Ministry of Environment of Denmark
Environmental
Protection Agency

WICE4Soil
Waste, Insects, and Circular Economy for Soil
-Et MUDP projekt

MUDP Report

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Miljøteknologisk Udviklings- og Demonstrationsprogram

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Photo front-page:Juvenile BSFL being separated from insect frass/DTI

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Contents

Preface

This report concerning the project WICE4Soil was supported by The Danish Environmental Protection Agency under the call of MUDP. The project has run from January 2019 to October 2021 in a close collaboration between Daka Refood A/S, Hannemann Engineering ApS and Danish Technological Institute. In addition, two Danish insect production companies have contributed to the work in WICE4Soil by a i) collaboration with Enorm Biofactory ApS on their parallel MUDP project[1], and with supply of pelletized technical frass from the production of ENTO-MASS ApS.

The purpose of this project is to evaluate the potential of insect frass as a soil fertilizer when Black Soldier Fly Larvae are reared on former foodstuff.

Picture: Technical frass (left) separated from impurities original in bio-pulp

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[1] MUDP project "Enorm Biofactory – Værdiforøgelse af restbiomasser gennem insektproduktion" (MST-117-00460)

Dansk opsummering

Affald, Insekter og Cirkulær Økonomi for jord
Tidligere fødevarer[2] (biopulp) er en værdifuld ernæringskilde, men bruges i dag kun til energiproduktion. WICE4Soil (Waste, Insects and Circular Economy for Soil) bygger oven på resultaterne i det tidligere WICE-projekt, som konkluderede at soldaterfluelarver (BSFL) kan leve på madaffald, og at de resulterende to produkter fra produktionen, nemlig larve-biomasse og larve ekskrementer (insektfrass), har et potentiale som henholdsvis dyrefoder (larver) og plantegødning (frass). I dette WICE4Soil projekt evalueres: *i*) kvaliteten og sikkerheden ved brug af biopulp til produktion af soldaterfluelarver og insektfrass, *ii*) produktionsoptimering af biokonvertering og *iii*) potentialet af insektfrass som landbrugsgødning.

Resultaterne fra WICE4Soil-projektet viser, at produktionen af soldaterfluelarver fra tidligere fødevarer og udnyttelsen af insektfrass som gødning er velegnet ud fra sikkerheds-, kvalitets- og økonomiske perspektiver. Således kan denne anvendelse føre til en bæredygtig up-cycling af tidligere madaffaldsstrømme.

Optimal produktion: 12 larver/cm², én fodringsevent med biopulp og tørstofindhold>20% → potentielt investeringsafkast på 4,7 år
Over en periode på 1½ år, blev der gennemført 20 enkeltforsøg i et pilotproduktionsanlæg, hvor der samlet blev omdannet 1400 kg biomasse til 239 kg larvemasse og 230 kg insektfrass. Larveproduktionen var stabil, uafhængig af årstidsvariation, med en biopulpudnyttelse på op til 95%. Dog havde 2020-forårets COVID-19 nedlukning en negativ indvirkning på kvaliteten af biopulp (→ højt fedtindhold og høj viskositet) og dermed også på produktionseffektiviteten.

En ny enhed, for hurtigere og mere præcis optælling af juvenile larver, blev udviklet i projektet. Driftsparametre for pilotproduktionen blev optimeret med hensyn til fodringsfrekvens, larvetæthed og biopulp-tørstof (TS%). Den endelige anbefaling er: udelukkende fodring ved opstart, larvetæthed på 12 larver/cm² og biopulp-tørstof >20%. Baseret på erfaringerne fra pilotprojektet, blev det beregnet, at et nyt produktionssted med opdræt af soldaterfluelarver fra biopulp vil have et investeringsafkast på 4,7 år under antagelse af en årlig produktion på ~13.000 ton soldaterfluelarver.

Biopulp og soldaterfluelarver produceret på biopulp kan indgå i fødevareværdikæden
Biopulpen havde et fedt- og proteinindhold, der var egnet til produktion af soldaterfluelarver og med en aminosyresammensætning svarende til kyllingefoder. Biopulp-tørstofindholdet var forholdsvis lavt, men kunne øges ved tilsætning af fx kornskaller, kerner og kaffegrums. Tungmetaller og indholdet af mikroorganismer i biopulp var under grænseværdien for dyrefoder. Soldaterfluelarver produceret på biopulp havde et fedt- og proteinindhold, der var egnet til videreforarbejdning til insektprotein- og olieproduktion. Både tungmetaller, *Escherichia coli* og *Enterococcus* spp., i frisk larve var alle under grænseværdien for dyrefoder. Indholdet af *Enterobacteriaceae* var dog over grænseværdien i de fleste enkeltforsøg, og vil derfor kræve et forarbejdningstrin (fx blanchering, tørring) før produktet kan anvendes som foder uden for fødekæden (fx foder til kæledyr).

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[2] Tidligere fødevarer er, af fødevarestyrelsen defineret som "*Animalske produkter eller fødevarer indeholdende animalske produkter, som ikke længere er bestemt til konsum af kommercielle grunde eller på grund af fremstillingsvanskeligheder, mangler ved emballagen eller andre fejl, der ikke indebærer nogen risiko for folke- eller dyresundheden*" fødevarestyrelsen

Definition på teknisk frass accepteret

Det var muligt, automatisk, at adskille insektfrass i en ren <2 mm fraktion og en affaldsfraktion. Den rene fraktion er defineret som teknisk frass uden urenheder. Baseret på resultaterne af WICE4Soil, blev denne definition godkendt af Fødevarestyrelsen i oktober 2019. Affaldsfraktionen, indeholdende urenheder, udgjorde kun op til 10 % af den samlede mængde frass.

Teknisk insektfrass rummer et potentiale som plantegødning

Kvaliteten af insektfrass med hensyn til NPK-indhold svarede til andre gødningstyper, som bruges til jordgødning. Tungmetaller, Salmonella og *E. coli* var alle under grænseværdien for økologisk godkendt gødning, mens *Enterococcus* spp var over grænseværdien i nogle enkeltforsøg.

Teknisk insektfrass rummer et potentiale som landbrugsgødning med hensyn til ydeevne, men håndteringen skal optimeres til anvendelse i stor skala. Under en mark-test blev pelleteret teknisk insektfrass tilført som for-gødning til majsvækst og sammenlignet med udbringning af mineralsk gødning. Den samlede mængde af tilført insektfrass var større end mineralgødningen for at nå det samme tilførte P-indhold, og udbringningen var derfor mere tidskrævende. Effekten på den initiale vækst af majs ved brug af insektfrass som for-gødning var enten lig med eller bedre end ved brug af mineralgødning.

Summary

Waste, Insects and Circular Economy for soil
Former foodstuff[3] (bio-pulp) are a valuable nutritional source, although today only used for energy generation. WICE4Soil (Waste, Insects and Circular Economy for Soil) builds on top of the findings in the former WICE project, which concluded that Black Soldier Fly Larvae (BSFL) can consume food waste, and that the final two products, BSFL biomass and residual substrate from the production (frass), have a potential as animal feed stock (BSFL) and plant fertilizer (frass). This present WICE4Soil considers the quality and safety from bio-pulp to BSFL and insect frass, production optimization of bioconversion, and the potential of insect frass as agricultural fertilizer.

Overall, the production of BSFL on former foodstuffs during WICE4Soil and the utilization of insect frass as fertilizer, was found to be suitable form both safety, quality and economical perspectives. The WICE4Soil project results indicate that such production system could lead to a sustainable up-cycling of former foodstuffs waste streams in the future.

Optimal production: 12 larvae/cm2, one feeding event with bio-pulp og DM>20%, potential RoI of 4.7 years
During 20 batches of pilot production over a time span of 1½ years, more than 1400kg bio-pulp was converted to 239 kg BSFL and 230 kg insect frass in a designed pilot production plant. A rather stable production of larvae was observed independent of seasonal variation with a bio-pulp consumption of up to 95%, with exception of the spring-2020 COVID-19 lockdown which had a negative impact on the pulp quality (high lipid content and high viscosity) and subsequently on the production efficiency. A new device for counting juveniles was developed for faster and more precise counting. Optimization parameters of the pilot production were tested regarding feeding frequency, initial larvae density, and bio-pulp dry matter (DM). The final recommendation is feeding only during start-up, 12 larvae/cm² density, and a rather high bio-pulp DM >20%. Summing up the learnings from a pilot production of BSFL reared on bio-pulp, a new production site producing ~13,000 tones BSFL per year would have a return of investment of 4.7 years.

Bio-pulp and BSFL produced on bio-pulp suitable for food chain use
The bio-pulp had a lipid and protein content suitable for BSFL production and with an amino acid composition similar to that of chicken feed. The bio-pulp dry matter content was rather low but could be increased by addition of e.g. husk, grains, and coffee grounds. Heavy metals and microbial content of bio-pulp were below threshold limit for animal feed. BSFL produced on bio-pulp had a protein and lipid content suitable for further processing to insect protein and oil production. Both heavy metals, *Escherichia coli* and *Enterococcus* spp., in fresh BSFL were all below the limit for animal feed. The *Enterobacteriaceae* content was above limit for most tested batches calling for a processing step (i.e. blanching, drying) before being used as feed outside the food chain (i.e. pet food).

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[3] According to the EU Catalogue of Feed Materials (Regulation (EU) No 2017/1017) former foodstuffs are: "*foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not present any health risks when used as feed*". EU-2017/1017 of 15 June 2017

Technical frass definition accepted

It was possible to automatically separate the insect frass into a clean <2-mm fraction, defined as technical frass without impurities, while the rest (accounting only for up to 10% of the total sample) was waste frass containing impurities. Based on the findings of technical frass, this definition was approved by the Danish Veterinary and Food Administration in October 2019.

Technical insect frass holds a potential as plant fertilizer

The quality of insect frass regarding NPK content was similar to other manure types used for soil fertilizer. Heavy metals, *Salmonella* and *E. coli* were all below the limit for eco-ladled fertilizers, while *Enterococcus* spp was above limit in some batches.

Technical insect frass holds a potential as agricultural fertilizer regarding performance, but handling must be optimized for large scale application. During a field test, pelletized technical insect frass was applied as pre-fertilizer for maize growth and compared to application of mineral fertilizer. The total amount of applied insect frass was larger than the mineral fertilizer to reach equal applied P-content, and application was therefore more time consuming, but the initial growth of maize on insect frass as a pre-fertilizer was either equal to or better than the mineral fertilizer.

1. Introduction

The conventional management practices of former foodstuffs are currently downcycling the resources primarily focusing on producing energy in the form of either steam during the incineration process, or methane and nutrient recovery during biogas production. The nutrient recovery of digest after bio-gasification is, however, associated with contamination risks as the bio-pulp is known to contain certain packaging impurities (i.e. plastics, metals, glass) and requires expensive logistics, due to its high-water content.

In natural systems, the recycling of organic material and soil formation is primarily governed by insects. Past studies have shown that different organic waste streams can be upcycled into high protein and lipid insect biomass, which leads to increased efficiency of the waste management system[4]. The organic fraction of food waste constitutes an important resource that Danish municipalities increasingly want to recycle in the best possible way. The basis for this approach is the Danish government's resource strategy 'Denmark without waste' by 2050, and focus is on the recycling of e.g. food waste. Such an ambitious goal calls for development of new circular technologies aiming at increasing the efficient use of resources and decreasing the use of raw materials[5]. Insects can degrade a large range of plant and animal biomass including industrial by-products and former food residues, ensuring efficient recovery of valuable nutrients from these otherwise wasted sources[4].

WICE4Soil paves the way for utilization of former foodstuffs (throughout the report also referred to as bio-pulp) in insect production and for the utilization of insect frass for soil improvements under the Circular Economy paradigm (Figure 1).

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[4] Diener et al., 201. Biological Treatment of Municipal Organic Waste using Black Soldier Fly Larvae. Waste and Biomass Valorization, 2, 357-363:https://link.springer.com/article/10.1007/s12649-011-9079-1

[5] Ministry of Environment and Food, 2018. Rethink – Reduce – Reuse- Recycle: http://en.mfvm.dk/filead-min/user_upload/MFVM/Miljoe/Cirkulaer_oekonomi/Advisory-Board-for-Circular-Economy-Report-2017-Content_Single_pages_WEB.pdf

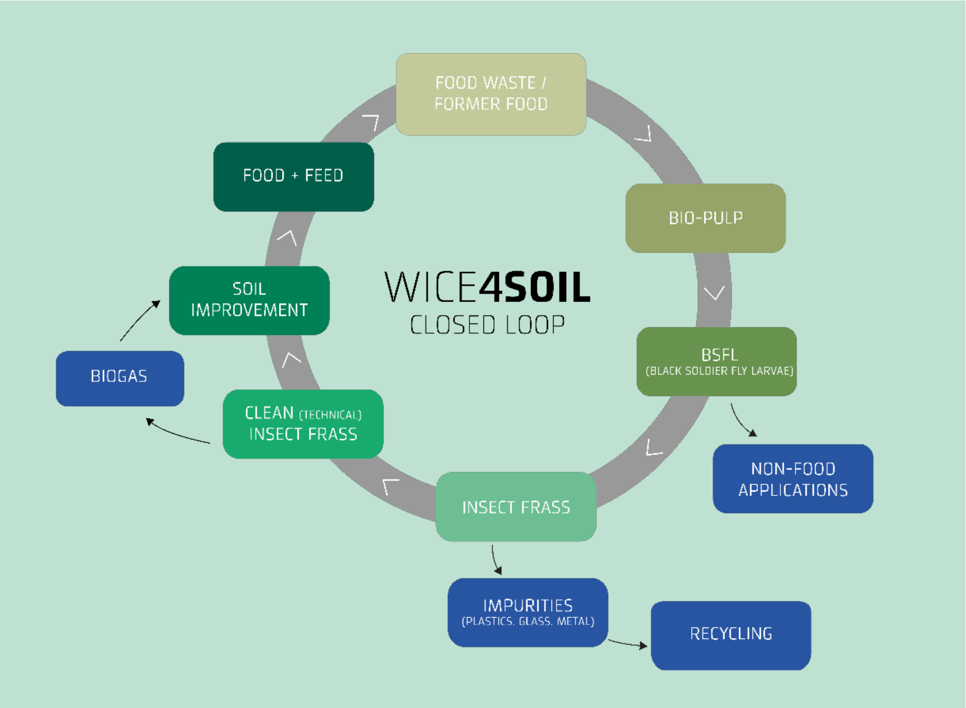


FIGURE 1. Diagram flow of the WICE4Soil project under the Circular Economy paradigm.

1.1 Objective

The main objectives of WICE4Soil (Waste, Insects and Circular Economy for soil) are to ensure that high-quality and complex macronutrients (i.e. protein and lipids) from former foodstuffs (bio-pulp) are safely and efficiently upcycled into similarly complex macronutrients - in the form of BSFL and to ensure high-nutrient recovery in the form of insect frass for further application as soil improvement.

The project was divided into subjects under different work packages (WPs) focusing on:
- Bio-pulp quality and safety
- Production and optimization of high-quality and -safety BSFL and insect frass
- Juveniles dosing, separation of insect frass, and removal of physical impurities
- Characterization and classification of insect frass
- Utilization of insect frass as soil amendment

Since former foodstuffs are currently banned from being used as feed in insect production, the WICE4Soil has obtained a special exemption from the Danish Veterinary and Food Administration.

2. Pulp quality and safety

Today, bio-pulp is not approved as feeding source for animal production. According to regulation (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials, only accepted feed can currently be used as insect feed.

Bio-pulp delivered from Daka ReFood is currently used for biogas production. The process of pulping is as follows: Former foodstuffs, including plastic containers (plastic bottles for ketchup, etc., and plastic bags), are loaded into a pulper that opens the bags and containers and releases the biomaterial. Then the biowaste is loaded into a separator where the bio-pulp is squeezed through a perforated separation plate. Organic material is forced through the separation plate, and a minor part of the inorganic material is transferred through the mesh. The bio-pulp including impurities (plastic film, plastic particles, metals, glass, etc.) in the bio-pulp is finally pumped to a large holding tank (Figure 2).

FIGURE 2. Processing from former foodstuffs to bio-pulp. From left: Former foodstuffs delivered to Daka ReFood; Final bio-pulp; Examples of impurities detected from final bio-pulp.

There is a concern that impurities of bio-pulp, heavy metals and microorganisms might be transferred to BSFL and/or insect frass during bioconversion. In the WICE4Soil project, the bio-pulp was analysed for its quality and safety regarding dry matter content, proteins and lipids, heavy metals, and microorganisms.

2.1 Methods

During the WICE4Soil Project, 20 batches of BSFL and insect frass were produced on bio-pulp from DakaReFood over a period of 1.5 years. The bio-pulp was collected during multiple visits at Daka ReFood and analysed for dry matter (DM), ash, and macronutrient (protein and lipid) content. A sample of 500 g was taken from all bio-pulp batches and stored at -18°C until being analysed for heavy metal and microbial content in an accredited laboratory. In addition, the amino acid profile of bio-pulp used to produce batch 18 was analysed and compared with the amino acid (AA) profile of chicken feed. An overview of the performed analyses is given in Table 1.

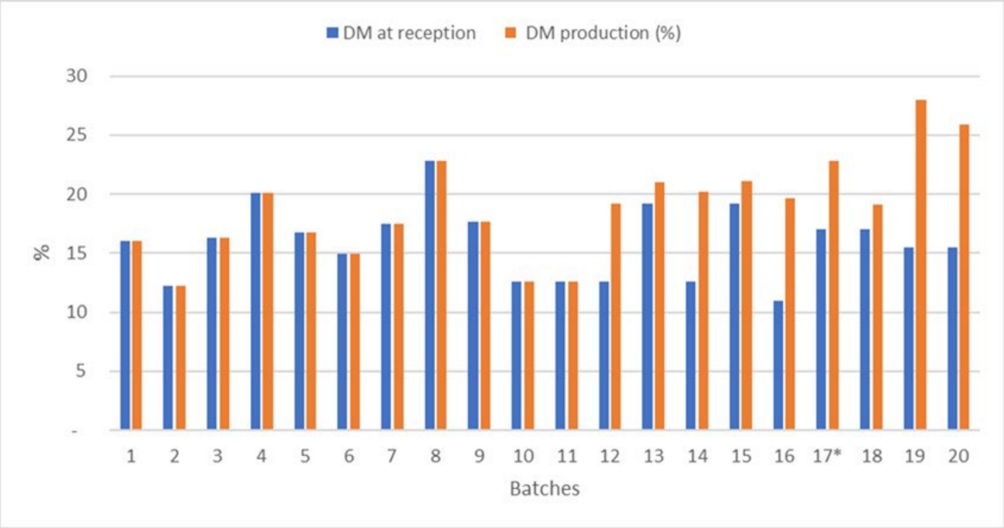
TABLE 1. Overview of analyses performed on bio-pulp.

	Performed analysis on pulp	Quantity of batches	Batches
Quality	Dry matter	20	All
	Ash content	20	All
	Protein	20	All
	Lipids	20	All
	Amino acid profile	1	18
Heavy metals	Lead	8	1-2, 14, 16-18
	Cadmium	8	1-2, 14, 16-18
	Chrome	3	1-2,
	Copper	3	1-2,
	Mercury	8	1-2, 14, 16-18
	Nickel	3	1-2
	Zinc	3	1-2
	Arsenic	5	14, 16-18
Microbiota	Salmonella	3	1-2
	Escherichia coli	10	1-2, 7-8, 11, 14, 16-18
	Enterococcus spp.	10	1-2, 7-8, 11, 14, 16-18
	Enterobacteriaceae	10	1-2, 7-8, 11, 14, 16-18

All batches and samples of bio-pulp were registered and stored at -18°C until used in the production.

2.2 Results and discussion

Overall, different batches of bio-pulp were successfully used in the production of BSFL at pilot scale. The pulp quality differed among the different batches as indicated by the DM (Figure 3).

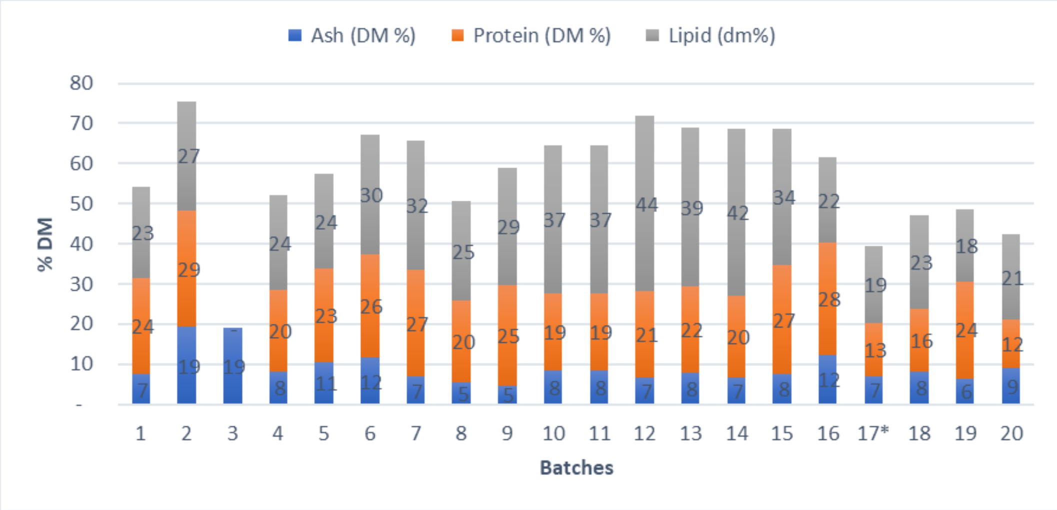


*The DM content belonging to batch 17 is an average value from different dietary treatments developed and tested during the production.

FIGURE 3. Dry matter of different pulps at reception and before utilisation in the production of BSFL. The DM of batches 1-11 is the same and the bio-pulp was used directly in the production.

In some cases, the DM of the received bio-pulp was very low (12-16%, batch 1-3, 6 and 10-11) whereas others had a naturally higher DM (19-23%; batch: 4 and 8). Some bio-pulp batches (batch 10-20) had a low DM (11-17%) or a very viscous consistency (batch 10-15) when received (Figure 3). Probably, the altered bio-pulp was due to the 2020-spring COVID-19 lockdown, when the production of bio-pulp relayed heavily on limited sectors (i.e. dairy production and retail). In order to increase DM and decrease viscosity, the bio-pulp was mixed with other waste streams (i.e. high fibre bio-pulp, wheat grain, husk, or coffee grounds).

The ash and macronutrients of the bio-pulp used in the production varied among different batches. Therefore, the ash varied between 5-19% among the batches, whereas the protein content in general was high and suitable for BSFL production (>15%) for most of the batches. The lipid concentration was relatively moderate (19-32%) and suitable for BSFL production for most of the batches, except for five batches (no. 10-15; 34-44%) with higher lipid concentration (Figure 4). It was expected that these batches were most affected by the COVID-19 lockdown, since their high viscosity could be attributed to high lipid content. However, the high lipid content can be seen mostly as a luxury problem, as it can be corrected by the inclusion of low-quality waste streams such as husk or high-fibre bio-pulp.



*The macronutrient and ash content belonging to batch 17 is an average value from different dietary treatments developed and tested during the production (see optimization section for explanation).

FIGURE 4. Ash and macronutrients profile of bio-pulp used in the production of BSFL.

The heavy metal analysis revealed that the bio-pulp can be safely utilized in the production of BSFL (Table 2).

TABLE 2. Heavy metals concentration in different bio-pulp used in the production of BSFL and the EU maximum limits.

Batches	B1	B2	B7	B14	B16	B17	B18	EU max. limit feed[6]
Lead mg/kg DM	< 2	< 2	0.8	1.1	0.7	1.1	0.6	10.2
Cadmium mg/kg DM	< 0.05	< 0.05	0.2	0.1	0.5	0.1	0.0	2.0
Mercury mg/kg DM	0.039	0.01	0.0	0.0	0.0	0.0	0.0	0.1
Arsenic mg/kg DM	NA	NA	1.6	0.8	1.1	0.0	0.0	2.0

NA: not available

Similarly, and as in the case of the heavy metals, the microbial analysis on the pulp revealed that the different bacteria species measured in the bio-pulp had a lower concentration than the maximum recommended levels (Table 3).

TABLE 3. Microbial concentration in different bio-pulps used in the production of BSFL and the recommended maximum limits.

Batches	B1	B2	B7	B8	B11	B14	B16	B17	B18	Max. limit
Salmonella (CFU/25g)	ND	ND								0[7]
Escherichia coli (CFU/g)	<100	<100	<10	<10	<10	<10	<10	<10	<10	500[7]
Enterococcus spp. (CFU/g)	>15,000	<100	>900	6,000	130	320	<10	347	<10	1mil[8]
Entero-bacteriaceae (CFU/g)	10	<10	<10	<10	130	<10	<10	411	<10	300[9]

ND: Not detected

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[6] EU directive 2002/32/EC on undesirable substances in animal feed: EUR-Lex - 32002L0032 - EN - EUR-Lex (europa.eu)

[7] Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=EN

[8] Fuka et al., 2017. Characterization of Enterococcal Community Isolated from an Artisan Istrian Raw Milk Cheese: Biotechnological and Safety Aspects. Food Technol Biotechnol., 55(3): 368–380. doi: 10.17113/ftb.55.03.17.5118

[9] Commission Regulation (EU) No 142/2011, on implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive.

The results of the heavy metal and microbial analyses were expected to be lower than the maximum limits as it was originally intended for food purposes and must comply with the required standards.

The essential AA profile of the bio-pulp (batch 18) was very similar to the AA profile of chicken feed, indicating that this type of substrate is of high quality (see Table 4).

TABLE 4. Essential amino acid (AA) profile in bio-pulp and commercial chicken feed.

Essential amino acids	Batch 18	Chicken feed*
Methionine	3.82	3.3
Threonine	7.93	6.31
Valine	11.34	7.91
Isoleucine	8.82	6.75
Leucine	14.87	12.58
Phenylalanine	8.17	8.18
Histidine	4.35	4.16
Lysine	10.05	9.94
Arginine	6.64	10.38
Tryptophan	2.1	2.06

*Chicken feed Pacostar 19

3. Production and Optimization of high-quality and -safety BSFL and insect frass

3.1 Methods - Production setup, quality and optimization

3.1.1 Overview

The production of BSFL and insect frass was conducted at pilot scale in trays of 40 x 60 cm (0.2 m2) under controlled laboratory conditions (temperature: 27±1°C, relative humidity: 45±9% and light to dark ratio: 14/10). Overall, an average of 10 trays/per batch were ensured during the pilot production with some batches counting only three trays, while others counted 18 or even up to 30 trays. The entire production was conducted in an approved laboratory at Danish Technological Institute. The approval from the Danish Veterinary and Food Administration allows for utilization of former foodstuff waste streams in the production of BSFL.

Four-day-old juveniles (approximately 3.5 mg/larval) were placed on individual trays consisting of bio-pulp. Multiple larval densities were tested during the production, and therefore, the number of juveniles used varied between 5,000/tray and 40,000/tray between different batches. The BSFL were fed for about 10 days until the first BSF prepupa (dark colour larvae) were observed in the trays, indicating that the larvae were ready for harvest.

When harvested, the larvae were separated from the insect frass while evaluating various separation strategies (Section 4.2), 1) a migration strategy, 2) manual separation, 3) mechanical sieve with multiple mesh sizes.

3.1.2 Quality

Post separation, as in the case of the bio-pulp, all batches of the BSFL and insect frass were analysed for dry matter and ash content. Samples of 500 g from both BSFL and insect frass were stored at -18 °C for later analysis of macro nutrients (NPK), heavy metals, and microbial analyses (see Table 5).

TABLE 5. Overview of analysis performed on the BSFL and insect frass.

Analysis performed on BSFL and frass		No. of batches	Batches
BSFL	Protein	9	1, 7-18, 20
	Lipid	6	1, 7, 14, 16-18
	Lead	6	1, 7, 14, 16-18
	Cadmium	6	1, 7, 14, 16-18
	Mercury	6	1, 7, 14, 16-18
	Arsenic	6	1, 7, 14, 16-18
	Escherichia coli	9	1, 7-8, 11, 14, 16-18,20
	Enterococcus spp.	9	1, 7-8, 11, 14, 16-18,20
	Enterobacteriaceae	9	1, 7-8, 11, 14, 16-18,20
Insect frass	N	8	1-2, 7, 14, 16-18
	P	8	1-2, 7, 14, 16-18
	K	8	1-2, 7, 14, 16-18
	Lead	8	1-2, 7, 14, 16-18
	Cadmium	8	1-3, 7, 14, 16-18
	Chrome	3	1-2
	Copper	3	1-2
	Mercury	8	1-2, 7, 14, 16-18
	Nickel	3	1-2
	Zinc	3	1-2
	Arsenic	5	7, 14, 16-18
	Salmonella	3	1-2
	Escherichia coli	10	1-2, 7-8, 11, 14, 16-18
	Enterococcus spp.	10	1-2, 7-8, 11, 14, 16-18
	Enterobacteriaceae	10	1-2, 7-8, 11, 14, 16-18

3.1.3 Optimization parameters

Optimization parameters of the BSFL- and insect frass-production reared on bio-pulp was evaluated using a series of key performance indicators.
Key performance indicators

1. BSFL and insect frass on a fresh weight (FW) basis
2. feed conversion rate (FCR) on a DM to DM and DM to FW basis
3. bio-pulp consumption on a FW and DM basis

Evaluated optimizations parameters

1. *Feeding frequency*: the BSFL were fed 1, 2, or 3 times with a total of 8 kg bio-pulp (Batch 1, DM:16%), while the other parameters were similar across the treatments.
2. *Various bio-pulps*: three batches of pulp (batch 1, 2, or 3), having different DM and ash contents were tested simultaneously, under similar conditions.
3. *Pulp dry matter and initial feeding*: identical bio-pulp was used at low (17%) and high (23%) DM in the production of BSFL during batch 7 and 8 and was provided either as 2 or 3 kg during the first feeding, while the other parameters were kept identical across treatments.
4. *Larval density*: two larval density experiments were conducted during this optimization (batch 9 and batch 19). Thus, during the two batches the larvae densities were 10, 15 and

20 larvae/cm^2 for batch 9, and 7, 10 and 12 larvae/cm^2 for batch 19. In batch 9, multiple feeding frequencies were used whereas during batch 19, the BSFL were only fed once.

5. *Density and bio-pulp enhancement*: two different densities (7 and 10 larvae/cm^2) and three different diets: Diet A was based on bio-pulp (80%) mixed with a high fibre pulp (20%); Diet B was based on bio-pulp (92%) and husk (8%), and Diet C was based on bio-pulp (80%) and coffee grounds (20%).

3.1.4 Greenhouse gas emission

The gas production of carbon dioxide (CO_2) and nitrous oxide (N_2O) was successfully assessed during batch 19 (September 2020), while methane (CH_4) ammonia (NH_3) and formaldehyde could not be measured due to cross-interference from volatile organic molecules in the pulp. The measurement was carried out at pilot scale in a closed climate room using an INNOVA photoacoustic gas analyser. Gases were sampled every ~10 minutes from 3 points in the chamber; the intake, close to the mixing fan (as a control of full mixing in the chamber), and in the outlet.

Gas production was calculated by subtracting the inlet from the outlet and recalculating from PPM to g/m^3. Knowing the amount of air exchanged from the chamber every hour, we calculated and plotted the hourly production of each of the gases and divided them by the biomass output in DM. This gave us the hourly production of all the gasses and the sum of this production was used as the total gas-specific production for the batch.

3.2 Production overview - Results and discussions

To produce 20 batches of larvae and insect frass (equal to 198 trays), 1,426 kg bio-pulp were used (Figure 5), and a total of 239 kg larvae and 230 kg insect frass were produced (Figure 6). The repeatability of the production indicates that bio-pulp can be successfully used in the production of BSFL.

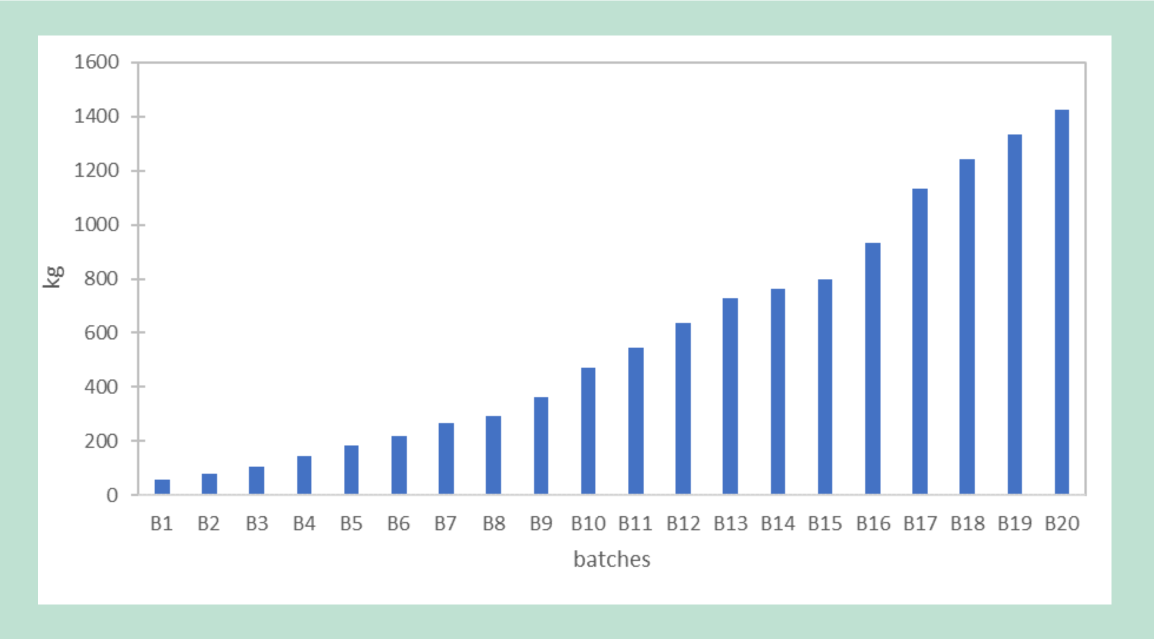

FIGURE 5. Cumulative bio-pulp used in the production of BSFL and insect frass during 20 batches (198 trays).

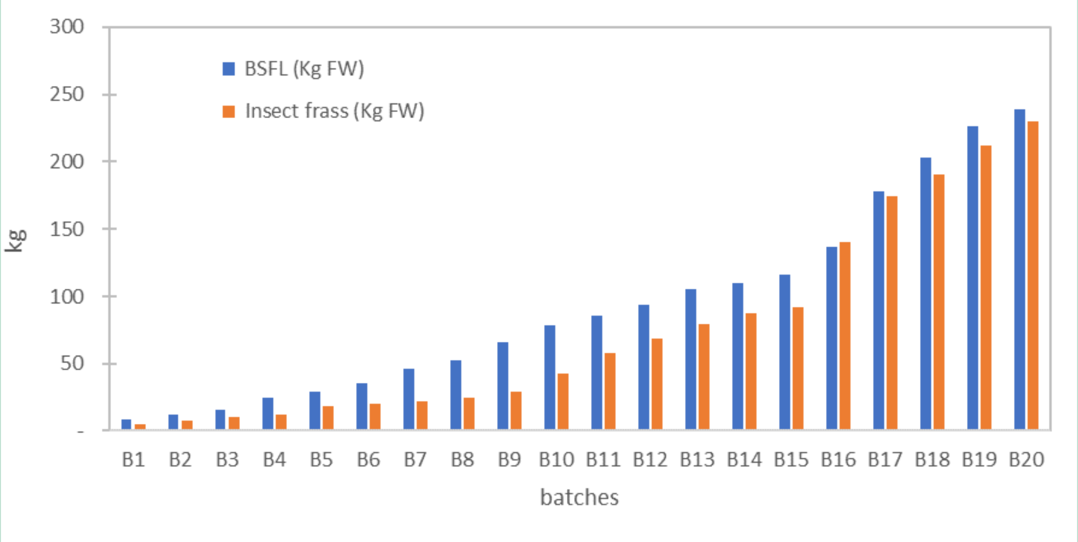

FIGURE 6. Cumulative BSFL and insect frass produced on bio-pulp.

Feed conversion rate (FCR) was calculated both on dry matter to fresh weight basis (Figure 7) and on dry matter to dry matter basis (Figure 8). The performance of BSFL as FCR was stable during the production period except for some of the batches produced during the 2020-spring COVID-19 lockdown (batch 10-15). The high variation within some of the batches is due to different experiments being conducted for optimization (e.g. initial larvae density, initial start feeding, and feeding frequency). The FCRs obtained during WICE4Soil were lower than FCRs obtained by other research of BSFL reared on various waste streams[10] but were similar to the FCRs obtained when BSFL are reared on catering waste[11]. The FCR results indicate that high-efficient BSFL production can be obtained on bio-pulp.

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[10] Surendra, K.C.; Tomberlin, J.K.; Van Huis, A.; Cammack, J.A.; Heckmann L.H.; Khanal, S.K. Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). *Waste Manag*. **2020**, *117*, 58–80.

[11] Gligorescu, A.; Fischer, C.H.; Larsen, P.F.; Nørgaard, J.V.; Heckman, L.-H.L. Production and Optimization of *Hermetia illucens* (L.) Larvae Reared on Food Waste and Utilized as Feed Ingredient. Sustainability 2020, 12, 9864. https://doi.org/10.3390/su12239864

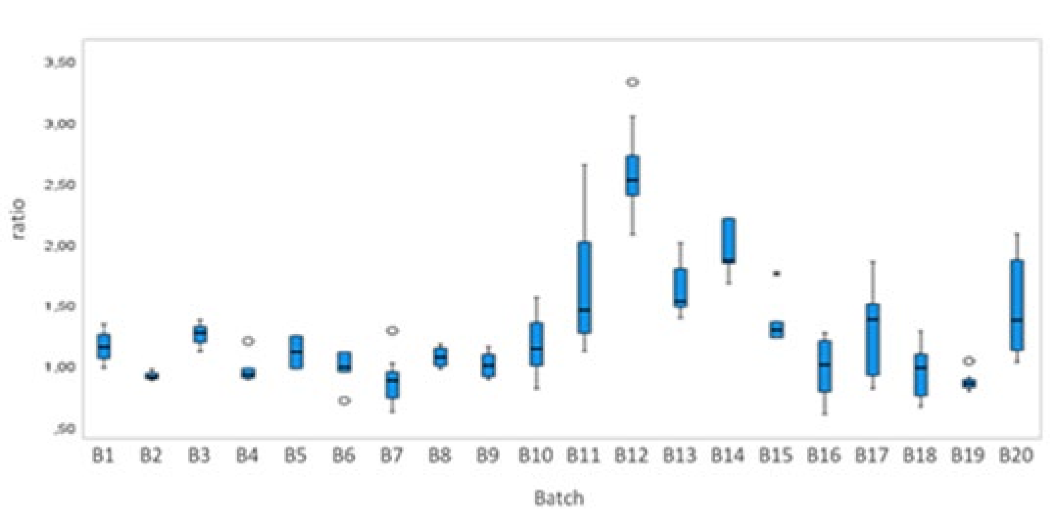

FIGURE 7. Feed conversion rate (FCR) obtained over 20 production batches. The FCR is displayed on dry to wet (DM/FW).

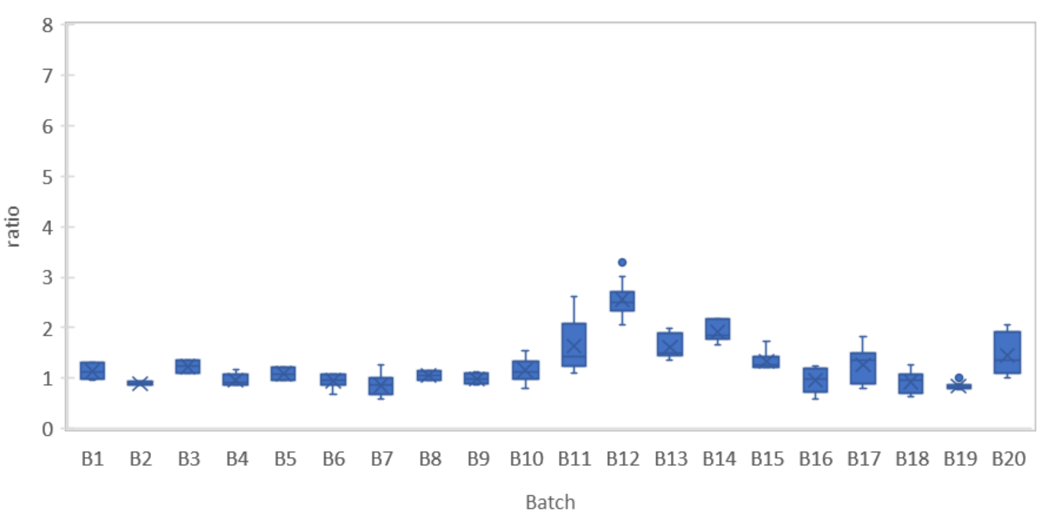

FIGURE 8. Feed conversion rate (FCR) obtained over 20 production batches. The FCR is displayed on dry to dry (DM) basis.

3.3 Quality and safety of BSFL and insect frass - Results and discussions

3.3.1 BSFL quality

The results show that BSFL produced on bio-pulp is suitable for insect protein and oil production in large quantities. The dry matter of BSFL (DM: 38±5%) was stable (15% coefficient of variation (CV)) during the production of 20 batches, the BSFL ash content was 13±5% (DM, basis), and the BSFL had a high protein (24-37%, DM, basis) and lipid (17-49%, DM, basis content (Figure 9).

The heavy metal concentration in the BSFL produced during the WICE4Soil project was lower than the EU maximum limits for feed, indicating a safe usage of the larvae (Table 6).

Most of the results of the microbial analysis reveal that BSFL fulfils the max. recommended limits of *E. coli*. The *Enterobacteriaceae* exceed the maximum limits for pet food for batch 8, 16, 17, 18 and 20. Furthermore, the *Enterococcus spp*. analysis reveals loads higher than 15,000, which is much less than the maximum recommended limit (Figure 7). Due to high concentration of *Enterobacteriaceae*, a thermal treatment should be included in order to reduce the microbial concentration in the BSFL-derived products (insect meal and oil) intended for pet food.

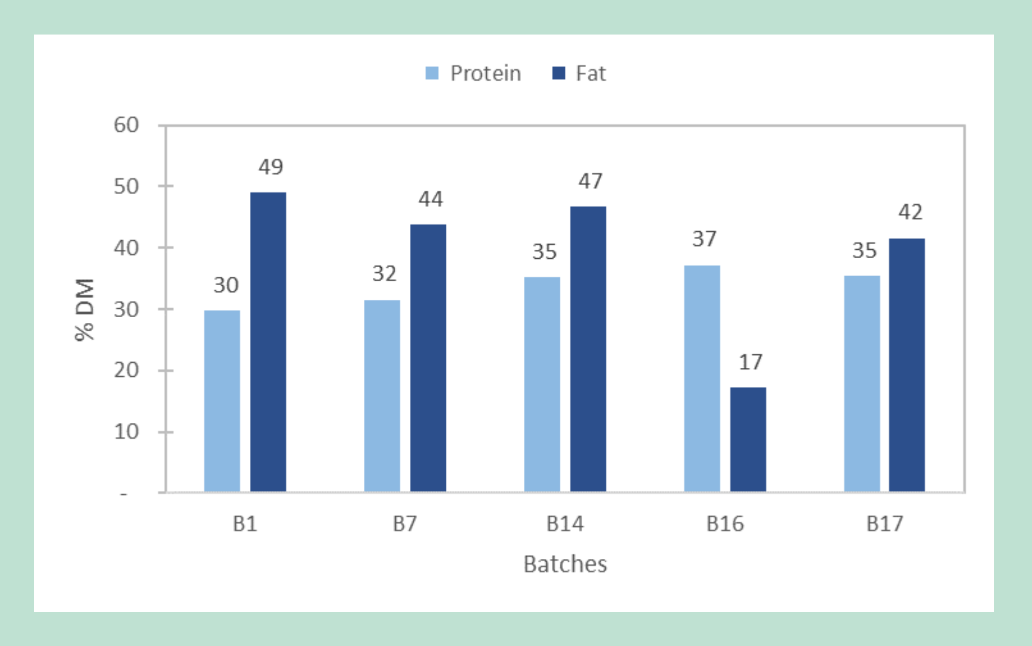

FIGURE 9. Protein and lipid of BSFL produced on bio-pulp during multiple batches.

TABLE 6. Heavy metal concentration in BSFL reared on bio-pulp and the EU maximum limits. Values in green cells are below EU maximum limit.

Batches	**B1**	**B7**	**B14**	**B16**	**B17**	**B18**	**EU max. limit feed**[6]
Lead mg/kg DM	0.3	0.1	0.2	0.14	0.30	0.35	10.2
Cadmium mg/kg DM	0.1	0.1	0.1	0.37	0.12	0.06	2.0
Mercury mg/kg DM	0.0	0.0	0.0	0.00	0.01	0.01	0.1
Arsenic mg/kg DM	0.5	0.7	0.3	0.30	0.27	0.26	2.0

TABLE 7. Microbial concentration.n in BSFL reared on bio-pulp and the recommended maximum limits. Values in green cells are below EU maximum limit

Batches	B1	B7	B8	B11	B14	B16	B17	B18	B20	Max. limit
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	10	362	< 10	<10	500[5]
Enterococcus spp. (CFU/g)	>15 k	>15 k	>15 k	140	91	>15 k	>15 k	>15 k	>9 k	1 mil[6]
Enterobacteriaceae (CFU/g)	430	27	>15 k	NA	18	3.9k	>9 k	>9 k	>9 k	5 k[7]

NA: not available; k: thousands.

Insect frass quality

The DM of insect frass for the 20 production batches was very high (55±16%), and the ash content was 17±9% (DM, basis). However, the DM and ash content varied between the different batches as suggested by the CV: 30% and 51%, respectively, probably due to differences in pulp dry matter and ash content as discussed in WP1 and illustrated in Figure 3 and Figure 4.

The NPK profile of the insect frass was similar to other manure types used as soil improvement across the globe (Table 8), indicating that insect frass from BSFL production on former foodstuffs could serve as potential for soil improvement.

Overall, the heavy metal analysis revealed that the insect frass obtained during the production of BSFL reared on bio-pulp is safe and has a lower concentration than the maximum limit of the EU Eco label soil improvement (Table 9).

The microbial analysis conducted on the insect frass revealed that Salmonella was not present, and the concentration of E. coli was below the maximum recommended limit for eco-labelled soil improvement. However, the concentration of Entrococcus spp. in insect frass was higher in multiple batches than the max. limit for manure (Table 10). The results of the microbial analysis reveal that a processing step aimed to reduce the Enterococcus loads should be considered before using the insect frass as soil improvement.

TABLE 8. The NPK (%) profile of insect frass produced during WICE4Soil compared to cow and poultry manure.

Batches	N (%)	P (%)	K (%)
B1	2.50	0.42	0.72
B2	1.80	2.10	1.07
B7	3.28	0.61	1.20
B14	3.08	0.83	1.40
B16	2.33	0.72	1.50
B17	2.24	0.27	0.54
B18	2.08	0.34	0.82

Batches	N (%)	P (%)	K (%)
Insect frass (avg.)	2.47	0.76	1.04
Cattle manure[12]	2.05	0.32	0.62
Poultry manure[12]	7.97	1.06	1.56

TABLE 9. Heavy metal concentration in insect frass obtained during production of BSFL reared on bio-pulp and the EU maximum limits of eco labelled soil improvement.

Batches	B1	B2	B7	B14	B16	B17	B18	EU Max. Eco label[13]
Lead mg/kg DM	< 2	< 2	0.34	0.97	1.36	0.59	1.04	100
Cadmium mg/kg	< 0.05	< 0.05	0.01	0.04	0.07	0.10	0.02	1
Chrome mg/kg	1.2	1.9	NA					100
Copper mg/kg DM	8.8	11	NA					100
Mercury mg/kg	0.04	0.01	0.01	0.01	0.02	0.01	0.02	1
Nickel mg/kg DM	1.1	1.7	NA					50
Zinc mg/kg DM	74	170	NA					300
Arsenic mg/kg			0.57	0.50	0.68	0.22	0.34	NA

NA: not available

TABLE 10. Microbial concentration in insect frass obtained from the production of BSFL reared on bio-pulp and the recommended maximum limits.

Batches	B1	B2	B7	B8	B11	B14	B16	B17	B18	Max. limit
Salmonella (CFU/g)	ND	ND								0[13]
Escherichia coli (CFU/g)	100	<100	<10	<10	<10	<10	<10	<10	<10	1k[13]
Enterococcus spp. (CFU/g)	>15k	3.2k	18	540	<10	470	>15k	> 15k	> 15k	1k[9]
Enterobacteriaceae (CFU/g)	>15k	420	18	540	<10	340	100	5.75k	120k	NA

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[12] Al-Suhaibani et al., 2020. Comparative Performance of Integrated Nutrient Management between Composted Agricultural Wastes, Chemical Fertilizers, and Biofertilizers in Improving Soil Quantitative and Qualitative Properties and Crop Yields under Arid Conditions. Agronomy, 10, 1503; doi:10.3390/agronomy10101503.

[13] EC 799/2006: Commission Decision of 3 November 2006 establishing revised ecological criteria and the related assessment and verification requirements for the award of the Community eco-label to soil improvers (notified under document number C (2006) 5369). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006D0799&from=EN

3.4 Optimization experiments - Results and discussions

During the production period a series of optimization studies were made in order to identify best feeding frequency, pulp quality and larval density to be used in the production of BSFL reared on former foodstuffs. Overall, good performances were obtained on the tested parameters, but maintaining a density of 12 larvae/cm^2, while feeding the larvae once (in the beginning of the production) with a relatively high dry matter (>20%) content bio-pulp, will ensure high efficiency.

3.4.1 Feeding frequency

The effect of the feeding frequency was evaluated for one, two or three feedings, all cumulating 8 kg identical bio-pulp per tray (batch 1) and with an initial larvae density of 9 larvae per cm^2. The effect of the feeding frequency is depicted as total production of larvae and insect frass (Figure 10), feed conversion rate DM to DM and DM to FW basis (Figure 11), and % pulp consumed (Figure 12).

All three figures show that production efficiency is independent of whether there are one or two feedings during eight days' growth, and further that three feedings resulted in a slightly less efficient production. The experiment indicates that a 1-time feeding strategy can be implemented in the production of BSFL reared on bio-pulp. This will reduce the handling and have an impact on the production costs.

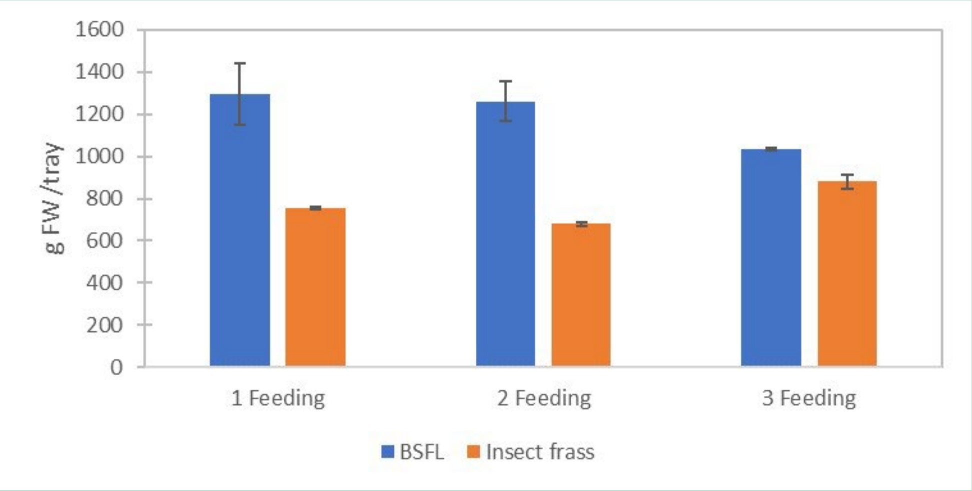

FIGURE 10. Larval and insect frass (FW) harvest as a function of feeding frequency (1, 2, or 3 feedings, with an equal cumulated food source), n= 3 for one and two feedings and 2 for three feedings.

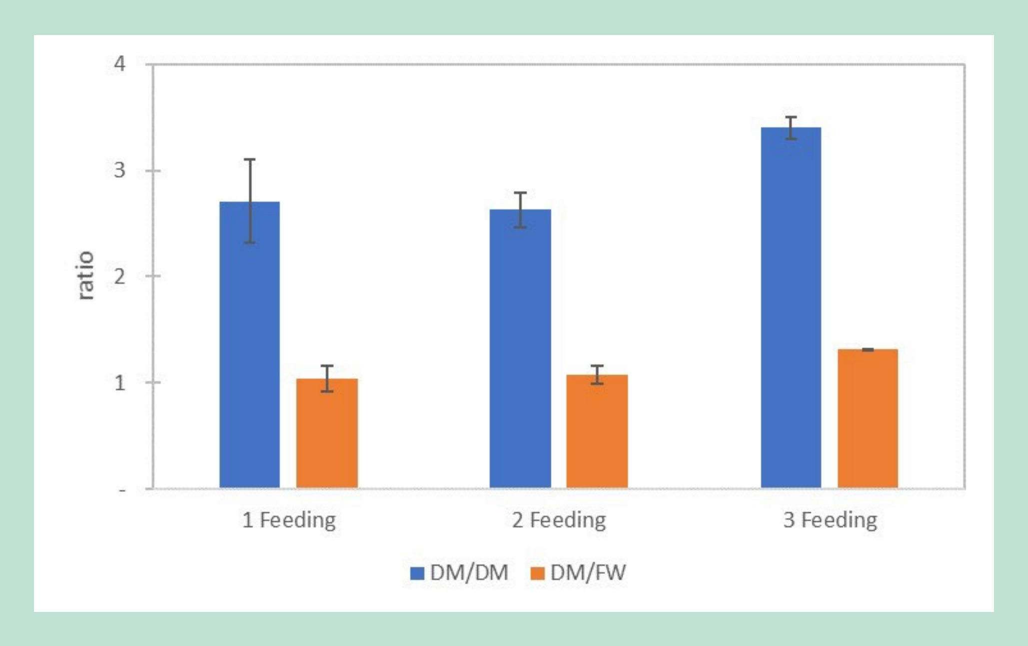

FIGURE 11. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of feeding frequency (1, 2, or 3 feedings, with an equal cumulated food source of 8 kg), n= 3 for one and two feedings and 2 for three feedings

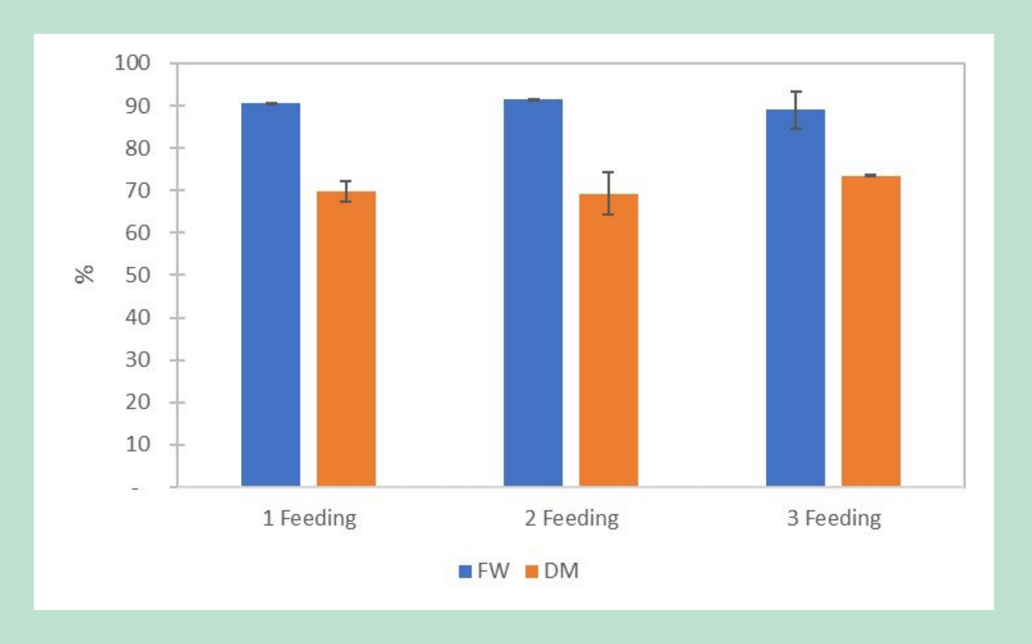

FIGURE 12. Pulp consumption on a fresh weight (FW) and dry matter (DM) as a function of feeding frequency (1, 2, or 3 feedings, with an equal cumulated food source).

3.4.2 Pulp quality

Three different bio-pulps belonging to batch 1-3 were tested in this experiment (Figure 3 and Figure 4 for pulp information). The larvae of those experiments all experienced 2 feedings (6 and 2 kg, respectively, with 8 days interval), the initial larvae density was 9 larvae/cm^2.

The results show that when three pulps were tested against each other, then there were no or limited differences in the production efficiency between batch 1 and 2 (Figure 13, Figure 14, and Figure 15). The bio-pulp used for batch 3 seems to perform less efficiently as the FCR obtained on this bio-pulp was higher than the other two FCRs obtained on bio-pulp from batch 1 and 2.

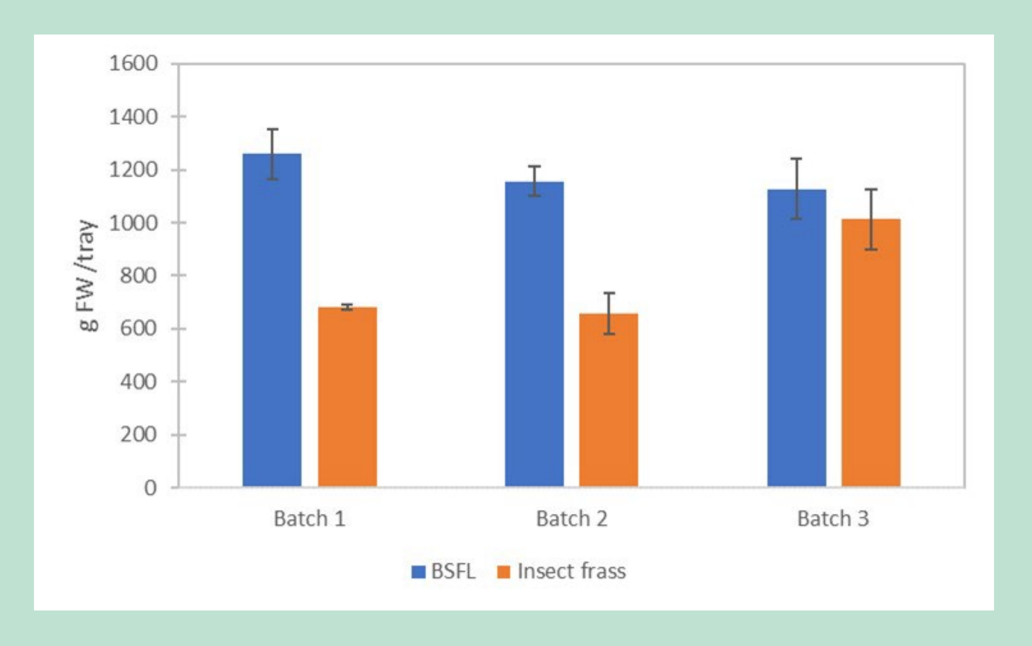

FIGURE 13. Larval and insect frass (FW) harvest as a function of three different pulps.

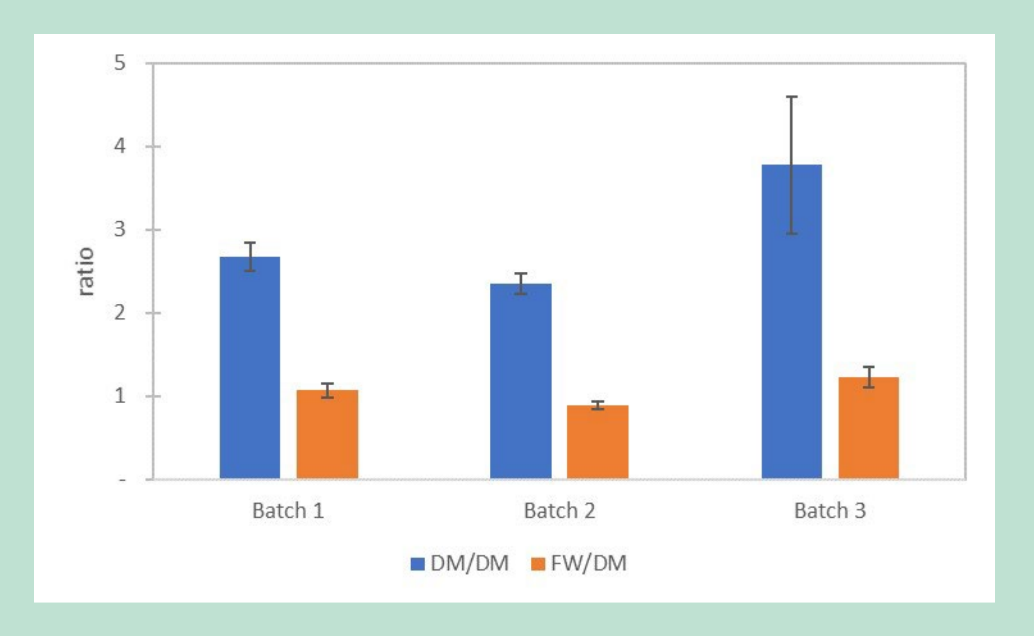

FIGURE 14. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of three different pulps.

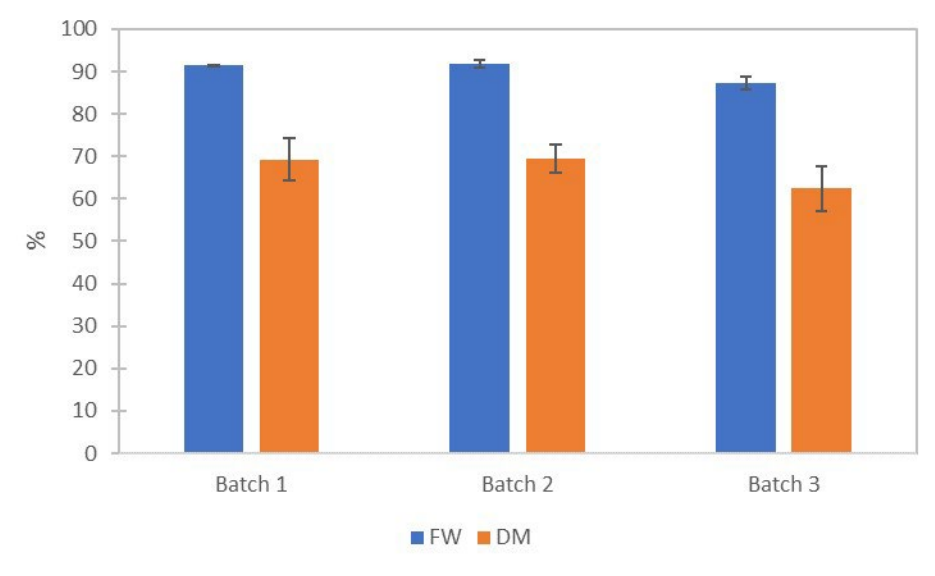

FIGURE 15. Pulp consumption on a fresh weight (FW) and dry matter (DM) as a function of three different pulps.

3.4.3 Pulp dry matter and initial feedings

Two different pulps (batch 7 and 8) were used to test if there was an individual or combined effect of pulp dry matter (17 and 23%, respectively) and initial amount of food applied. In total, 6.5 kg pulp was applied per tray over four feedings with the initial feeding at either 2 or 3 kg. The production efficiency measured by total harvest and FCR shows a slightly improved production when the initial feeding is 2 rather than 3 kg. However, there was no effect of the pulp dry matter content of 17 or 23% (Figure **16**, Figure 17, and Figure 18). The combined effect of the initial 3 kg pulp at a low dry matter showed the poorest production performance.

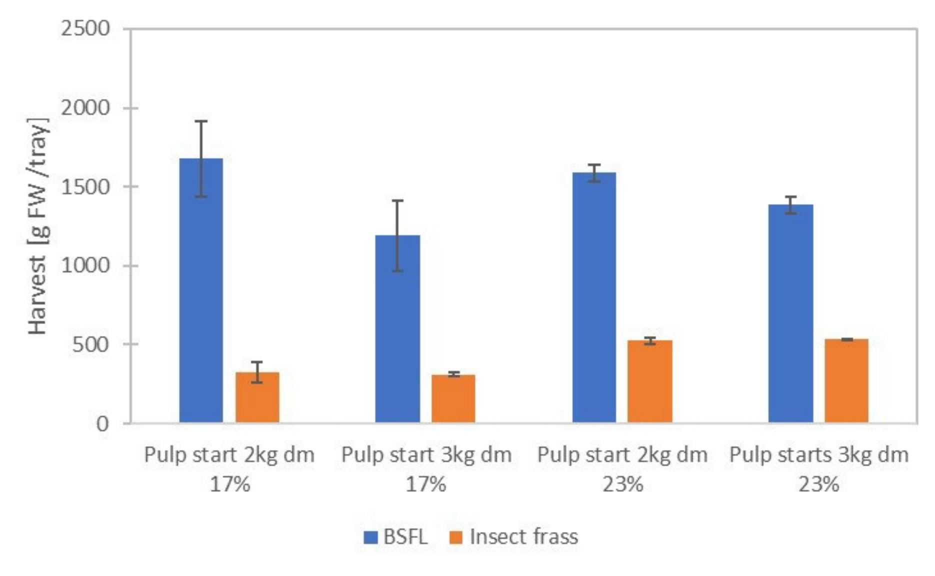

FIGURE 16. Larval and insect frass (FW) harvest as a function of initial pulp addition (2 or 3 kg, out of a total addition of 6.5 kg) and as a function of dry matter in pulp (17 or 23%).

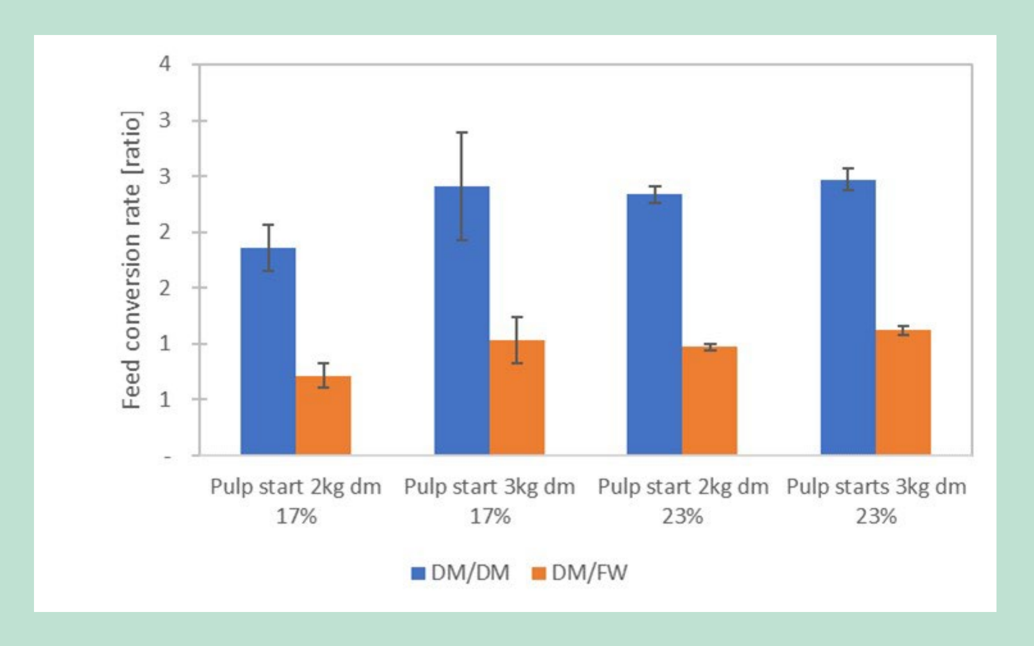

FIGURE 17. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of initial pulp addition (2 or 3 kg, out of a total addition of 6.5 kg) and as a function of dry matter in pulp (17 or 23%).

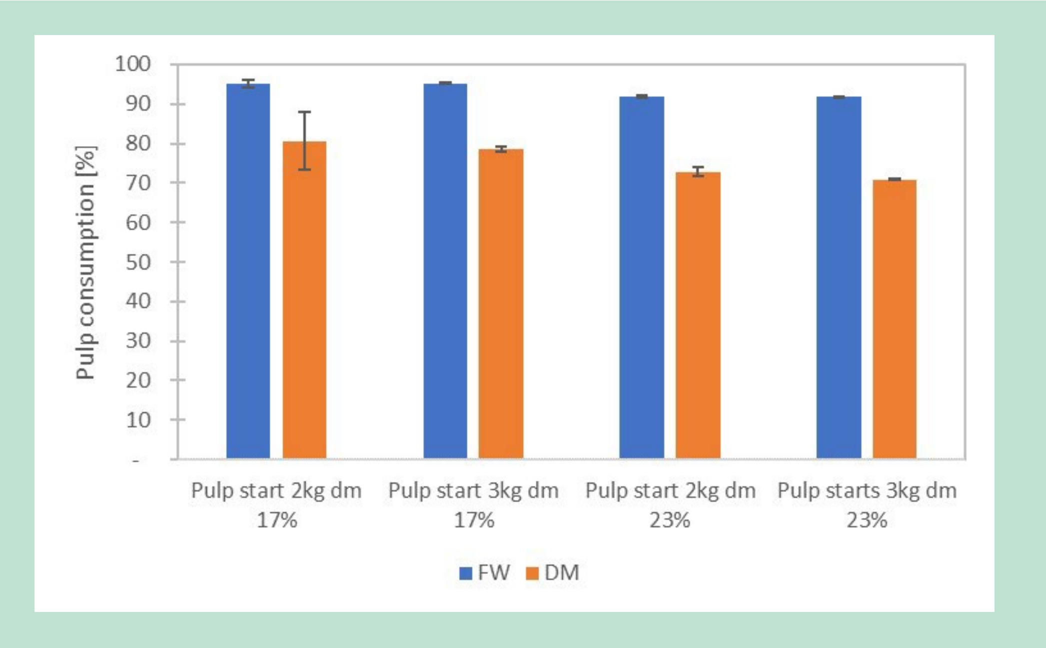

FIGURE 18. Pulp consumption on fresh weight (FW) and dry matter (DM) as a function of initial pulp addition (2 or 3 kg, out of a total addition of 6.5 kg) and as a function of dry matter in pulp (17 or 23%).

3.4.4 Density experiment 1

The production of BSFL and insect frass was found to rise with increased larval density (Figure 19), whereas the FCR was less efficient (higher values) due to increasing larval density (Figure 20). This is believed to be highly associated with high mortality and high migration of larvae observed during the experiment at high densities (Survival rates: 10 larvae/cm²: 67±1; 15 larvae/cm²: 51±2; 20 larvae/cm²: 37±4) indicating that the carrying capacity of the trays

(60x40) was exceeded at 15 and 20 larvae per cm². The consumption of pulp was high across all densities (Figure 21).

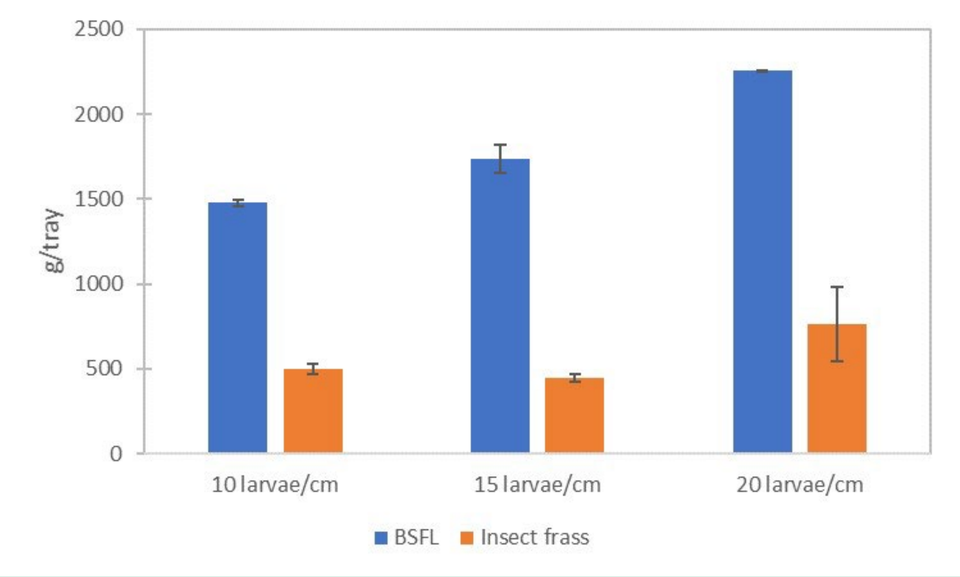

FIGURE 19. Larval and insect frass (FW) harvest as a function of three different densities (10, 15, and 20 larvae/cm²).

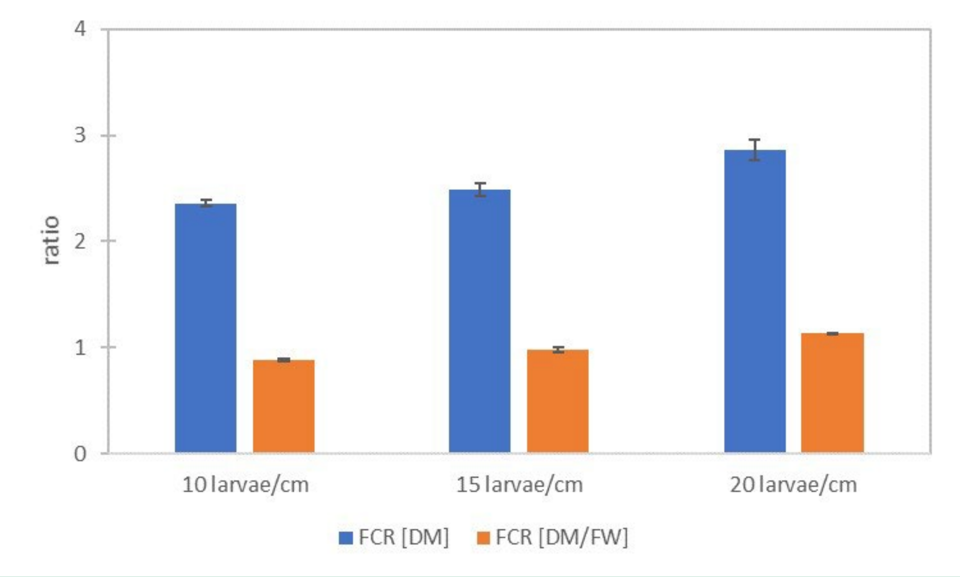

FIGURE 20. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of three different densities (10, 15, and 20 larvae/cm²).

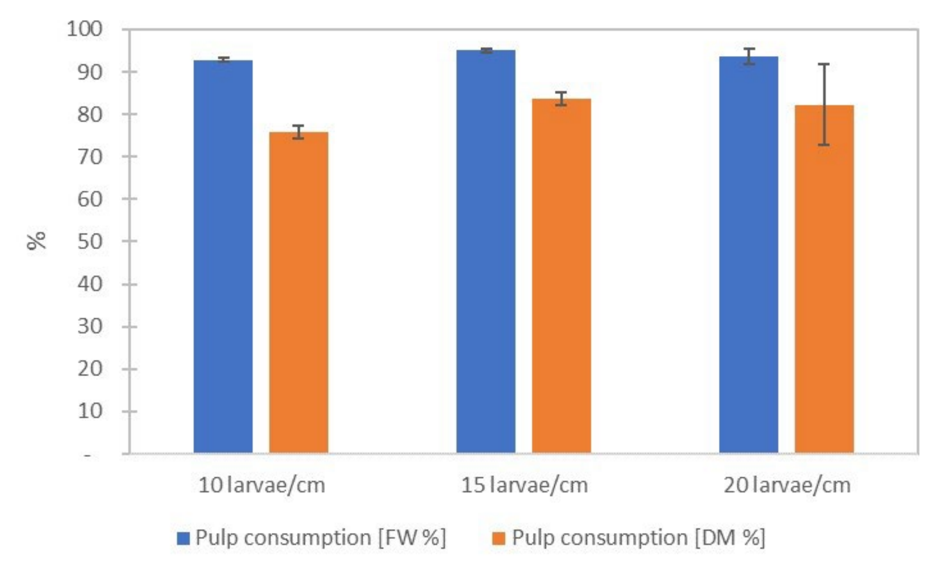

FIGURE 21. Pulp consumption on a fresh weight (FW) and dry matter (DM) as a function of three different densities (10, 15, and 20 larvae/cm²).

3.4.5 Density experiment 2

A second density study revealed that an increase in density from 7 to 10 and 12 larvae/cm², respectively, increases the production of BSFL and insect frass biomass (Figure 22), while having no effect on FCR (Figure 23) and pulp consumption (Figure 24). The survival rate during harvest of the second density experiment of 7, 10, and 12 larvae per cm^2 were 47, 73, 85%, respectively. The results indicate that when produced on 60 x 40 cm trays, a density of 12 larvae/cm/² can be used.

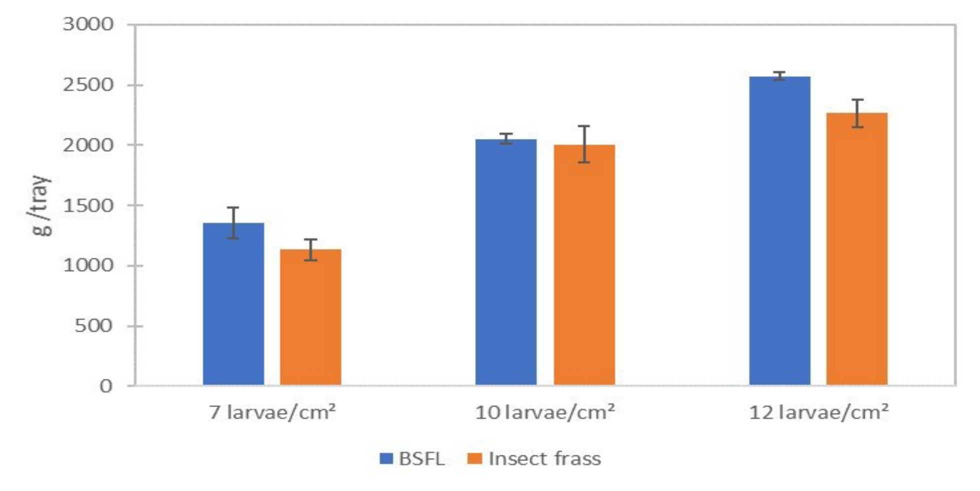

FIGURE 22. Larval and insect frass (FW) harvest as a function of three different densities (7, 10, and 12 larvae/cm²).

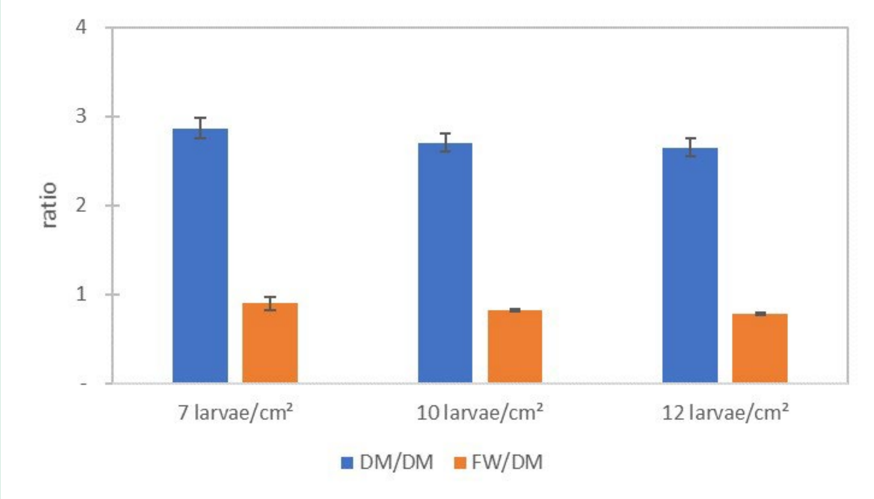

FIGURE 23. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of three different densities (7, 10, and 12 larvae/cm²).

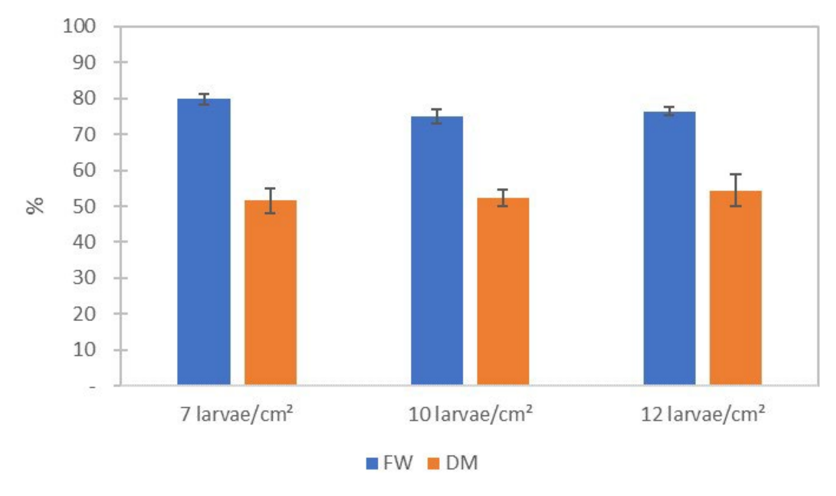

FIGURE 24. Pulp consumption on a fresh weight (FW) and dry matter (DM) as a function of three different densities (7, 10, and 12 larvae/cm²).

3.4.6 Density and bio-pulp enhancement

The production of BSFL and insect frass was found to be higher when bio-pulp was mixed with a high-fibre pulp from Daka ReFood, indicated as diet A, followed by diet B (bio-pulp mixed with husk) and diet C (bio-pulp mixed with coffee grounds) (Figure 25). Similarly, the FCR revealed a higher efficiency and a higher pulp consumption, when larvae were fed on diet A, followed by diet B, and finally diet C (Figure 26 and Figure 27).

The increase in density led to higher larval and insect frass production and better efficiency for all three diets (Figure 25, Figure 26, and Figure 27). Therefore, the results of the experiment indicate that mixing bio-pulp with a high-fibre substrate such as fibrous pulp from Daka Re-Food can ensure high BSFL production and overtake problems related to either DM and/or viscosity of the pulp.

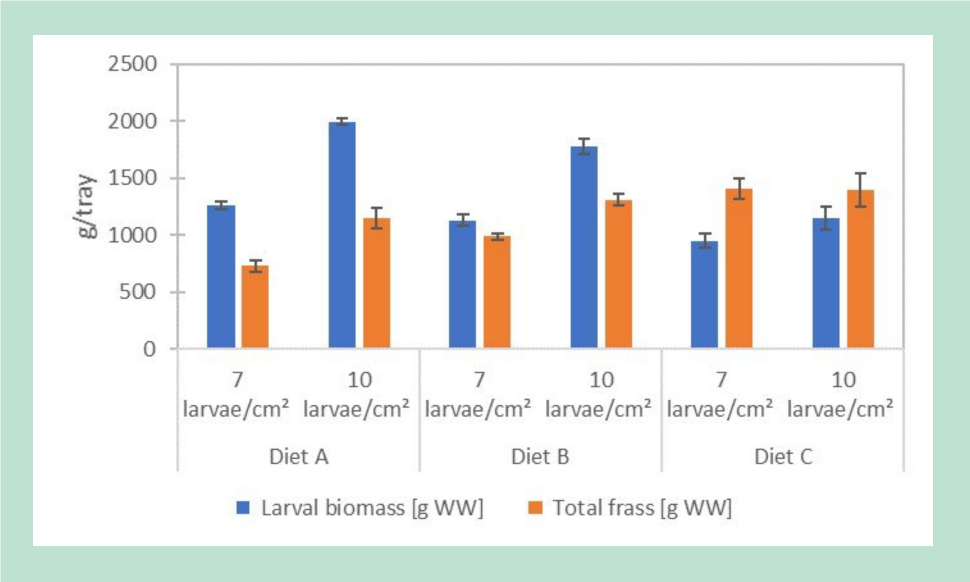

FIGURE 25. Larval and insect frass (FW) harvest as a function of two different densities (7 and 10 larvae/cm²) and three diets.

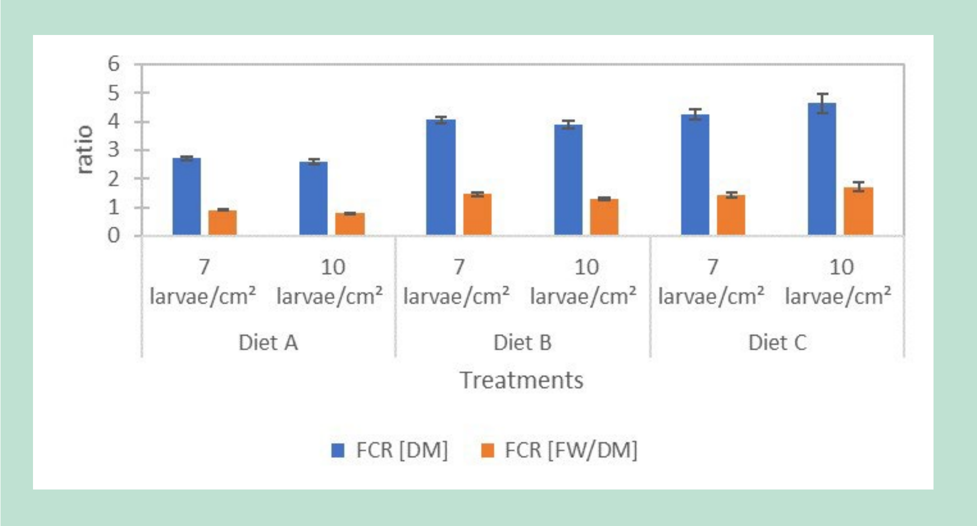

FIGURE 26. Feed conversion rate (FCR) (DM/DM and FW/DM basis) as a function of two different densities (7 and 10 larvae/cm²) and three diets.

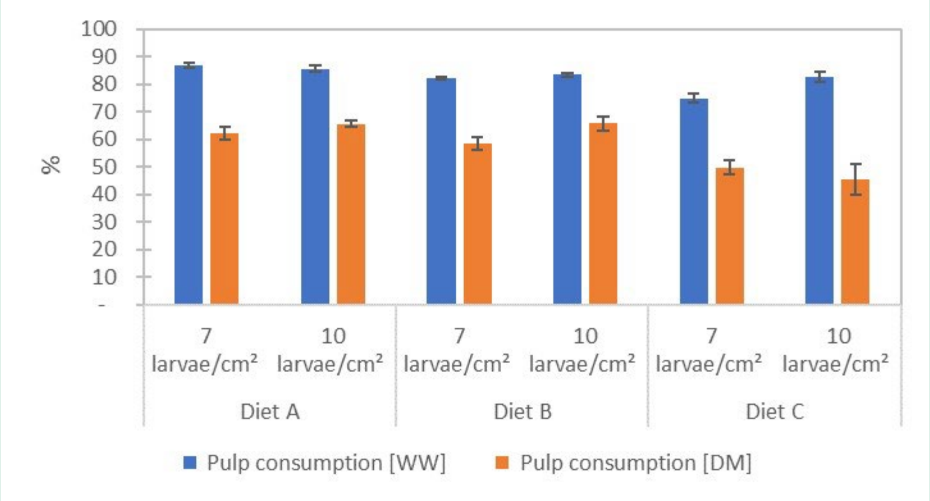

FIGURE 27. Pulp consumption on a fresh weight (FW) and dry matter (DM) as a function of two different densities (7 and 10 larvae/cm²) and three diets.

3.5 Gas emissions - Results and discussions

The gas production of the greenhouse gases showed a total of 934 g of carbon dioxide (CO_2) pr. kg DM produced while nitrous oxide (N_2O) was 0.04 g/kg DM. Comparison of carbon dioxide and nitrous oxide (N_2O) pr. kg BSFL DM produced on bio-pulp was similar to other studies (Table 11).

The production of CO2 showed the characteristic production pattern, with low production in the beginning and a substantial increase after (under these temperature and RH conditions) approx. 4 days, lasting around 2.5 days. Following this peak during day 6, the production of CO2 starts steadily declining as the biological activity goes down. This decline continues until around day 10, when the larval biomass was harvested.

The N2O production was extremely low compared to CO2 and is not a concern in theses insignificant concentrations, neither regarding ventilation nor environmental impact. Some N2O is released during the period of high biological activity (day 0-6.5) and a decline is detectable following day 6, before settling around 0 following biomass harvest (day 10). Please note that the noise of the measurements is relatively high, due to the extremely low concentrations.

TABLE 11. Production of gases and Global Warming Potential (GWP) from the WICE4Soil pilot production in comparison with GHG emissions from other studies.

Studies	**Carbon dioxide (CO_2) [g/kg DM]**	**Nitrous oxide (N_2O) [g/kg DM]**
WICE4Soil	934	0.04
Ermolaev et al. 2019[14]	1,750	0.02

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[14] E. Ermolaev, C. Lalander, B. Vinnerås, 2019. Greenhouse gas emissions from small-scale fly larvae composting with Hermetia illucens, Waste Management, Volume 96, 65-74, https://doi.org/10.1016/j.wasman.2019.07.011.

Studies	**Carbon dioxide (CO_2) [g/kg DM]**	**Nitrous oxide (N_2O) [g/kg DM]**
Mertenat et al, 2019[15]	NA	0.12
Pang et al, 2020[16]	1,394	0.01
Parodi et al., 2020[17]	2,750	0.05

* Global Warming Potential (GWP) was expressed as g CO_2 equivalents based on the GWP100 of CH_4 (34) and N2O (298) with carbon feedback.

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[15] Mertenat, A., Diener, S., Zurbrügg, C., 2019. Black Soldier Fly biowaste treatment e assessment of global warming potential. Waste Manag. 84, 173e181. https://doi.org/10.1016/j.wasman.2018.11.040

[16] Pang, W., Hou, D., Chen, J., Nowar, E.E., Li, Z., Hu, R., Tomberlin, J.K., Yu, Z., Li, Q., Wang, S., 2020. Reducing greenhouse gas emissions and enhancing carbon and nitrogen conversion in food wastes by the black soldier fly. J. Environ. Manag. 260, 110066.https://doi.org/10.1016/j.jenvman.2020.110066.

[17] Parodi, Alejandro & Boer, I.J.M. & Gerrits, Walter & van Loon, Joop & Heetkamp, M.J.W. & Schelt, Jeroen & Bolhuis, J. & Zanten, Hannah. (2020). Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing – A mass balance approach. Journal of Cleaner Production. 271. 122488. 10.1016/j.jclepro.2020.122488.

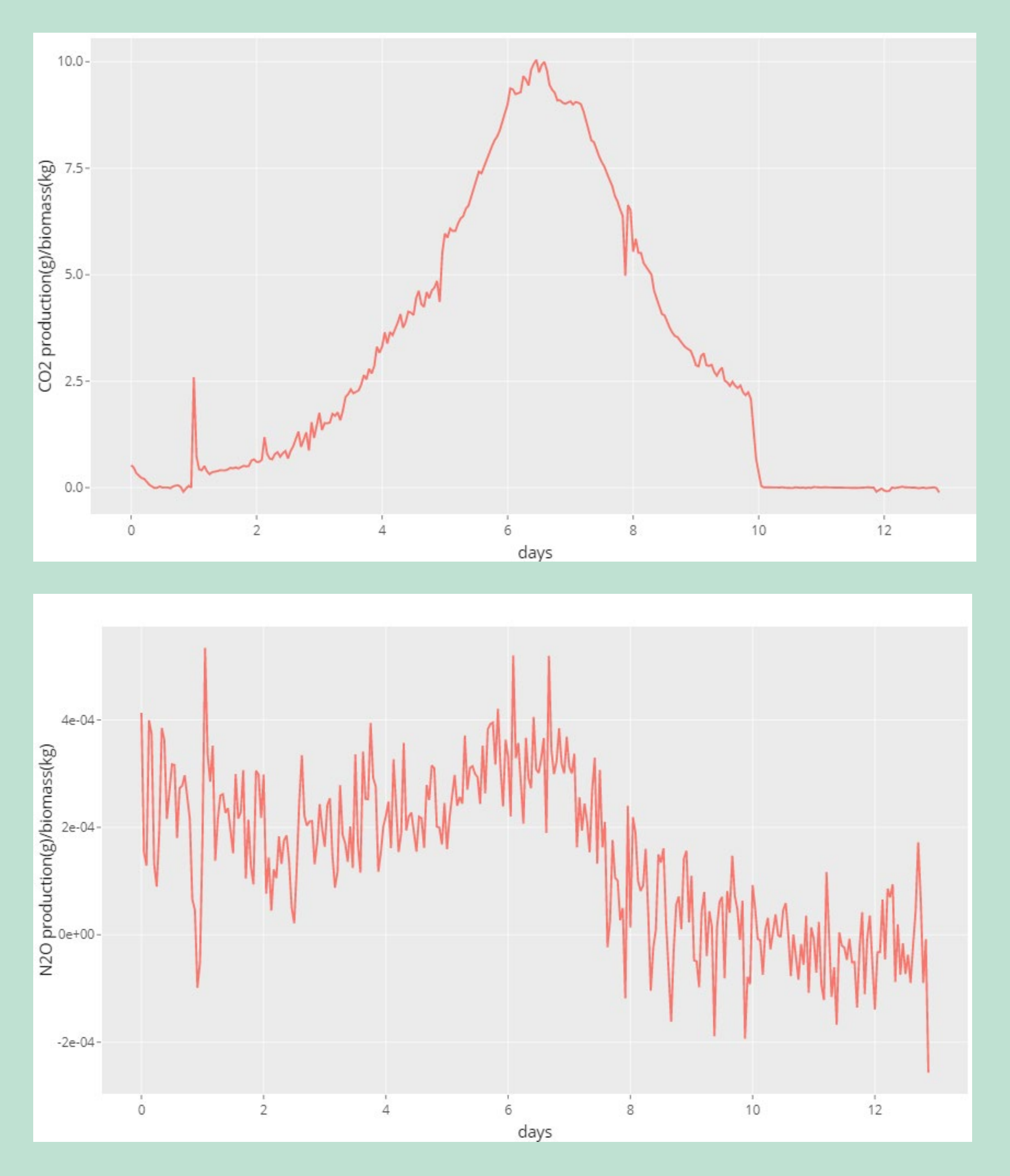

FIGURE 28. Time series of production of the carbon dioxide (CO2, top) and nitrous oxide (N2O, bottom) during the production of BSFL, starting at day 0 and running for 10 days before harvest. For accumulated gas production, see Table 11. The production is depicted as g of GHG per kg biomass (DM) produced.

4. Juvenile counting technology

4.1 Juveniles counting technology

4.1.1 Available solutions

Today, counting of juveniles (5-7DOL, days old larvae) most often takes place by using a weighing approach (total weight/average weight). This procedure is labour intensive due to manual handling during the weighing and dosing process (distribution in the production trays), especially at industrial scale, where approximately 100,000 5-7DOLs are required per tray, leading to high variation in larval density across the production trays. Systems aiming at a 100% counting technology, obviating the weighing procedures, would optimize and uniformize production.

Current method relying on light detection for fast and reliable BSFL-counting after hatching (neonates counting), however, this method is only applicable for samples without impurities of e.g. exoskeletons or insect frass and in the earlier stage of development. *Hannemann Engineering* has earlier developed a proof-of-concept of a vision system generated for a simpler picture technology that overcomes the issue related to the presence of impurities while counting only the 5-7DOLs. This initial proof-of-concept relied on an algorithm that could not handle larvae size variation between trays.

4.1.2 4.1.2 Hannemann Engineering BSL 5-7DOL counter

During this project, *Hannemann Engineering* has developed an improved inline vision-based automatic counting device that relies on image recognition. The device uses a wide variety of technologies for dynamic and automatic image identification and analysis, and each contributes to a more precise and accurate count than the former proof-of-concept solution. The developed automated vision-based solution can correctly identify and count 5-7DOLs while overtaking issues associated with feed residues or exoskeleton.

Currently, the 5DOL counter is unique, no one else is able to count 5-7DOL's at this precision and speed. Hannemann Engineering ApS is now looking for investors to finalize the 5-7DOL counter. Due to a huge interest from several large BSF producers, we are sure that this solution will add higher efficiency to the production of BSF's.

The following requirement specifications were set for a successful 5-7DOL counter:

- Count BSF 5-7 DOL's – Fulfilled (see Figure 29 and Figure 30)
- Precision -10% +0% - Fulfilled (Figure 29 and Figure 30)
- Counting 1.000 DOL's/s (work in progress, half-way to final goal)
- Counting DOL's in samples with impurities, e.g. frass, exoskeletons and feed - Fulfilled (see Figure 30)
- Counting alive DOL's excluding dead (solution idea developed although not yet implemented)

The user of this new device will initially place a subsample of a known weight on the tray and start the device. When the process is started, the device will capture images that are processed on a server, and the software will calculate the number of insects. For quality purposes, the information and pictures are fed back into the user interface, where the end-user can see the processed image and determine if the insects are too close to each other for a proper counting. When the count is determined, the user interface will display the count details with specifications of counting precision as well as potential improvement measures to be carried out by the user.

The method is tested against manual count and showed a deviation of maximum 5%. For a sample without contamination the 5-7DOL counter counted 517 larvae compared to 545 counts manually, thereby a deviation of -5% (Figure 29). And for a sample with impurities, counting was 101 BSL by the 5-7DOL counter device against 102 BSL by manual count, hence a deviation -1% (Figure 30).

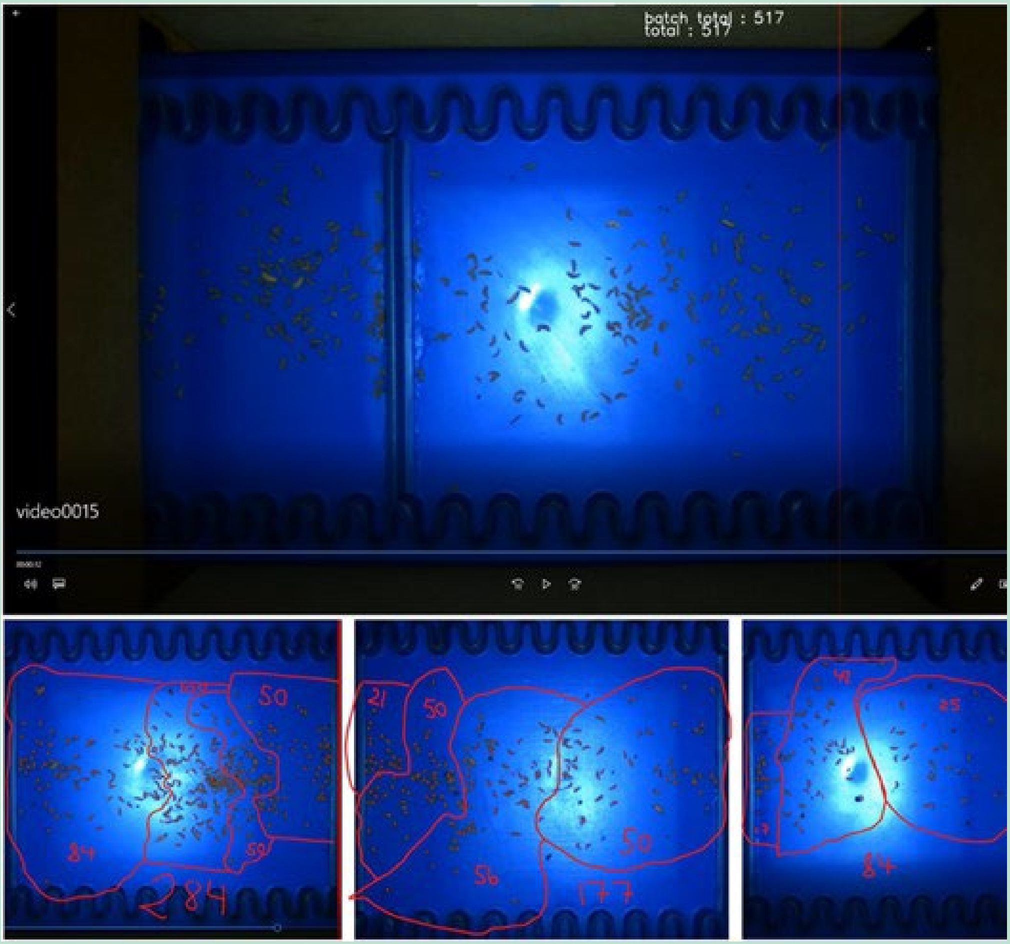

FIGURE 29. Evaluation of counting presicions for a batch of 5-7 days old larvea (5-7DOL) nearly without contamination.

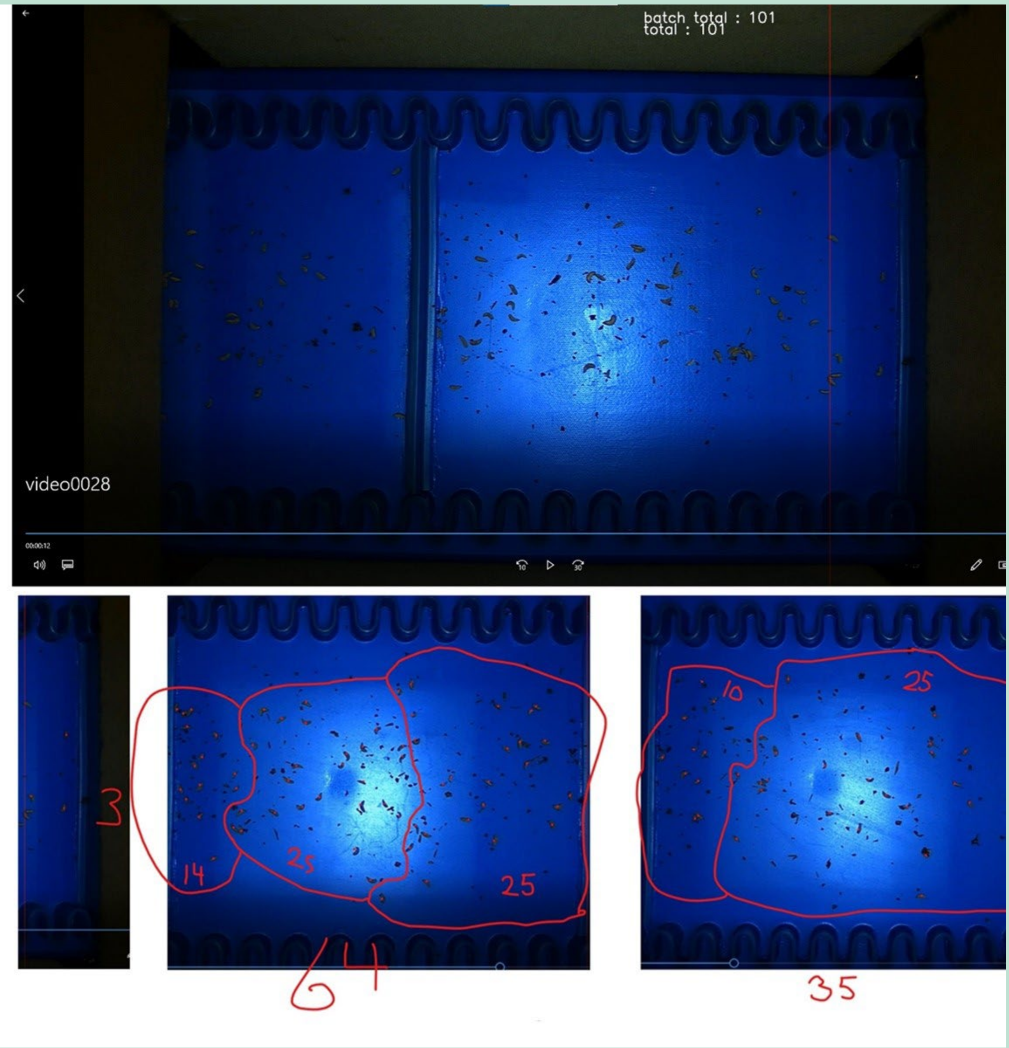

FIGURE 30. Evaluation of counting precisions for a batch of 5-7 days old larvae (5-7DOL) with contamination of part of substrate, dead larvae and frass.

5. Upscaling of BSFL and insect frass production

5.1 Application of HACCP principles

The implementation of the principles of Hazard Analysis and Critical Control Points (HACCP) in the production of BSFL reared on former foodstuffs is designed to prevent the occurrence of problems, correct deviations and provide the records for further analysis[18].

HACCP is defined by 7 principles:
1. Conduct hazard analysis.
2. Determine critical control points (CCPs).
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective action.
6. Establish verification procedures.
7. Establish record-keeping and documentation procedures.

The WICE4Soil pilot production of BSFL larvae and insect frass for soil fertilizer reared on bio-pulp was conducted according to the HACCP principles in order to provide a Best Practise Guide for upscaling.

During the WICE4Soil project, the individual batches were registered at arrival and deposited in a freezer dedicated to the project before being used in the project. A tracking procedure was developed based on batch number to ensure that the bio-pulp, BSFL, and insect frass from the production were easily identifiable for individual batches (Table 1 and Table 5) according to Principle 7. This procedure was further extended and implemented for different samples used for the quality and safety analysis.
Samples of bio-pulp, BSFL, and insect frass were taken and used to analyse the heavy metals and microbiota throughout the entire production process (i.e. bio-pulp, BSFL, and residue bio-masses (insect frass)) following Principle 1. The results from the analysis were compared with the EU maximum limits (Principle 3) as described in WP1 and WP2. Based on the analysis, a series of hazards was identified as presented in Table 12.

TABLE 12. Identification of hazards through the production of BSFL and insect frass reared on former foodstuffs.

Hazard type	Bio-pulp	BSFL	Insect frass
Chemical: heavy metals	Lower than limit	Lower than limit	Lower than limit
Biological: microbiota	Lower than limit	Enterobacteriaceae multiple batches	High Entrococcus spp multiple batches
Physical impurities	Packaging residues in very low limits	Potential microplastics	Very low loads, potential microplastic

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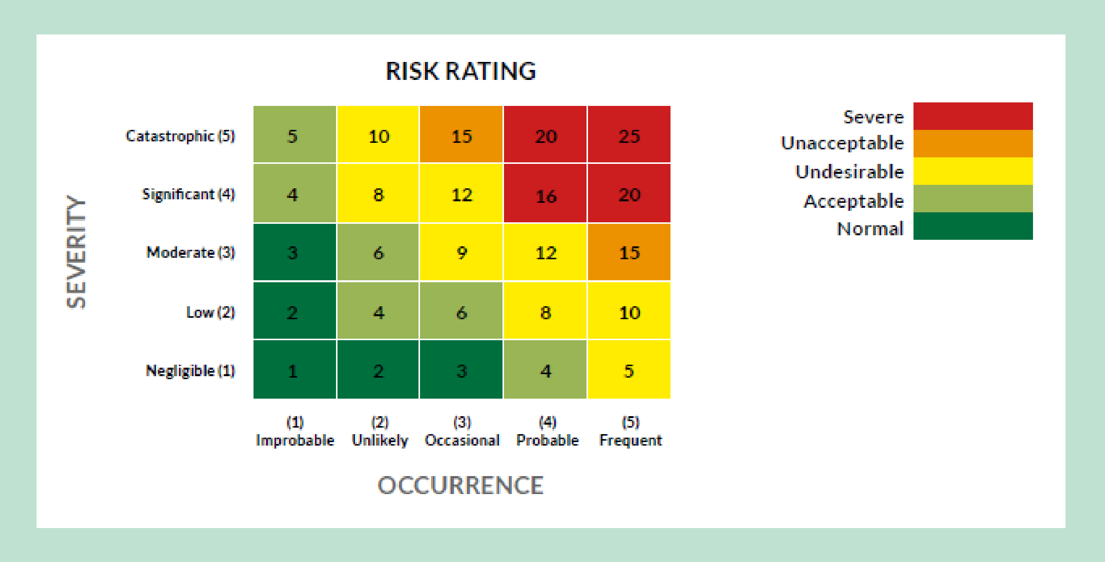
[18] IPIFF, 2020. Guide on Good Hygiene Practices. https://ipiff.org/wp-content/uploads/2019/12/IPIFF-Guide-on-Good-Hygiene-Practices.pdf

Based on the hazard identification, the CCP (Principle 2) targeting BSFL and insect frass should be established when produced on bio-pulp. BSFL will be slaughter (e.g. blanching) before being defatted and dried and we recommend that the CCPs are applied on the final products (insect defatted meal and insect oil) instead of the larvae as considered during this project. A thermal processing step (e.g. composting) should be considered for the frass to reduce the *Enterococcus* loads. Moreover, the implementation of residues detection (e.g. metal detectors) should be used to analyse the bio-pulp. Consequently, a pre-treatment of bio-pulp removing potential loads of packaging materials should be considered. A risk assessment diagram should be developed for individual products and hazards (Figure 31).



RISK RATING

SEVERITY	(1) Improbable	(2) Unlikely	(3) Occasional	(4) Probable	(5) Frequent
Catastrophic (5)	5	10	15	20	25
Significant (4)	4	8	12	16	20
Moderate (3)	3	6	9	12	15
Low (2)	2	4	6	8	10
Negligible (1)	1	2	3	4	5

OCCURRENCE

Severe	🟥
Unacceptable	🟧
Undesirable	🟨
Acceptable	🟩
Normal	🟩

FIGURE 31. Example of risk assessment matrix[19].

The pulp as well as the defatted insect meal, insect oil, and processed frass should be monitored for microbial concentration and physical impurities (Principle 4) on a regular basis (Principle 6) to ensure high hygiene standards. If higher levels of chemical and biological contaminants are detected in the products, a series of corrective actions (e.g. isolation of the batch, pulp analysis, disinfection of production unit) (Principle 5) should be considered.

5.2 Large-scale BSFL and insect frass production

The production of BSFL and insect frass at large scale was modelled using Excel, and the Return on Investment (RoI) was calculated. The model consists of input and output, and calculations (Table 13).

TABLE 13. Legend of different commands used in the model.

Input
Calculation
Output

The model was developed on a series of input from the pilot production, optimization and separation experiments, market price estimation and background knowledge from Daka ReFood, DTI, and Hannemann Engineering ApS.

The cost of the bio-pulp (former foodstuffs) was calculated based on the solvency of a biogas plant for this specific product revealed by Daka ReFood. In 2020, the Danish biogas industry could invest 1.2 – 3.5 DKK/m^3 methane produced from this product. The value of bio-pulp is mainly controlled by three factors:

1. content of easily degradable biomass.
2. the accessibility of biomass with a high biogas potential for the specific biogas plant.
3. the ability of the waste management plant to sell expensive biogas certificates.

Based on the above considerations, the value of bio-pulp produced from former foodstuffs in 2020 was between 0.10 and 0.38 DKK/kg bio-pulp with 17% DM. In order to ensure a realistic cost of the bio-pulp, while ensuring a higher dry matter content required by the BSFL, the bio-pulp was calculated as follows:

$$Price\ tonne\ biopulp\ (DM\ 23\%) = \left[\frac{100\ dkk * 380\ dkk}{2} * 23\%\right] / 17\%$$

Following this approach, the cost of bio-pulp at a 23% DM content was estimated to 325 DKK/tonne.

The cost associated with main products from the BSFL production: defatted insect meal and insect oil were calculated based on the available soymeal, fish meal and palm oil prices (see Table 14). The cost of insect oil was assumed to equal palm oil, whereas the cost of defatted insect meal was estimated to be 10% higher than the average value between the soymeal and fish meal. The insect frass price was assumed to be 1,000 DKK/tonne.

TABLE 14. Market prices for soymeal, fish meal and palm oil and the estimated price for defatted insect meal, insect oil (similar to palm oil) and insect frass.

	DKK/tonne
Soymeal[19]	3,122
Fishmeal[10]	9,082
Palm oil[10]	5,777
Defatted insect meal*	6,712
Insect oil*	5,777
Insect frass*	1,000

*Expected

The model consists of 6 sections: 1) Pilot production and experiment data, 2) Factory data and estimated yield, 3) CAPEX, 4) OPEX, 5) Revenues and 6) Return on Investment.

The utilization of bio-pulp and the production of BSFL and insect frass were calculated on a FW basis for 1m² based on the results from the pilot production and optimization experiments as presented in Table 15.

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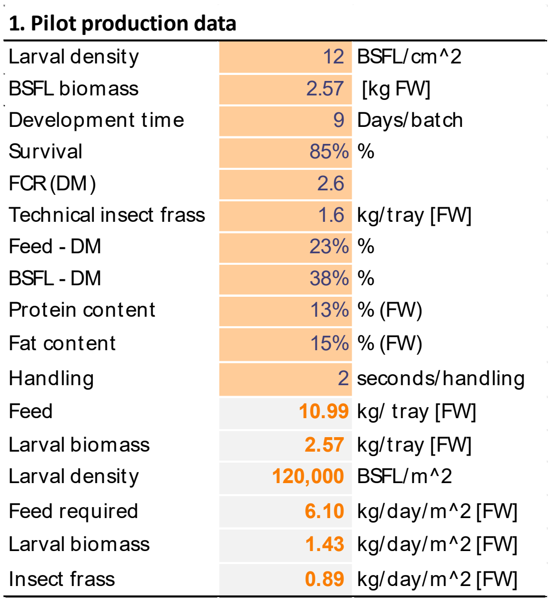
[19] Index Mundi, 2021. Market price of soymeal, fish meal and palm oil in 2021: Soybean Meal vs Fishmeal - Price Rate of Change Comparison - IndexMundi; Palm oil - Monthly Price (Danish Krone per Metric Ton) - Commodity Prices - Price Charts, Data, and News - IndexMundi

TABLE 15. Pilot production data, consisting of input from the pilot production and optimization experiment and calculation on production.

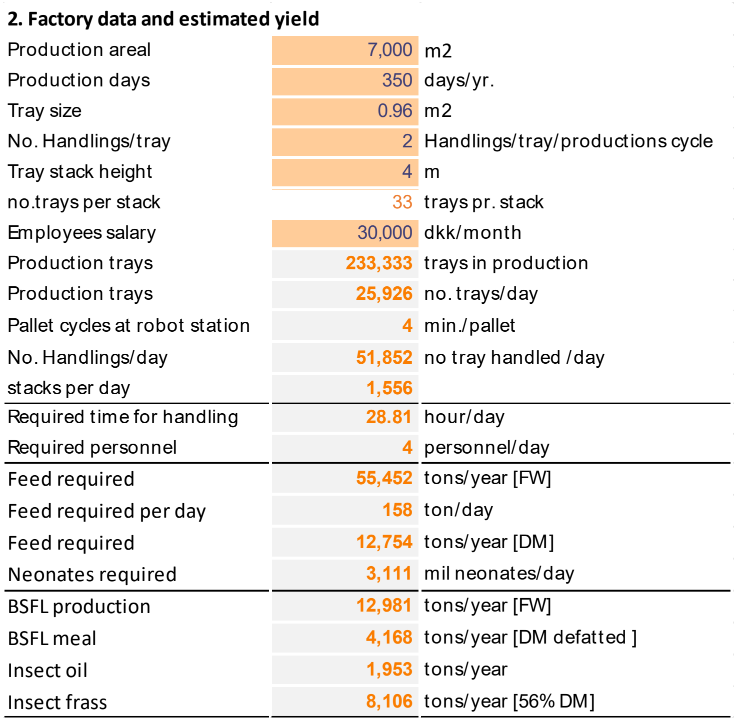


1. Pilot production data

Larval density	12	BSFL/cm^2
BSFL biomass	2.57	[kg FW]
Development time	9	Days/batch
Survival	85%	%
FCR (DM)	2.6	
Technical insect frass	1.6	kg/tray [FW]
Feed - DM	23%	%
BSFL - DM	38%	%
Protein content	13%	% (FW)
Fat content	15%	% (FW)
Handling	2	seconds/handling
Feed	10.99	kg/ tray [FW]
Larval biomass	2.57	kg/tray [FW]
Larval density	120,000	BSFL/m^2
Feed required	6.10	kg/day/m^2 [FW]
Larval biomass	1.43	kg/day/m^2 [FW]
Insect frass	0.89	kg/day/m^2 [FW]

The input and calculations from Table 18 were further used together with a series of factory data (i.e. production area, days in production, tray size, amount of handling per tray and employee salary) to calculate a series of requirements and estimate the yearly amount of insect meal, insect oil and insect frass production (Table 16).

TABLE 16. Factory data input and estimated yield for large-scale BSFL and insect frass production.



2. Factory data and estimated yield

Production areal	7,000	m2
Production days	350	days/yr.
Tray size	0.96	m2
No. Handlings/tray	2	Handlings/tray/productions cycle
Tray stack height	4	m
no.trays per stack	33	trays pr. stack
Employees salary	30,000	dkk/month
Production trays	233,333	trays in production
Production trays	25,926	no. trays/day
Pallet cycles at robot station	4	min./pallet
No. Handlings/day	51,852	no tray handled /day
stacks per day	1,556	
Required time for handling	28.81	hour/day
Required personnel	4	personnel/day
Feed required	55,452	tons/year [FW]
Feed required per day	158	ton/day
Feed required	12,754	tons/year [DM]
Neonates required	3,111	mil neonates/day
BSFL production	12,981	tons/year [FW]
BSFL meal	4,168	tons/year [DM defatted]
Insect oil	1,953	tons/year
Insect frass	8,106	tons/year [56% DM]

It is estimated that an investment of 23m DKK is required to establish a BSFL and insect frass production of 13 tonnes/year reared on former foodstuffs. The costs include the following materials: three handling robots, three vibrating sieves (like the one used in the project), processing system for defatting, drying and oil extraction, a direct pipe for pumping bio-pulp into the production, six automated guided vehicles (AVG), control panel, cooling systems, counting and dosing of neonates and initial building (Table 17).

TABLE 17. CAPEX estimation of large-scale BSFL and insect frass production.

3. CAPEX	Price in dkk	
production area (building)	12,600,000	dkk (ca. 1800-2200dkk/m2)
Handling Robot	3,600,000	dkk (3x1.2mio.)
Sieving x3	168,000	dkk
Processing machinery	2,000,000	Oil press + drying
direct pipe	200,000	
AGV (MIR)	1,560,000	260.000 / each
Control panel	500,000	
cooling systems	1,500,000	
Counting and dosage neonates	750,000	
Total	**22,878,000**	dkk

The OPEX of the BSFL factory were calculated using the following assumptions: The price of the juvenile larvae received from a third party was estimated to 20 DKK per 1m juveniles, the feed was calculated based on input from Daka ReFood (325 DKK /tonne), and 5% of CAPEX was expected for operational costs. Furthermore, the personnel costs per year were estimated according to a monthly average salary of 30,000 DKK and the required personnel (Table 18).

TABLE 18. OPEX estimation for large-scale BSFL and insect frass production.

4. OPEX		
Production of neonates larvae	21,802,667	20 dkk. pr. 1million neonates
Feed	18,021,984	@325 dkk/tone
Operational and maintenance	1,143,900	5% of the CAPEX
Personnel cost	1,481,481	salary cost/year
Total	**42,450,032**	dkk/year

5. Revenues		
BSFL meal	**27,973,139**	6102 dkk/tonne, avg. of soy and fishmeal
Insect oil	**11,280,135**	5777 dkk/tonne
Insect frass	**8,106,054**	1000 dkk/tonne
Total	**47,359,328**	dkk/year

Return on Investment for BSFL production reared on former foodstuffs was estimated to 4.7 years, providing a good business case if former foodstuffs are approved as feed in the BSFL production (Table 19). However, this model has limitations, and a few assumptions require a more critical approach when considered.

TABLE 19. Return on Investment (RoI) from the production of BSFL and insect frass reared on former foodstuffs.

6. Return on investment		
Revenues	47,359,328	dkk/year
OPEX costs	42,450,032	dkk/year
Difference	4,909,296	dkk/year
CAPEX	22,878,000	dkk/year
Return on investment	**4.7**	years

6. Development of concept for optimal insect frass utilization

Successful production of BSFL and insect frass depends on the input parameters (juvenile larvae and bio-pulp configuration), the production output (larval biomass, insect frass), and the final separation process. The input (yellow) and output (red) of a BSFL production feeding on former foodstuff is depicted in Figure 32.

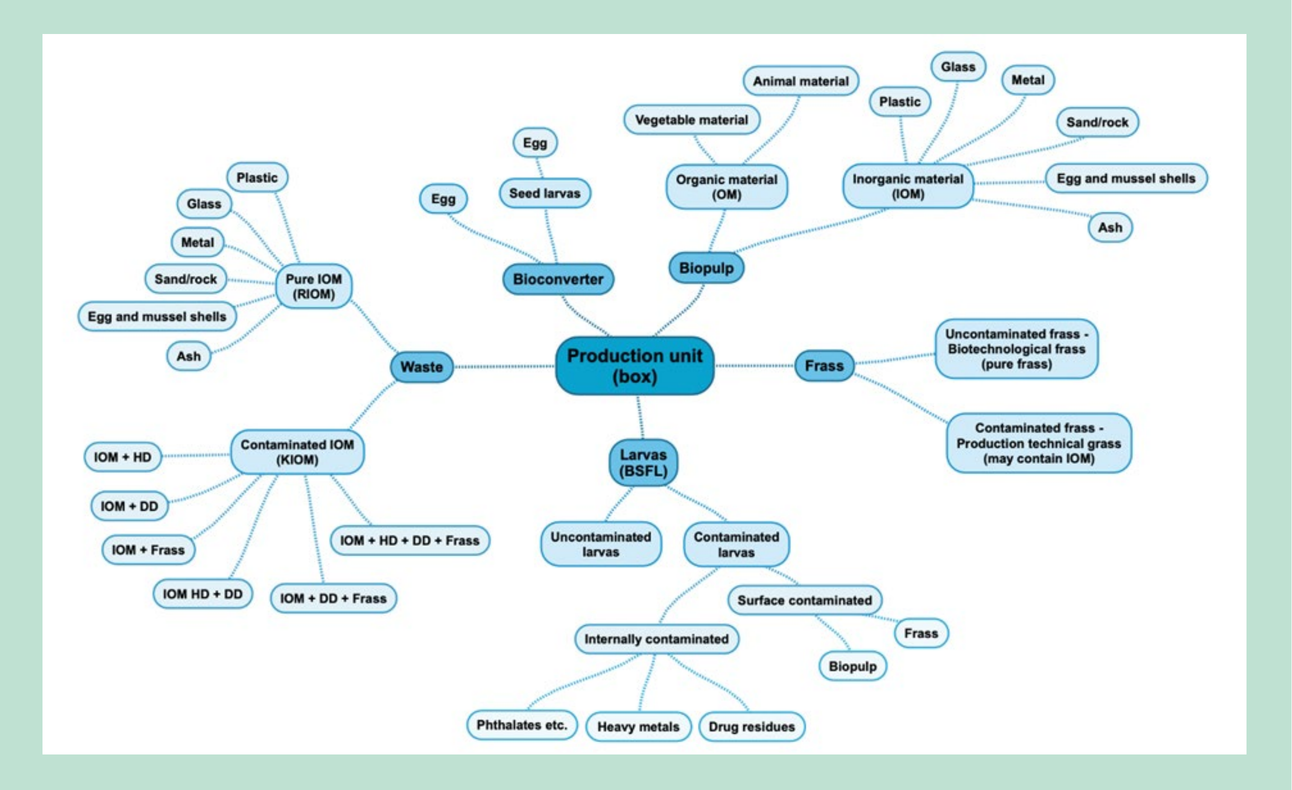


FIGURE 32. BSFL and frass production overview. Input above the yellow line, output after the red line.

6.1 Separation and removal technology for insect frass without impurities

The overall target of this part of separation is to ensure a clean frass product usable as a fertilizer on agricultural land. This implies that the level of impurities present in the final frass product is below the level prescribed by law. In general, we use the Danish executive order no. 760 of 30/07/2019 that focuses on storage and application of fertilizers from animal husbandry, but it is irrelevant when focusing on insect production. The utilisation of insect frass could contain remains of BSFL (dead or exoskeleton parts) and therefore violates the (EC) No. 1774/2002

Animal by-products Regulation[20]. If insect remains are present in the frass, sterilization is mandatory according to Regulation (EU) No 952/2013[21]. This procedure is expensive and will undermine the business case. Several insect producers have a permission to sell frass as fertilizer with the condition of ensuring that no insect remains are found in the final product intended for soil improvement applications.

The bio-pulp delivered by Daka ReFood is currently intended for biogas production, and the production of bio-pulp is briefly described in the following:

Former foodstuffs, including full packed and products that have expired, are collected and loaded into a pulper and grinded. The former foodstuffs are passed through a separator, where the bio-pulp is squeezed through a separation sieve, which implies that most organic material is transferred through the separation plate, while most packaging is retained and removed. However, a small fraction of packaging material (mainly of plastic origin) will pass through the sieve together with the organic material and end up in a large holding tank.

To avoid that packaging material ends up in the field while using the current bio-pulp production procedure, two different approaches could be considered: pre-treatment of bio-pulp or separation after bioconversion.

6.1.1 Pre-treatment of bio-pulp

Three methods for bio-pulp pre-treatment and their respective challenges were considered. Utilisation of a single- or multiple-step **hydro cyclone separation technology** is often used for removal of impurities and relies on density variation. In this case, however, the bio-pulp's organic fraction has a density of app. 1,200 kg/m³, while the plastic varies from 10 to 2,150 kg/m³, making the use of a hydro cyclone technology inappropriate. **Sieving by squeezing** the bio-pulp through a sift of lower mesh sizes than for the initial separation will remove hard plastic, but the foil will slip through due to its folding ability. A **vibrating sifting technology** might get rid of plastic foil and larger plastic objects such as low density and flexible plastic (e.g. EPS), however, the sift will easily clog due to the large organic fractions, especially fibres. This may be solved using specially designed self-cleaning sieves during online operation, although not considered in detail in this project.

6.1.2 Post-harvest treatment (sieving)

Removal of impurities is in most cases easier after bioconversion when the insect frass is relatively dry. After bioconversion of bio-pulp with BSFL, a typical tray will contain a mixture of BSFL and residuals (insect frass, exoskeletal parts, packaging materials (e.g. plastics, cardboard, etc.)). The goal is to separate the residual mixture into clean BSFL, technical frass (clean frass <2mm) and unusable waste.

Three separation methods were investigated for their efficiency *i*) Migratory, *ii*) Manual sifting and *iii*) Mechanical sifting.

i) Migratory approach: The migratory approach was used when the resulting fraction after bioconversion was very sticky and clustered together, making it impossible to separate through a sieve. The content of a tray was placed on a sieve of 7-mm mesh size and left for the larvae to

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[20] Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32002R1774

[21] Regulation (EU) No 952/2013 of the European Parliament and of the Council of 9 October 2013 laying down the Union Customs Code OJ L 269. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0952&from=EN

migrate through the meshes and into a collection tray (see Figure 33). With this method, the larvae are separated but without further separation of the residual into minor fractions.

FIGURE 33. Separation of BSFL and insect frass using a migratory approach.

ii) Manual sieving: The manual sieving consisted of a modular sieve (4- and 2-mm mesh size) to separate the content of a tray into a clean BSFL fraction, an unusable waste fraction, and a technical frass fraction. The content of a production tray was placed on the top of the modular sieve and manually sieved into a fraction consisting of BSFL and large particle residues on the top of the 4-mm mesh and a technical frass fraction passing through the 2-mm mesh (**Figure 34**). Hereafter, the sieve with the 4-mm mesh size containing the mixed fraction was placed on the top of a collecting tray and maintained steady until most of the BSFL migrated through the 4-mm mesh into the tray. This procedure was implemented on most of the production batches.

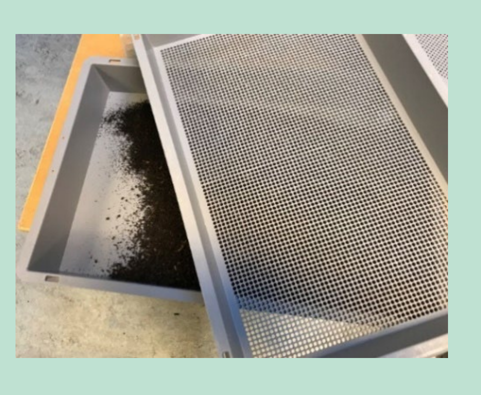

FIGURE 34. Example of technical frass separation using 2-mm mesh during manual sieving tray.

iii) Mechanical sieving
A large advantage of using a vibrating industrial sieve is that it works very well as a continuous inline process. The mechanical sieving used for this test consisted of a rotational vibrating modular screen with 4- and 2-mm mesh sizes. With the final 2-mm mesh sift, a technical frass fraction was obtained. As in the case of manual sieving, the sieve was stopped and the fraction consisting of BSFL and large particle residues from the top of the 4-mm mesh were collected and placed on a steady 4-mm mesh until most of the larvae migrated and dropped into a collecting container (Figure 35).

FIGURE 35. Separation of BSFL and insect frass using mechanical sieving.

The mechanical sieving was used for Batch 17. Under the density and bio-pulp enhancement optimization, the content of a tray belonging to different dietary treatments was divided into fractions of high-value BSFL biomass, technical frass, and low-value unusable waste (Figure 36).

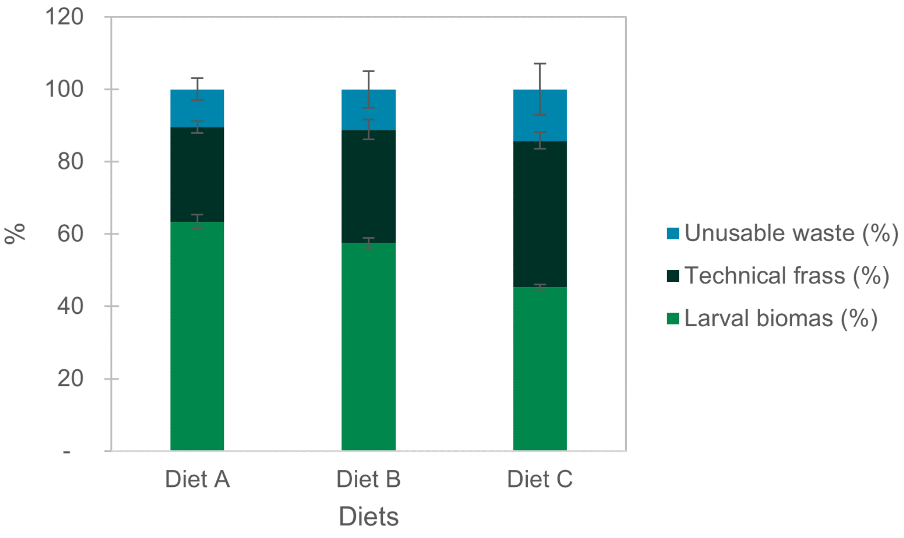

FIGURE 36. The separation of a tray content into different fractions with mechanical sieving: BSFL, technical frass, and unusable waste.

6.1.3 Conclusion

The manual and mechanical sieving procedures showed that the content of a production tray can be separated into three fractions: BSFL, technical frass, and unusable waste intended for incineration (10%), providing a relevant usage of the nutrient-rich insect frass. A fine tuning of the vibrating sieve and more tests of the sifting technique will give an even better result than already obtained.

6.2 Characterisation of the physical impurity content in insect frass

The technical frass obtained after separation of larvae and impurities during the production of batch 1-3 and 17 was characterised by the frass' physical structure and content of impurities (dead and living larvae, packaging residues and larval exoskeleton). The content of living and dead larvae as well as exoskeleton content was low, representing 6% for batch 1, 5% for batch 2 and <2% for batch 3 (Figure 37). The frass from batch 1 was very viscous and sticky resulting in high larval mortality. Moreover, it could not be separated using a sieve, instead a migration approach was used for separation (Figure 37).

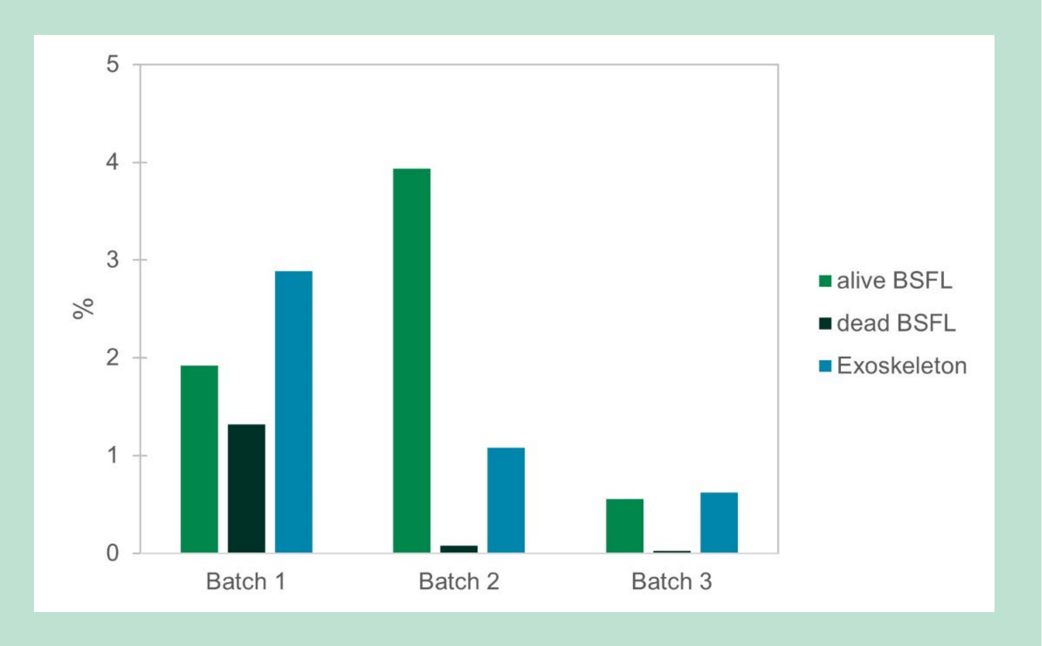

FIGURE 37. Characterisation of technical frass based on percentage of living and dead BSFL and exoskeleton found in the frass of batch 1-3.

Based on these first characterisation and production results (batch 1-3), the bio-pulp was further enhanced with other waste streams (high-fibre pulp, husk, and coffee grounds) to ensure high larval survival, high larval and insect frass production, and easier and better separation. The frass from batch 17 was separated into different fractions (>4mm, <4mm, <3 mm, and <2mm) using the mechanical sieving procedures (Figure 38).

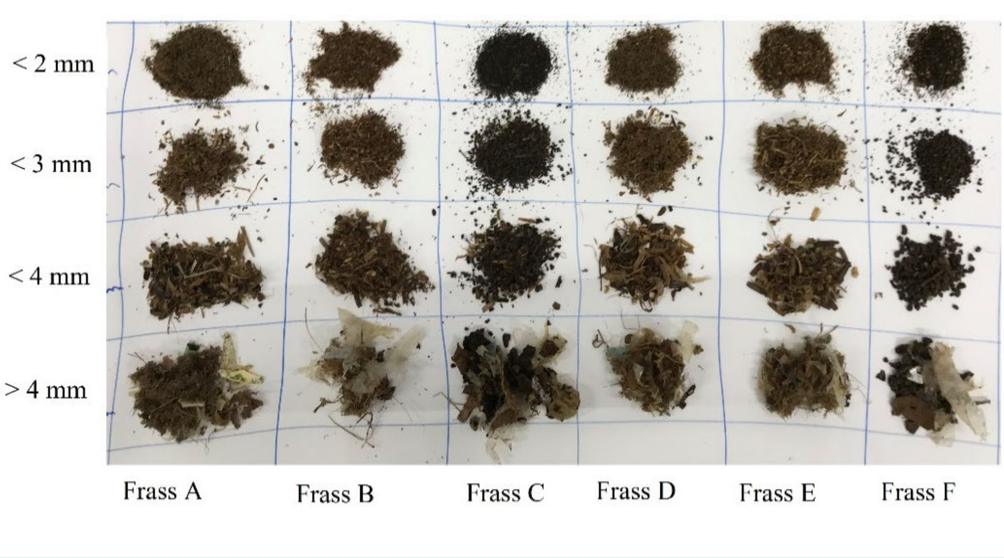

FIGURE 38. Example of insect frass separated by mechanical sieving.

The separation of frass (batch 17) in different fractions was very successful. 60-80% of the frass passed through all sieves, reaching a particle size of <2 mm (technical insect frass) as seen in Figure 39. Overall, the highest fraction of frass recovered from the treatment was associated with Diet B (bio-pulp 92% and husk 8%), followed by Diet A (bio-pulp 80% and high-fibre bio-pulp 20%), and finally by Diet C (bio-pulp 80% and coffee grounds 20%) (Figure 39).

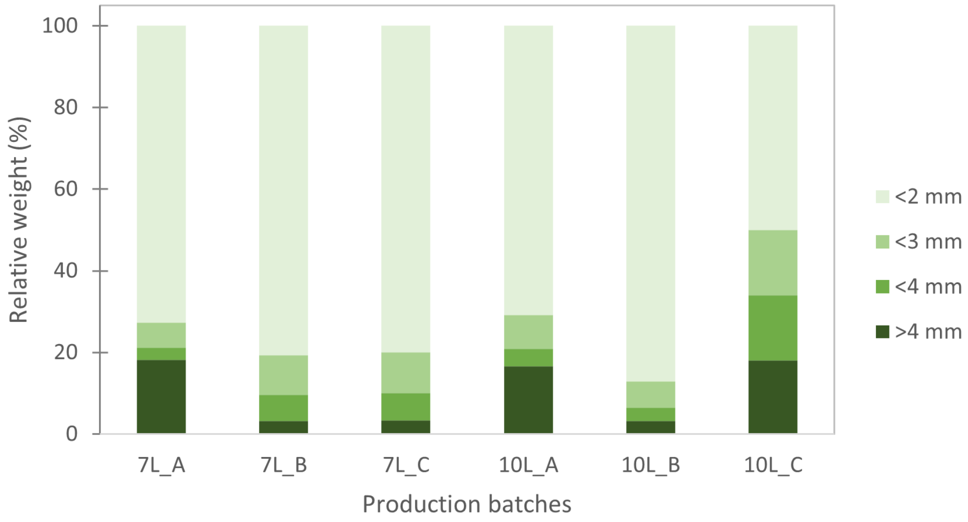

FIGURE 39. Separation of frass into four different fractions, using mechanical sieving.

The technical frass (<2 mm) was further characterised for content of impurities (packaging residues and living or dead BSFL). As seen in Figure 40, the content of impurities was very low (less than 3%) indicating that this fraction of frass is of high quality. Moreover, the presence of living BSFL (0.5%) was only observed in the technical frass belonging to Diet C at a density of 7 larvae/cm²(7_C). Although the results are promising, a visual inspection under a microscope is needed to determine if the technical frass contains exoskeletons or other small impurities such as microplastics and other micro-residues.

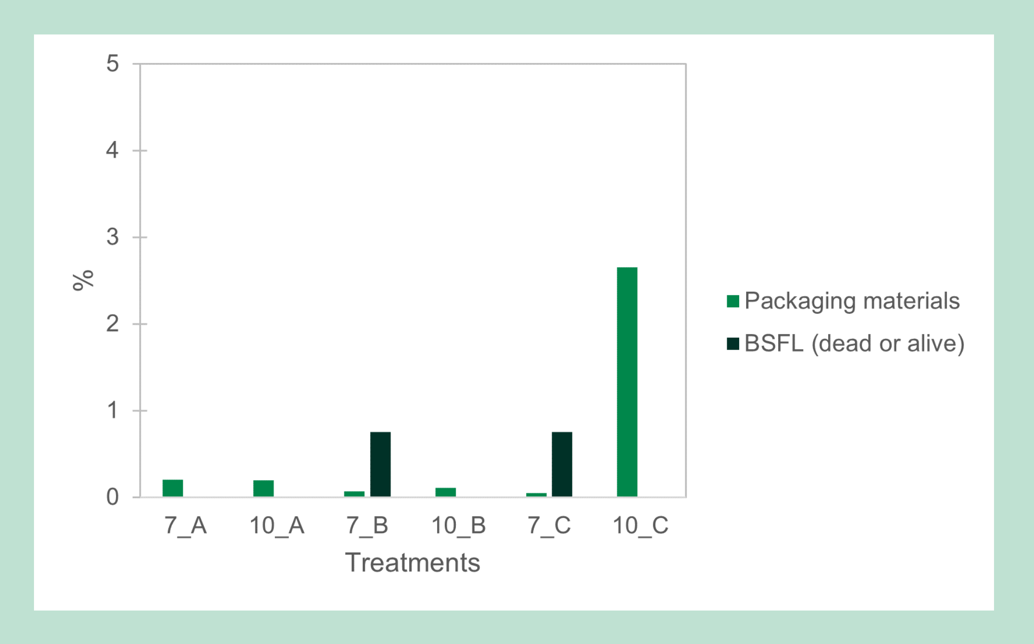

FIGURE 40. Impurity content of insect technical frass produced during batch 17, after bio-pulp enhancement.

6.3 Contribution to insect frass legislation

In order to develop an economical and sustainable applicability of insect frass as soil amendment, input and documentation developed during WICE4Soil were provided for future harmonization of the EU rules on the processing and use of insect frass as a fertilizer product.

The high-nutrient content observed during WICE4Soil shows that insect frass is a valuable soil fertilizer. In principle, insect frass is a mixture of faeces, feed residues, exoskeletons, and dead larvae. According to the Animal By-Product Regulation this mixture was a category 2 product, when this project was initiated, and requires pressure sterilization before being used for fertilizer purposes. Therefore, this project together with the MUDP project Enorm Biofactory[22] jointly provided documentation and knowledge to contribute to more pragmatic and realistic framework conditions for the categorization and handling of insect frass. In February 2019, a team was formed with participants from Enorm Biofactory, Daka ReFood, DTI, and DTU, led by Dorte Lau Bagges, advising the Danish Veterinary and Food Administration, and participating in EFSA's European Food Safety Authority. The aim was to prepare a plan for how insect frass can be recycled in an efficient and safe manner. This work was carried out in close collaboration with the Ministry of Food, Agriculture and Fisheries and the Danish Veterinary and Food Administration, as well as DC SANTE, the EU Commission's body for food safety. By November 8th, 2021, this IPIFF has submitted a press release, that welcomes the adoption of the Regulation that paves the way for the first EU-wide insect frass standards[23].

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[22] MUDP project “Enorm Biofactory – Værdiforøgelse af restbiomasser gennem insektproduktion” (MST-117-00460)

[23] Nov-08-2021-IPIFF-PR-frass-Regulation-Commission-adoption.pdf

The risk of parameters such as heavy metals and pathogens as well as the characterization of form and properties were analysed after production of BSFL reared on either former foodstuff (this project) or legal feed substrates in the production at Enorm Biofactory.

A new definition of insect frass was proposed based on the analyses and tests conducted, and the possible part in terms of legislation, where insect frass <2 mm is defined as technical frass to be used directly as fertilizer and soil improvement on agricultural land. The fraction >2 mm (which may contain dead BSFL lichens) must be handled as an ABP category 2 product and handled by e.g. Daka ReFood for further treatment in parallel with other dead animals. This proposed definition was approved by the Danish Veterinary and Food Administration in October 2019. The Danish Agency for Agriculture does not accept direct spreading of technical frass without a specific permit for each individual field frass, because technical frass is not listed in the appendix of the Waste to Land regulations as part of the Danish Environmental Protection Agency resort. Such a permit is quite expensive in terms of preparation for companies as well as municipalities. Instead, Enorm Biofactory applied and was granted a permit to classify their technical frass as a technical by-product of waste that can be sold and used freely as fertilizer and soil improver. With the approval of technical frass, considerations regarding processing methods of insect frass are redundant.

6.4 Comparison of insect frass and manure sources: GHG, odour emission, dry matter, and impurity levels

6.4.1 Methods

The emission of gases from two different types of insect frass and three different types of manure types were measured:

- Insect frass from BSFL reared on chicken feed (Frass CF)
- Insect frass from BSFL reared on former foodstuffs (Frass FF)
- Pig manure
- Acidified pig manure
- Horse manure

Emissions of CO_2, CH_4, N_2O and NH_3 were evaluated from a 100 g sample placed in a 1 L container on day one and day three using INOVA. The odour emissions were evaluated on a highly diluted sample (x251) to avoid NH_3 interference and only succeeded on the Frass CF on day three, which had a relatively low ammonia emission. Furthermore, the dry matter and impurity levels of insect frass and other manure types were compared.

6.4.2 Results

Overall, the emission of CH_4 was much higher from the manure biomasses that are very liquid, anoxic and have a high anaerobic microbial activity producing methane. The CO_2 emission was higher from the insect frass. The N_2O emissions from Frass CF was higher during day one, when compared with the rest of the substrates. The N_2O emissions from Frass FF were similar to the different manure types. Ammonia (NH_3) emissions from the two frass types were comparable to the acidified manure but lower than the horse and pig manure (Table 20).

TABLE 20. Emissions of CO_2, CH_4, N_2O, and NH_3 of insect frass from BSFL reared on chicken feed (Frass_CF) and bio-pulp (Frass_FF) and three types of manure.

	Days	CO_2 (ppm)	N_2O (ppm)	NH_3 (ppm)	CH_4 (ppm)
Frass _CF	Day 1	32,094	10.42	114	29
	Day 3	11,244	0.88	73	14
Frass _FF	Day 1	7,269	0.98	130	29

	Days	CO_2 (ppm)	N_2O (ppm)	NH_3 (ppm)	CH_4 (ppm)
	Day 3	3,528	0.27	175	37
Horse manure	Day 1	3,936	0.59	563	26
	Day 3	3,730	0.32	825	46
Acidified pig manure	Day 1	4,170	0.53	182	1,279
	Day 3	1,137	0.71	192	50
Pig manure	Day 1	3,538	0.48	403	621
	Day 3	1,246	0.38	338	44

A series of odour-related compounds available in manure used as fertilizer were identified in the insect frass, and most compounds had lower levels than the odour emissions from manure (Table 21).

TABLE 21. List of odour components found in insect frass (Frass CF) compared to levels found in manure intended for soil amendment.

Component	ppm	Typical levels found in manure
Acetic acid	1800	higher
Limonene	1134	NA
3-methylindole	890	higher
Hydrogen cyanide (HCN)	755	NA
Ammonia (NH3)	134	lower
Dimethylformamide	580	lower
Trimethylamine	518	higher
4-methyl-phenole	428	higher
Methanol	236	NA
Dimethyl sulfide	198	lower
Hydrogen sulfide (H2S)	143	higher
Methanethiol	60	higher

Overall, the dry matter content of the two types of tested frass were similar when compared to each other, and - as expected - much higher than the manure types. The impurity levels in Frass FF were higher when compared with the other substrates, but they were still low (2%). The impurity levels in Frass CF were similar to the one of horse manure (Table 22).

TABLE 22. Comparison of dry matter and impurity levels between two types of insect frass and two types of manure.

Sample	Dry matter [%]	Impurities [% DM basis]
Frass_CF	53.2	0.1
Frass_FF	49.5	2.0
Horse manure	22.1	0.2
Pig manure Horsens Bioenergi	6.7	0.1

n=2

7. Pelletized technical insect frass tested as pre-fertilizer for maize

The objective of this test was to evaluate the use of insect frass as a fertilizer in field scale, both regarding the fertilizer effect and the general use of insect frass. In this trial it was applied to the soil before sowing of maize.

7.1 Method

Palletized insect frass produced in Denmark by EntoMass ApS was applied as pre-growing fertilizer with a corresponding amount of phosphorus in mineral fertilizer, diammonium phosphate (DAP) spread before sowing compared to zero start fertilizer (Table 23). The amount of fertilizer applied with insect frass was calculated based on insect frass analysis (Table 24). Hence, treatment DAP-5 and frass-5 (low level), DAP-15 and frass-16 (middle level), DAP-25 and frass-26 (high level) are considered to have received equivalent amounts of phosphorus.

TABLE 23. Treatments in the trial.

Treatment	Phosphorus (P) Kg P applied per ha	Applied Kg fertilizer per ha
Control	0.0	0
DAP-5	5.0	25
DAP-15	15.0	75
DAP-25	25.0	125
Frass-5	5.2	720
Frass-16	15.6	2170
Frass-26	26.1	3620

TABLE 24. Nutrient content of pelletized insect frass and DAP mineral fertilizer

	Trial analysis – Frass
Dry matter	86.8
Total nitrogen (N)	29.5 kg/ton
Ammonium N	8.7 kg/ton
Phosphorus (P)	7.2 kg/ton
Potassium (K)	11.3 kg/ton

The field trial was laid out on a field in Southwest Jutland at Ålbæk near Bramming (Figure 41).

FIGURE 41. Location of the field trial at Ålbæk near Bramming. The soil type is coarse sand. Organic matter content is 2.8 % in the soil, 0-25 cm depth. The soil pH was a bit low (Rt = 5,6/pH = 5.1) while P-status (Pt = 6,89) and K-status (Kt = 10.5) was at a high level. The field was applied 60 tons slurry per ha mid-May.

The disease and weeds pressure were low in the field. The experiment was stopped with the last plant sample on July 27th. May 2021 was characterized by low temperatures and heavy rain. In June and July, the temperature and precipitation were just above the norm (1991-2020) (DMI, 2021).

Mineral fertilizer was distributed with a hand pushed trial fertilizer spreader, while the insect frass was spread manually by hand since the amounts were too big to handle by the spreader. All treatments were conducted on May 18th. Maize was sown on May 30th.

The trial was laid out as a complete block trial, 1 factor (randomized). There were 4 repetitions of each treatment. See table 25 for specification of treatments.

TABLE 25. Trial design.

Plot	Row1.	Row2.	Row3.	Row4.
1	Buffer	Buffer	Buffer	Buffer
2	6	4	5	7
3	2	6	2	1
4	7	1	7	6
5	1	5	6	3
6	4	2	4	5
7	5	3	1	2
8	3	7	3	4
9	Buffer	Buffer	Buffer	Buffer

Two times during the growing season, the plant biomass has been measured with NDVI/NDRE. These data have been collected with a multispectral camera mounted on a drone. Simultaneously, plant samples were taken to measure the nutrient status of the crop.

7.1.1 Biomass index (NDVI, NDRE)

NDVI and NDRE biomass indexes are measurements for N uptake, however this is not quantified in kg N and it can only be interpreted as relative differences between treatments.
For description of the biomass, two different indices were used: NDVI (Normalized difference vegetation index) and NDRE (Normalized difference red edge index). The indices are calculated based on data collected with a multispectral camera mounted on a drone. The wavelengths used to calculate the indices are NIR (Near infrared), Red (Red, visible) and RE (Red Edge). The reflectance gives information about the green reflection of the crops, thus the amount of chlorophyll.

$$\mathrm{NDVI} = \frac{(\mathrm{NIR} - \mathrm{Red})}{(\mathrm{NIR} + \mathrm{Red})} \qquad \mathrm{NDRE} = \frac{(\mathrm{NIR} - \mathrm{RE})}{(\mathrm{NIR} + \mathrm{RE})}$$

NDVI is the preferred index when the crops are small and NDRE when the crops are larger, and leaves overlap[24].

7.1.2 Plant samples, nutrient content

At the same dates as the drone flights, leaf samples were taken and analysed for content of following nutrients (not shown): nitrogen (N), phosphorus (P), potassium (K), sulfur (S), manganese (Mn), boron (B), molybdenum (Mo), copper (Cu), zinc (Zn) magnesium (Mg). iron (Fe) and calcium (Ca).

Plant sampling was made by collecting last fully developed leaves. The leaves were washed and dried before analysis. Nitrogen was analysed by Dumas method and all other nutrients were determined by use of ICP-OES.

7.2 Results and discussion

In the current study, pelletized insect frass was tested in field scale by applying it as a start fertilizer to maize before sowing compared to corresponding content of phosphorus in DAP, mineral fertilizer.

NDVI and NDRE indexes show only small differences in biomass in treatments with and without insect frass (link to fertilizer results). According to this observation insect frass does not harm the maize growth and has at least equal effectiveness compared to low and middle mineral fertilizer.

In middle and high fertilizer doses, pelletized frass showed significantly higher concentration of several essential nutrients in leaf tissue taken 37 days after sowing (link to fertilizer results), and this also accounts for P-uptake, despite the comparable applied P at low, middle and high fertilizer treatments. But did on the other hand result in less Potassium (K) uptake. Whether the extra uptake is due to nutrient supply by applied insect frass or if insect frass in some other ways help nutrients in the soil to become available to the maize crop is not given in this trial. This release of nutrients could be explained by acidification of the soil that facilitate access to nutrients or it could be a kind of a bio-stimulant effect. Both positive and negative effects on the nutrient content seem to fade over time, since the effects are almost gone at second sampling 58 days after sowing.

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[24] Bonfil, D. J. (2017). Wheat phenomics in the field by RapidScan: NDVI vs. NDRE, Israel Journal of Plant Sciences, 64(3-4), 41-54.

The field trial shows that insect frass could serve as fertilizer for plant production. A high amount of insect frass is, however, necessary when applied as fertilizer alone, which makes annual large-scale application to agricultural fields inconvenient. Instead, insect frass could serve as a nutrient addition: *i*) in the production of small-scale production such as household plants, green house vegetables and mushrooms, *ii*) as an additive to cattle manure, and *iii*) as P source for soil restauration equal to sludge application.

8. Conclusion

This status report for WICE4Soil was written in a close collaboration between Daka ReFood A/S, Hannemann Engineering ApS and Danish Technological Institute. Some of the activities was performed in good collaboration with Enorm Biofactory on their parallel MUDP project[25], and EntoMass kindly delivered pelletized insect frass for agricultural field test.

The purpose of WICE4Soil was to evaluate the potential of insect frass as a soil fertilizer when Black Soldier Fly Larvae are reared on former foodstuff. The project evaluated the quality and safety from bio-pulp to BSFL and insect frass, production optimization of bioconversion and the potential of insect frass as agricultural fertilizer.

The pilot production of BSFL was optimized and both produced larvae and insect frass had a product quality and safety approved for usage as both animal feedstock (BSFL) and agricultural fertilizer (technical frass). A juvenile counting solution was developed for faster processing. Summing up the learnings for a pilot production of BSFL reared on bio-pulp, a new production site producing ~13000 tonnes of BSFL per year would have a RoI of 4.7 years.

The technical frass (<2 mm) was obtained with mechanical sieving and the definition was approved by the Danish Veterinary and Food Administration in October 2019. Pelletized insect frass was tested on field pilot scale for their effect as a pre-fertilizer for maize production. Insect frass holds the potential as plant fertilizer but is more time-consuming in the application process.

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[25] MUDP project “Enorm Biofactory – Værdiforøgelse af restbiomasser gennem insektproduktion” (MST-117-00460

WICE4Soil. Waste, Insects, and Circular Economy for Soil
Tidligere fødevarer (biopulp) er en værdifuld ernæringskilde, men bruges i dag kun til energiproduktion. WICE4Soil (Waste, Insects and Circular Economy for Soil) bygger oven på resultaterne i det tidligere WICE-projekt, som konkluderede at soldaterfluelarver (BSFL) kan leve på madaffald, og at de resulterende to produkter fra produktionen, nemlig larve-biomasse og larve ekskrementer (insektfrass), har et potentiale som henholdsvis dyrefoder (larver) og plantegødning (frass). I dette WICE4Soil projekt evalueres: i) kvaliteten og sikkerheden ved brug af biopulp til produktion af soldaterfluelarver og insektfrass, ii) produktionsoptimering af biokonvertering og iii) potentialet af insektfrass som landbrugsgødning.
Resultaterne fra WICE4Soil-projektet viser, at produktionen af soldaterfluelarver fra tidligere fødevarer og udnyttelsen af insektfrass som gødning er velegnet ud fra sikkerheds-, kvalitets- og økonomiske perspektiver. Således kan denne anvendelse føre til en bæredygtig up-cycling af tidligere madaffaldsstrømme.

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