their organic matter content, NPK content and the presence of heavy metals. The results of the analysis of GB, GB+BD, SFIS, PS+BD, PS+GB and CS are shown in Annex 3. Results shown for reference CS were obtained from the manufacturer of the feed.

Lastly, the frass of the BSF larvae can possibly be reintegrated in the nutrient cycle as crop fertilizer. Dry matter content, organic matter content and nutrients have been determined in frass and substrates before and after the BSF experiment. A complete overview of the analyses is shown in Annex 3. A summarized overview is shown in Table 6.

Table 6 The summarized results of the fertilizer analyses of the frass and several other products for reference.

Product	DM (g/kg)	OM (% DM)	N_t (g/kg DM)	C/N ratio	P2O5 (g/kg DM)	K2O (g/kg DM)	Reference
Frass variants							
GB – frass	182	166	6.0	14	22.9	19	This report
GB + BD – frass	352	319	8.7	18	16	23	This report
SFIS – frass	267	222	20.8	5	80.2	35	This report
PS + GB – frass	535	522	3.3	80	4.4	4.1	This report
PS + BD – frass	576	566	2.1	136	3.0	3.5	This report
Black soldier fly frass	-	-	4.54	-	1.23	2.44	(Chavez & Uchanski, 2021)
Black soldier fly frass	-	-	1.27	-	0.46	2.79	(Chavez & Uchanski, 2021)
Black soldier fly frass	-	-	4.4	-	5.2	4.1	(Chavez & Uchanski, 2021)
Other products							
Solid cow manure (straw)	267	155	7.7	10.1	4.3	8.8	Van Geel et al (2019)
Poultry manure	562	416	28.4	7.3	23	19.2	Van Geel et al (2019)
VGF-compost	696	242	8.9	13.6	4.4	7.9	Van Geel et al (2019)
Green compost	599	179	5.0	17.9	2.2	4.2	Van Geel et al (2019)
Wheat straw	850	765	5.8	65.9	1.6	8.6	Van Geel et al (2019)

To evaluate the fertilizer value of the obtained frass the following parameters are of interest: organic matter content and it's stability, nutrient content in the frass and the presence of other valuable components, such as chitin. An indication of the stability of a product (is it rather a fertilizer or a soil improver?) can be derived from the CO_2 production rate in incubation studies with frass and direct testing in the field. In practice, the C/N quotient is often used as an indication of the stability of a product. It is generally assumed that the higher the C/N the more stable the product, which corresponds to a lower mineralization rate. This is however, not always true.

The diet of the BSF has a large effect on whether the frass is considered as a soil improver or an organic fertilizer. GB frass and GB +BD frass have C/N ratios comparable with VGF-compost and Green Compost.
Looking to the C/N ratio of the SFIS frass (Table 6) it suggests that it is possibly a fast working organic N (P) fertilizer (the low C/N ratio suggests that NH_4-N is present in the sample). GB + BD has a C/N ratio of 18 and may release some N. PS + GB and PS +BD frass have very high C/N ratios (table 6), even higher than wheat straw. In practice these would be regarded as soil improvers with a low mineralization rate.
In a separate gas production test (not shown in this report, more information Wim Bussink (NMI), 2022) the decay rates (CO_2 production) from several products have been measured (frass, BD, sludge, compost and others). Frass (PS+GB) showed much higher CO_2 production rates than that of compost or frass obtained from Bestico (Berkel en Rodenrijs, the Netherlands) despite the high C/N quotient. This matches with results of (Smith, 1986) who observed high CO_2 production rates, independent of the C/N quotient. The results in this separate gas production test showed that both tests (field trials or CO_2 production measurements) are necessary for judging the stability of frass products.
Furthermore, the frass obtained from larvae reared on SFIS is very rich in N and P, and has a low C/N ratio. Watson et al.. (2021) reported C/N ratios for BSF frass of 16 . GB and GB+BD are in the same range, whereas PS + GB and PS + BD have a relatively high C/N ratio.

Also the phosphate and potassium contents of GB frass and GB +BD frass are low in comparison to the other products. SFIS frass has a high P content. Under Dutch law, the amount of P_2O_5 is taken into account for 50% in fertilizing programs because compost has a high mineral (sand) content. In frass generally the ash content is relatively low when larvae are fed on typical organic substrates. This would mean that in case of frass the total P amount has to be taken into account. The results show that the diet of the BSF large determines if the frass could be applied a soil improver or an organic fertilizer (or an intermediate). One of the advantages of frass could be the presence of chitin. This has however not been determined and therefore it is not clear if there is a difference between treatments.

The presence of heavy metals in the substrate and frass (before and after BSF rearing) was also analyzed (Table 7).

Table 7 Heavy metal concentrations and concentration factors for frass relative to substrate in experiments with BSF feeding on SFIS. N.D. = not determined.

Element	Before (mg/kg DM)	After (mg/kg DM)	Concentration
Cadmium	0.38	0.49	1.3
Chrome	9.8	14	1.4
Copper	37	58	1.6
Mercury	<0.04	0.04	N.D.
Nickel	10	17	1.7
Lead	<6.6	<6.4	N.D.
Zinc	332	496	1.5
Arsenic	1.6	2.5	1.6

From these results, heavy metal concentration factors were calculated to be between 1.3 and 1.7 in the frass relative to the substrate, which is in this case SFIS. If it is assumed that all metals will stay in the substrate, and with a dry matter breakdown percentage of 40%, a concentration factor of 1.67 would be expected. This suggests some heavy metals accumulation in the larvae biomass. The concentration of larvae was not analyzed in this report and an accumulation of heavy metals in the BSF larvae can thus not be confirmed. Biancarose et al. (2017), however confirmed this suspicion. BSF larvae were reared on substrates enriched with seaweed, which naturally contains high concentrations of heavy metals and arsenic. They indeed found an increase in heavy metal and arsenic concentrations in the larvae, when increasingly more seaweed was added to their feeding substrate (Biancarose et al., 2017). The content of heavy metals in the BSF larvae is a key factor determining its potential use as feed for i.e. chickens. Besides, legislation has to be adapted before the BSF larvae, reared on substrates that are considered waste, can be fed to e.g. chickens.

4 Conclusions

Results across the different substrates were compared, resulting in Table 9. Table 8 shows an assessment per substrate for substrate breakdown, larval growth, ease of residue separation and potential risks, displayed as +/- ratings. For substrate breakdown, we compared WRI values (calculated from frass only). For larval growth, we compared growth rates per day and for residue separation we compared ease of separation at the end of the experiment.

The boundaries for each parameter are described in Table 8, below.

Table 8 *Boundaries of the parameters linked to a score (+/-).*

	--	-	+-	+	++
Range growth rate	0-1	1-3	3-5	5-7	7-9
Range WRI	2-4	4-6	6-8	8-10	10-12
Range separation	Not possible to separate	Difficult to separate	Takes effort, but separable	Easy and sievable	Separation can be done, sieving only once

The rankings for each substrate are described in Table 9 below.

Table 9 Rating of different aspects of waste stream bioconversion by BSF larvae. The range is between ++ and --, where ++ is best, +- is neutral and -- is worst. The substrates are germinated barley (GB), germinated barley with barley dust (GB+BD), secondary food industry sludge (SFIS), primary food industry sludge with barley dust (PS+BD) or germinated barley (PS+GB) , pig manure solids (PMS) and chick starter feed (CS) as a reference.

	Substrate breakdown (WRI)	Larval growth (mg/d fresh weight)	Residue separation (visual inspection)	Risks
GB	++	+	-	Moist and sticky material, difficult to separate
GB+BD	N.D.	++	-	Moist and sticky material, difficult to separate
SFIS	N.D.	+	+-	Rinsed instead of sieved
PS+BD	+	-	+	Low larval growth rate
PS+GB	++	+	+	Larval growth rate is moderate
PMS	+-	+-	+	Top layer of substrate dries out, small larvae
CS (reference)	++	++	+	Larval growth rate is moderate

Some of the applied waste streams have potential for bioconversion by BSF larvae. The substrates of interest for continuous experiments are Germinated Barley + Barley Dust, Secondary Food Industry Sludge of Holland Malt and Primary Sludge of Avebe + Germinated Barley, providing that residue/larvae separation can be improved. It is shown that the larvae have a high nutritional value regarding protein and fat content. Growth rates of the larvae were moderate when compared to other know researches. The relation between the substrate composition (protein and fat content) and the nutritional value of the BSF larvae needs some further research. It is concluded that BSF frass can be nutrient rich depending on the diet. The means that it is possible to obtain frass with the characteristics of an organic fertilizer as well as a soil improver. The substrate mixtures look promising, however they can still be further optimized in future studies.

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Annex 1 Substrates at the start of the experiment


Figure a Germinated barley


Figure b Germinated barley + barley dust


Figure c Secondary food industry sludge

Figure d Primary sludge + barley dust


Figure e Primary sludge + germinated barley


Figure f Pig manure solids


Figure g Reference, chick feed

Annex 2 Residue separation, pictures of substrates, larvae and frass at different times during the tests

Residue separation in the different substrates;
GB: Difficult to sieve, material is wet and sticky. A small dry top layer formed.
GB +BD: Difficult to sieve, some barley grains are present. The material is wet and sticky. Barley dust is not eaten.
SFIS: One of the triplicates is easy to sieve, the other two can be rinsed instead of sieved. Rinsing was easy.
PS +BD: The frass is easy to sieve. The larvae and barley need to be separated further, by an additional sieving step. A small dry top layer formed.
PS +GB: The frass is easy to sieve. The larvae and barley need to be separated further, by an additional sieving step. A small dry top layer formed.
PMS: The frass is easy to sieve, on the condition that the pig manure solids are eaten completely by the larvae. Additional moisture was added, since the turds dry out easily.
CS: The frass is easy to sieve, on the condition that the moisture content is optimal and the larvae have a propriate size.

Table A1 Pictures of the germinated barley and frass during the experiment

1. Germinated barley (GB)		
During	End of test	End: Frass + substrate
		

Table A2 Pictures of the germinated barley + barley dust and frass during the experiment

2. Germinated Barley + Barley Dust (GB+BD)		
During	End of test	End: Frass + substrate
		

Table A3 Pictures of the secondary food industry sludge and frass during the experiment

3. Secondary food industry sludge (SFIS)		
During	End of test	End: Frass + substrate
		

Table A2 Pictures of the primary sludge + barley dust and frass during the experiment

4. Primary Sludge + Barley Dust (PS +BD)		
During	End of test	End: Frass + substrate
		

Table A3 Pictures of the primary sludge + germinated barley and frass during the experiment

5. Primary Sludge + Germinated Barley (PS+GB)		
During	End of test	End: Frass + substrate
		

Table A4 Pictures of the pig manure solids and frass during the experiment

6. Pig Manure Solids (PMS)		
During	End of test	End: Frass + substrate
		

Table A7 Pictures of the chick starter feed and frass during the experiment

7. Reference, Chick Starter feed (CS)		
During	End of test	End: Frass + substrate
		

Annex 3 Analysis results

Table B1 *The analysis results or the starting substrate, the frass (the product of BSF larvae rearing) or residue (residual non-eaten substrate). The dry matter, the organic content, the nutrient content and the heavy metals are determined from each of the samples. N.D. stands for non-detectable, meaning that the concentration was below the detection limit. A '-' means that the analysis was not performed, or the substance was not present in the analysed sample.*

	Germinated barley			Germinated barley + Barley dust			Secondary food industry sludge			Primary food industry sludge + Barley dust			Primary food industry sludge + Germinated barley			Chick starter (reference)		
	Start	Frass	Residue	Start	Frass	Residue	Start	Frass	Residue	Start	Frass	Residue	Start	Frass	Residue	Start	Frass	Residue
Dry matter (g/kg)	149	182	477	236	352	-	146	267	-	421	576	689	369	535	711	310	-	-
Organic matter (% of DM)	93.6	91.3	91.5	94.3	90.7	-	88.6	83	-	98.1	98.2	97.8	97.9	97.6	95.2	58	-	-
Ash g/kg DM	64	87	85	57	93	-	114	170	-	19	18	22	21	24	48	61	-	-
pH	5.5	5.9	5.8	5.3	6.1	-	6.2	6.3	-	4.4	5.7	5.7	4.8	5.4	6.4	-	-	-
EC (mS/cm 25°C)	-	11.39	7.86	10.62	9.23	-	10.75	20.48	-	2.22	1.55	2.43	2.89	2	2.55	-	-	-
N-total (g/kg DM)	42	33.2	27.4	33.2	24.7	-	86.8	77.8	-	5.2	3.6	6.3	8	6.1	8.6	-	-	-
Phosphate P2O5 (g/kg DM)	13.7	22.9	13.7	11	16	-	50.4	80.2	-	3.21	2.98	3.21	3.66	4.35	3.89	-	-	-
Potassium K2O (g/kg DM)	14	19	14	17	23	-	28	35	-	4	3.5	4.3	4.1	4.1	5.3	-	-	-

S-total (g/kg DM)	3.1	4.6	3.6	2.3	3	-	6	8.6	-	0.4	0.3	0.4	0.6	0.5	0.8	-	-	-
Magn. MgO (g/kg DM)	2.5	3	1.6	2.5	3	-	8.3	10	-	N.D. (<0.77)	0.77	0.81	N.D. (<0.75)	0.86	2.5	-	-	-
Chloor (g/kg DM)	3.7	6.4	4.6	3.5	5.7	-	1.4	1.1	-	0.7	N.D. (<0.57)	N.D. (<0.56)	0.61	1	0.74	-	-	-
Cadmium* (mg/kg DM)	-	-	-	-	-	-	0.38	0.49	-	N.D. (<0.23)	N.D. (<0.12)	-	-	-	-	-	-	-
Chrome (mg/kg DM)	-	-	-	-	-	-	9.8	14	-	N.D. (<3.4)	N.D. (<3.4)	-	-	-	-	-	-	-
Copper (mg/kg DM)	-	-	-	-	-	-	37	58	-	N.D. (<4.6)	N.D. (<4.6)	-	-	-	-	18	-	-
Mercury* (mg/kg DM)	-	-	-	-	-	-	N.D. (<0.04)	0.04	-	N.D. (<0.04)	N.D. (<0.04)	-	-	-	-	-	-	-
Nickel (mg/kg DM)	-	-	-	-	-	-	10	17	-	N.D. (<2.9)	N.D. (<2.9)	-	-	-	-	-	-	-
Lead* (mg/kg DM)	-	-	-	-	-	-	N.D. (<6.6)	N.D. (<6.4)	-	N.D. (<6.8)	N.D. (<6.8)	-	-	-	-	-	-	-
Zinc (mg/kg DM)	-	-	-	-	-	-	332	496	-	26	21	-	-	-	-	54	-	-
Arsenic* (mg/kg DM)	-	-	-	-	-	-	1.6	2.5	-	N.D. (<1.2)	N.D. (<1.2)	-	-	-	-	-	-	-

*maximum level for Pb, Cd, Hg and As according to RICHTLIJN 2002/32/EG: 10, 1 or 2, 0.1, 2 or higher depending on the exact definition (Europees Parlement en de Raad, 2002).

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