Processing of food industry waste streams by compost worms

Experiments in 2021

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This report describes five experiments to observe the growth of *Dendrobaena veneta* and *Eisenia fetida* on substrates originating from Avebe (potato starch and protein producer), Holland Malt (maltings) or others (kitchen and garden wastes). The substrates include Tarra (Avebe), Secondary food industry Sludge (Holland Malt and Avebe), Barley Dust (Holland Malt), Germinated Barley (Holland Malt), Bokashi (Jansen Wijhe), Potato Peels (van Vulpen voeders), vegetable fruit and garden waste (van der Wal and Ogar), Apple pulp (van Vulpen voeders), Champost (Koolen champignons CNN) and Carrot and Pumpkin Peels (van Vulpen voeders). The compost worms' growth was monitored for 4-6 weeks. The compost worms were able to convert the substrates into dry, light and powdery vermicompost high in nutrients and organic matter. The compost worms themselves increased in biomass and produced eggs. The protein content of the compost worms ranged between 57.1 and 68.3 % of DM, whereas the fat content ranged between 7.8 and 9.1% of DM. Tarra and Secondary food industry Sludge (Avebe) are suitable substrates for vermicomposting, when mixed with other substrates. Germinated Barley and Secondary Food Industry Sludge (Holland Malt) are less suitable for vermicomposting, due to substrate instability, even when mixed with other substrates. Additions of barley dust and chalk make the substrates' texture and composition more suitable for the worms to feed on.

Keywords: *Eisenia fetida, Dendrobaena veneta,* compost worms, vermicompost, food industry waste streams, potato starch, maltings, kitchen waste, garden waste.

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Preface

ACRRES is involved in the Dutch pilot project 'Kringlooplandbouw Veenkoloniën' (circular agriculture in the Veenkoloniën) which is aimed at closing agricultural cycles on regional level. The project ran in 2021. By closing nutrient cycles, emission levels can be lowered and secondly, soil quality can be maintained or improved by applying local organic waste streams to the soil. Organic waste streams can be converted/processed by insect larvae and earthworms, resulting in a protein rich stream (insect larvae and worm biomass) but also in frass and vermicompost (i.e. insect larvae and worm faeces) respectively. Both streams are used in agricultural practices on a small scale. With the help of Avebe and Holland Malt, suitable organic waste streams were identified for the cultivation of insect larvae and worms. Within this project it was investigated whether those waste streams can benefit agricultural practices in the region 'Veenkoloniën'. This project is a part of the project 'Innovatie biodiversiteit Veenkoloniën' (Innovation biodiversity peatlands).

More information:

- https://www.nmi-agro.nl/2020/12/08/pilot-kringlooplandbouw-veenkolonien/
- https://anog.nl/innovatie-biodiversiteit-veenkolonien
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Summary

This report describes five different experiments to observe growth of *Dendrobaena veneta* and *Eisenia fetida* on substrates originating from Avebe (potato starch and protein producer), Holland Malt (maltings) or others (kitchen and garden wastes). The substrates include Tarra (Avebe), Secondary Food Industry Sludge (Holland Malt and Avebe), Barley Dust (Holland Malt), Germinated Barley (Holland Malt), Bokashi, Potato Peels, VFG, Apple pulp, Champost and Carrot and Pumpkin Peels.

The five experiments all had a slightly different set-up. The first experiment (Experiment 1) was done in small plastic trays (1L) with 25 worms, whereas the second experiment (Experiment 2) contained 10L of substrate with 1 kg of worms. The third experiment (Experiment 3) was done in chicory racks each containing 50L of substrate with 5 kg of worms. The vermicompost and the compost worms were sent to a commercial lab for analyses. The protein content of the compost worms ranged between 57.1 and 68.3 % of DM, whereas the fat content ranged between 7.8 and 9.1% of DM.
The compost worms' growth was monitored for 4-6 weeks during the experiments. The compost worms were able to convert the substrates into dry, light and powdery vermicompost high in nutrients and organic matter. The compost worms themselves increased in biomass and produced eggs. Some substrate combinations stood out above the rest, these included combinations of tarra, barley dust, secondary food industry sludge (Avebe), potato peels and bokashi or vegetable fruit garden waste (VFG). Tarra and secondary food industry sludge (Avebe) are suitable substrates for vermicomposting. Tarra however cannot be used as the single substrate for worm cultivation, due to its acidity. Chalk should be added to balance the pH when tarra is used as a substrate. Germinated barley and secondary food industry sludge (Holland Malt) are less suitable for vermicomposting, due to the quick degradation of the substrates. Addition of barley dust makes the substrates drier, thereby adjusting the consistency which makes the substrate more suitable for the worms to feed on.
The fourth experiment focused on good growth and reproduction of the worms. Additional substrates (e.g. bokashi and VFG compost) were added, to test their ability to substitute peat in the substrate mixes. The experiments were performed in chicory racks. The racks containing VFG compost showed no worm production and this stream is therefore believed to be not adequate for worm growth. A mixture of tarra, barley dust, secondary food industry sludge (Avebe), potato peels and bokashi showed the best worm growth an reproduction rates. The final experiment was again focused on good growth and reproduction of the worms and performed in the 20 m long worm beds. Each bed contained 300 kg of worms, to which a varying amount of substrate was added. Visual observation showed that mixtures of tarra, secondary food industry sludge (Avebe), champost, potato, apple, carrot and pumpkin peels were completely eaten by the worms.

The vermicompost produced in the experiments had a good structural consistency. The products obtained in Experiment 3 were analyzed for their organic matter (OM), N, P and K content, and for the presence of heavy metals. It was concluded that only products originating from substrates TARRA III (containing tarra, barley dust and secondary food industry sludge (Avebe)) and TARRA40 IV (containing tarra and peat) were considered vermicomposts, due to the lack of worm growth in substrates TSS I (containing peat, barley dust and secondary food industry sludge (Holland Malt)) and TARRA2 II (containing tarra, barley dust and secondary food industry sludge (Holland Malt)).
Throughout the different tests substrates were identified with potential for good worm growth. In conventional worm industries, worms are usually grown on peat mixtures. Peat is not a sustainable substrate in the long term and should be replaced by better alternatives. The waste streams of Avebe (mixed with other substrates) seemed promising and can potentially be good alternatives for peat.

1 Introduction

Vermicomposting is a method of valorizing organic waste streams into (vermi)compost and new worm biomass. It is considered an environmentally friendly method for biowaste treatment. *Eisenia fetida* and *Dendrobaena veneta* are well-known and often used compost worm species, due to their ability to convert all sorts of biowaste, including animal manure, industrial organic waste, municipal waste and sewage sludge. Moreover, *E. fetida* and *D. veneta* are able to grow in a wide temperature range, are highly capable of processing organic wastes and are readily handled (Geremu et al., 2020; Haiba et al., 2014). The major benefit of using earthworms for composting is that the composting happens in the gut of the earthworm by microorganisms, resulting in a faster composting process and a very stable organic end product. The vermicompost can be used as fertilizer, thereby enhancing crop growth in several studies (Blouin et al., 2019; Lim et al., 2015; Wang et al., 2017). The final product is a fine humus like product. In addition, bulk density, pH, water holding capacity, heavy metal levels, nitrogen, phosphorus and potassium content are promoted by vermicomposting over traditional composting (Aslam, 2021; Geremu et al., 2020). Some recently studied substrates for producing earthworms include bakery industry sludge (Yadav & Garg, 2019), cattle solid wastes (Rini et al., 2020), pineapple waste (Zziwa et al., 2021), malting sludge (Hanc et al., 2020) and municipal solid waste (Ramprasad & Alekhya, 2021). The second useful product obtained from vermicomposting are the earthworms themselves. The worms are a feed source which can potentially be used in poultry and fish farming. They contain a large amount of protein (between 55 and 70 % of the dry matter (DM) with a higher content of essential amino acids compared to fish or meat meal (Parolini et al., 2020).

In this report, different organic waste streams originating from potato starch and malting industries were used as substrates for the earthworms to convert into vermicompost and biomass. Five experiments in different set-ups were conducted with the main aim of finding novel substrates for the production of vermicompost and biomass. The experiments were performed at WormsSystems in Oostwold, the Netherlands. The substrates were adjusted after each previous experiment and adapted to the worms preferences. The worms and the vermicompost were analyzed for their nutritional and fertilizer value.

2 Materials and methods

2.1 Compost worms and substrates

In these experiments compost worm species *Dendrobaena veneta* and *Eisenia fetida* were used in the approximate ratio 7:3. The compost worms originated from the breeding and test facility of WormsSystems located in Oostwold, the Netherlands.
The worm substrates all originated from Avebe (potato starch and protein producer, located in Gasselternijveen, the Netherlands), Holland Malt (maltings located in Eemshaven, the Netherlands) or were kitchen and garden wastes (**Table 1** for names, abbreviations and origins of the substrates). The abbreviations are derivates from the names, the capitals in the name form the abbreviation e.g. Secondary Food Industry Sludge; SFIS. All waste streams are written with capitals throughout this report, in order to make it easier to understand the abbreviations.

Table 1 *Overview of the substrates with abbreviations and origins.*

Name of the substrate	Abbreviation	Place of origin
Germinated Barley	GB	Holland Malt
Barley Dust	BD	Holland Malt
Secondary Food Industry Sludge (Holland Malt)	SFIS	Holland Malt
Primary food industry Sludge (Avebe)	PSA	Avebe
Secondary food industry Sludge (Avebe)	SSA	Avebe
Tarra	Ta	Avebe
Peat*	Pe	Veenbaas (Drachten)
Bokashi (from leaves)	Bok	Jansen Wijhe (Wijhe)
Potato Peels	PP	van Vulpen voeders (Tiel)
VFG compost	VFG1	van der Wal (vegetable fruit garden waste)
VFG compost	VFG2	Ogar (vegetable fruit garden waste)
Apple pulp	Ap	van Vulpen voeders (Tiel)
Carrot and Pumpkin Peels	CPP	van Vulpen voeders (Tiel)
Champost	Cham	Koolen Champignons CNN (Marum)

* *Peat is used as a standard material for breeding earthworms. It is mixed with more nutritious streams and eaten by the worms. It is considered a bedding material.*

The compositions of the Holland Malt and Avebe substrates as provided by the manufacturers are shown in **Table 2**.

Table 2 *Compositions of the starch (Avebe) and malting (Holland Malt) industries substrates (as provided by the manufacturers). All components are based on dry matter. The composition of tarra (Avebe), was unknown, only pH was measured (pH 3).*

Component	Germinated Barley Holland Malt	Barley Dust Holland Malt	Secondary food industry Sludge Avebe	Secondary Food Industry Sludge Holland Malt
g/kg product	49	865	48-53	115
Ash g/kg	98	85	137-156	103
Protein g/kg	409	101		?
Fibre g/kg	214	211		?
N g/kg			100-106	96 g TKN/kg MS*
P g/kg			16-17	21
K g/kg			16	
As mg/kg			3.4	<1.0
Cd mg/kg			3.0-4.2	0.3
Ca mg/kg				6900
Cr mg/kg			<11-12	7.4
Cu mg/kg			116-117	41
Pb mg/kg			24-31	1.7
Hg mg/kg			0.19-0.21	0.07
Ni mg/kg			<5.7-<6.4	10
Zn mg/kg			365-386	310

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

All percentages and calculations in this report are shown on fresh weight basis. Calculations or numbers on a different basis are indicated where necessary.

2.2 Experimental setup

2.2.1 General experimental setup

All the experiments were performed in the test facility of WormsSystems in Oostwold. Test conditions for all experiments were similar at a temperature of around 18 °C. A total of 5 experiments were performed in several container sizes. The batch of tarra was pretreated, the water on top of the tarra was drained and the substrate airdried some more over the span of a couple of days. The (compound) substrates were mixed by hand and left for a while, until present liquids were absorbed by the more solid substrates. To some substrates, barley dust (Holland Malt) or chalk (Dolokal, 7 kg/m^3) were added to stabilize them. A varying amount of worms was added to each substrate, depending on the type of experiment. The worms were monitored on week days. The duration of the test varied between experiments. Each of the experiments is described individually below.

2.2.2 Experiment 1

The first experiment was performed as a quick scan for evaluating worm behavior in the different (compound) substrates. This experiment was conducted in small plastic trays (1L), each containing 1 kg (wet weight unless stated otherwise) of substrate and 25 compost worms, which were placed in a larger container to prevent escape (**Figure 1**). Six different substrate combinations were prepared to observe growth in the worms. The substrates were Pe+GB, Pe+SFIS, Pe, Ta, Pe+SS and Ta+Pe. The combinations were all on a 50/50 wet weight basis. After 10 days of growth, the earthworms were evaluated on their performance and health/survival. The results are based on a single event.

Figure 1 *Set up of Experiment 1 in small plastic trays*

2.2.3 Experiment 2

The second experiment was conducted in bigger plastic trays (60x40x15 cm), containing 10L of substrate each. Fifteen different mixtures of substrates were made based on wet weight (kg) (**Table 3**). To each tray, 1 kg of worms was added. Experiment duration was 10 days. The experiment was performed without replicates.

Table 3 *Composition of the substrates in Experiment 2. The numbers represent the volume ratios of the mixed substrates. For example, substrate mix #1 is composed of two parts peat (Pe), 1 part Secondary food industry Sludge (SSA) and 2 parts of Barley Dust (BD) until the total volume was 10L.*

# of substrate	Peat (Pe)	Secondary food industry Sludge (SSA)	Secondary Food Industry Sludge (SFIS)	Tarra (Ta)	Barley Dust (BD)	Germinated Barley (GB)	Water
1	2	1			2		
2		0.5		1	1		
3			1	1	1		
4	1			4	1		
5	1		1		1		
6	1		4				
7	1				4		1
8	1				2		?
9	1		2		2		
10	1			4			
11	1		2				
12	1			2			
13	1	1			1		
14		1		2	2		
15	9					1	

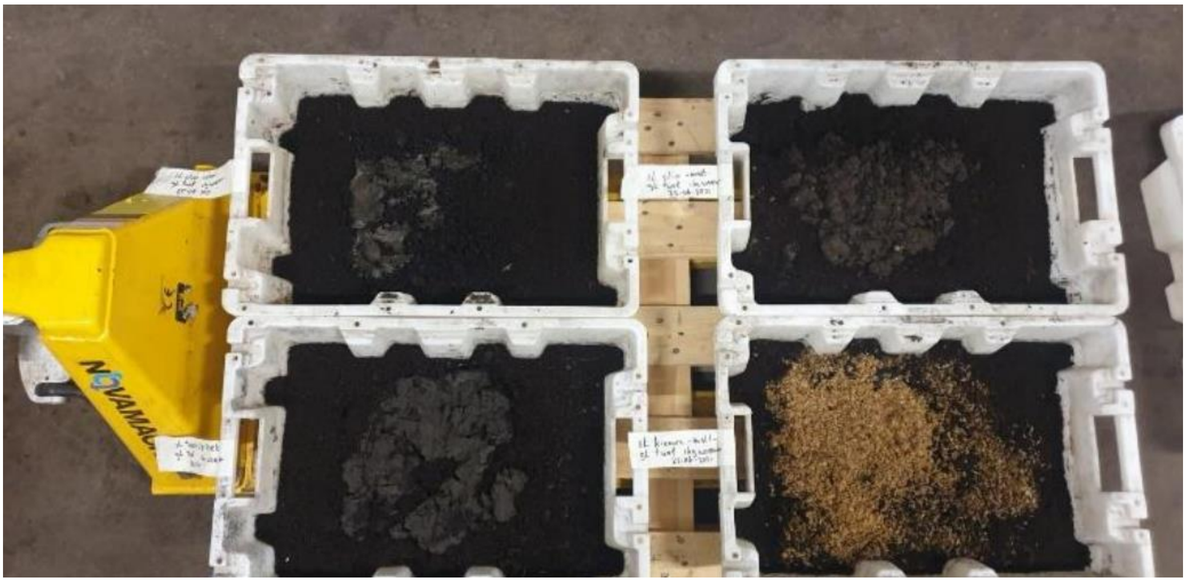

Figure 2 *Set up of Experiment 2, in big plastic trays each containing 10L of substrate.*

2.2.4 Experiment 3

Based on Experiment 2, four different mixes of substrates were selected and further tested in a climate cell (18 °C), in chicory racks of 90x120x15 cm (see **Figure 3**) each containing 50 L of substrate in total (**Table 4**). The experiment was performed in quadruplicates.

Figure 3 *The difference between the big plastic white trays (green arrow), used in Experiment 2 and the chicory racks (red arrow) used in Experiment 3.*

Table 4 *Substrate mixtures in Experiment 3. The amounts are shown in kilogram and add up to a total volume of 50L. Barley dust was added to make the substrate drier. Chalk was added to substrate TARRA2 II, TARRA III and TARRA40 IV to neutralize the pH.*

Substrate code	Peat (Pe) kg	Tarra (Ta) kg	Barley Dust (BD) Kg	Secondary food industry Sludge Avebe (SSA) kg	Secondary Food Industry Sludge Holland Malt (SFIS) kg	DM of substrates start (%)	pH start
TSS I	9.0		2.0		17.7	47.1	-
TARRA2 II		23.3	2.0		17.7	63.1	-
TARRA III		40.1	2.0	4.5		86.7	5.6
TARRA40 IV	4.5	46.6				83.8	6.6

To substrates TARRA2 II, TARRA III and TARRA40 IV, chalk was added to increase the alkalinity of the substrate, making the substrate more suitable for worms to feed on. The substrates were mixed by hand and 5 kg of worms were added to each tray. The duration of the experiment was six weeks.

2.2.5 Experiment 4

Experiment 4 continued upon the results from Experiment 3. The focus was on good growth and reproduction (cocoon production) of the worms. At several occasions in this experiment extra substrates were added to the substrates of the previous experiments, e.g. bokashi and VFG compost were added to evaluate if the peat in previous experiments could be replaced. The experiment was done in chicory racks, each containing a mix of the substrates (**Table 5**). All waste streams were first mixed by hand and left for a while, until all moisture was absorbed by the solid components. Experiment 4 was a single experiment. To each of the trays, 250g of worms was added. The duration of the experiment was one month, the worms were monitored on week days.

Table 5 *Mixing ratios of the substrates in experiment 4*

Substrate code	Ta (L)	BD (L)	SSA (L)	PP (L)	Bok (L)	VFG1 (L)	VFG 2 (L)	pH start
Rack 1	1	1	2	1	5			7
Rack 2	1		2		7			7
Rack 3	2	4	4					7
Rack 4	1	1	2			1		7
Rack 5	1	1	2				3	7
Rack 6	1	1	2	1			5	6.5

2.2.6 Experiment 5

Experiment 5 (singular) was done in a worm bed of 20 m long, 1.6 m wide and 0.8 m tall. One bed contained around 300 kg of worms. The focus was on good growth and reproduction (cocoon production) of the worms. The substrates were divided in two similar portions (based on volumes) and administered at two random spots on the surface of the bed (first half at the start of the experiment, second half the day after) and the experiment was monitored for 25 days. If the worms favor the substrate, they will move towards it and feed on it. The behavior of the worms was scored throughout the test.

Figure 4 *Worm beds at WormsSystems*

*__Table 6__ Composition of the substrates for Experiment 5. Ta = tarra; BD = Barley Dust; SSA = Secondary food industry Sludge (Avebe); PP = Potato Peels; VFG2 = VFG from Ogar; Ap = Apple pulp; CPP = Carrot and Pumpkin Peels; Cham = Champost. *egg shells were added to create a neutral pH.*

Substrate code	Ta (L)	BD (L)	SSA (L)	PP (L)	VFG 2 (L)	Ap	CPP	Cham	Total amount of substrate (L)	pH at start
Bed 1	5		2	1	5				13	6.5
Bed 2		23	6	3		7	7		46	6.5
Bed 3	6		2	3		1	1		13	6.5
Bed 4*	1		2	1				14	18	6.5
Bed 5*	2		4			2		12	20	6.5

2.3 Sampling and analysis

At the end of each test, the contents of the containers were separated in worms and vermicompost. Both worms and vermicompost were weighed. Worms were scored on appearance, health signs and activity.

Only from Experiment 3 samples (worms and vermicompost from 1 tray for each substrate) were sent in for analysis. The worms were dried at 70°C before shipping. Nutritional value of the worms was analyzed by Agrolab, Kiel, Germany. Fertilizer values and heavy metals were analyzed by Eurofins, Wageningen, the Netherlands.

2.4 Short overview of the experiments

__Table 7__ An overview of the parameters of the different experiments executed in this report.

Experiment	Total volume of substrate (L)	Amount of worms (kg)	Used trays	Duration of the experiment (days)	Amount of different substrate combinations	Comments
1	1	25 worms	Small plastic trays	10	6	No replicates
2	10	1	Big plastic white trays	10	15	No replicates
3	50	5	Chicory racks	42	4	Quadruplicates were pooled before analyses. The compost worms and the vermicompost were sent in for analysis
4	Proportions	0.2	Chicory racks	30	6	No replicates
5	Varying quantities	300	Worm bed	25	5	No replicates A small amount of substrate was randomly distributed over the worm bed, to see if the worm favored the substrate.

3 Results and discussion

Results described in this section are grouped per experiment. Overall, the compost worms converted the substrates into dry and powdery vermicompost (**Figure 5**).

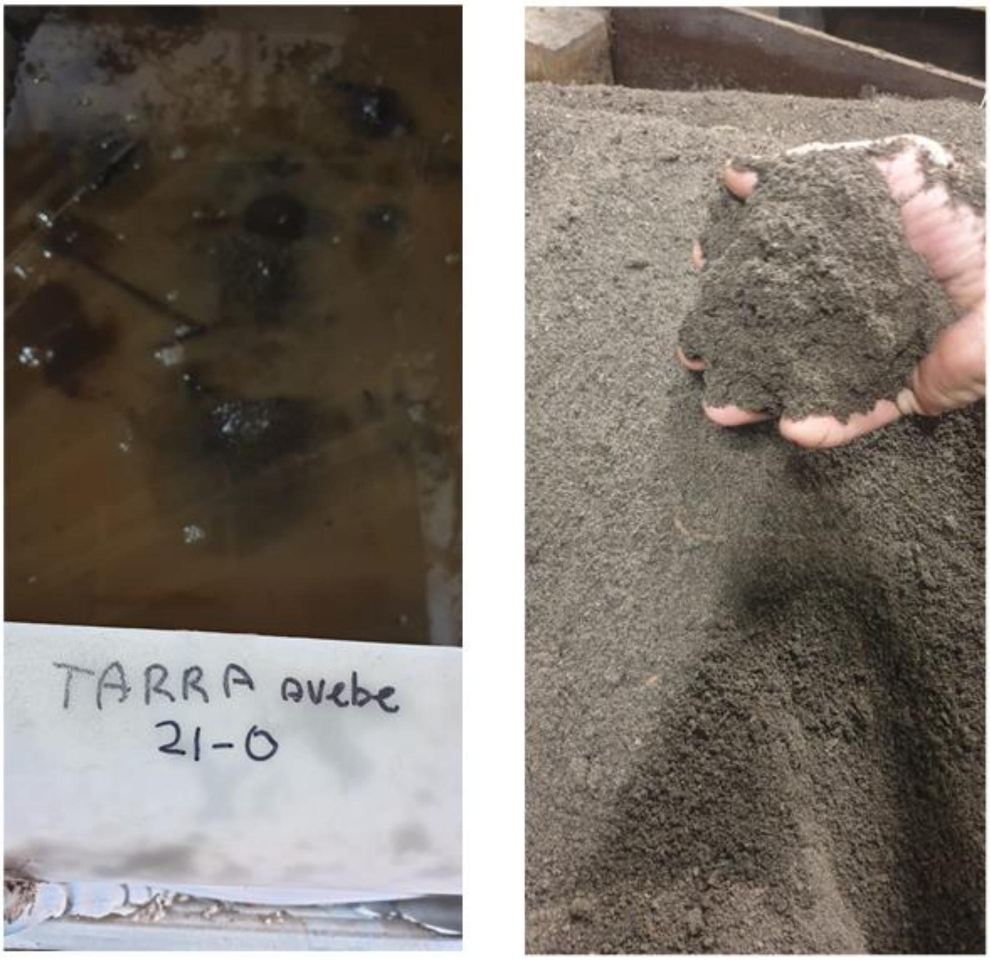

Figure 5 *On the left, non-drained tarra substrate originating from Avebe. On the right, tarra after conversion into vermicompost by the compost worms, resulting in a dry nutrient rich soil.*

Experiment 1 was a quick test to observe the behavior of the worms in the substrates. Results are shown in the table below.

Table 8 *Results of Experiment 1 where the worms were grown in substrate combinations. Pe = peat; Ta = Tarra; BD = Barley Dust; SSA = Secondary food industry Sludge (Avebe); SFIS = Secondary Food Industry Sludge (Holland Malt); GB = Germinated Barley.*

Substrates	# of worms at start	# of worms final	Conclusion
Pe+GB	25	25	Reasonable biomass increase
Pe+SFIS	25	23	Good biomass increase
Pe	25	25	Good biomass increase
Ta	25	2	Stopped after 24 hours
Pe+SSA	25	24	Big worms, good biomass increase
Ta+Pe	25	23	Weak worms

It is not possible to grow worms on tarra only because of the high acidity (pH 3) of this substrate. The worms died within 24 hours. The worms grew best on a mixture of peat with secondary sludge obtained from Avebe.

Results of Experiment 2 are shown below. Fifteen different combinations of the substrates were made. Starting total wet weight of the worms was 1 kg per substrate.

Table 9 *Results of Experiment 2, each tray started* with 1 kg of worms. Pe = peat; Ta = tarra; BD = Barley Dust; SSA = Secondary food industry Sludge (Avebe); SFIS = Secondary Food Industry Sludge (Holland Malt); GB = Germinated Barley. Trays highlighted green indicate positive results.

Tray no.	Substrate	Worm biomass compared to start (%)	Remarks
1	(2xPe, 1xSSA, 2xBD)	105	Growth, compost difficult to sieve
2	(0.5xSSA, 1xTa, 1xBD)	100	No growth, experiment stopped
3	(1xTa, 1xSFIS, 1xBD)	60	Decrease in amount of worms, experiment stopped
4	(1xPe, 4xTa, 1xBD)	115	Good growth
5	(1xPe, 1xSFIS, 1xBD)	50	Decrease in amount of worms, experiment stopped
6	(1xPe, 4xSFIS)	50	Decrease in amount of worms, experiment stopped
7	(1xPe, 4xBD, 1xWater)	110	Weed growth, experiment stopped
8	(1xPe, 2xBD, ?Water)	110	Weed growth, experiment stopped
9	(1xPe, 2xSFIS, 2xBD)	60	Decrease in amount of worms, experiment stopped
10	(1xPe, 4xTa)	125	Good growth, easily sievable
11	(1xPe, 2xSFIS)	60	Product expires
12	(1xPe, 2xTa)	125	Good growth, easily sievable
13	(1xPe, 1xSSA, 1xBD)	60	Decrease in amount of worms, experiment stopped
14	(1xSSA, 2xTa, 2xBD)	110	Good growth
15	(9xPe, 1xGB)	70	Product expires

Tarra had a low pH (3), which made the addition of chalk necessary in some substrates to neutralize pH. Germinated Barley (GB) expired quickly and resulted in the presence of maggots from flies laying eggs in the GB during transport and storage. No further experiments were conducted with this substrate. Growth of the worms, ease of sieving and shelf life of the substrates are important parameters in this experiment. Looking at these parameters, it was concluded to move forward with substrate #4, 10, 12 and 14, sometimes with small additions of secondary Holland Malt sludge.

Figure 6 *In the substrate Germinated Barley, flies laid eggs during transport and storage, resulting in maggots.*

Experiment 3 was executed in quadruplicates. At the end of the experiment, the four different trays with the same substrate were pooled. The results of Experiment 3 are presented in **Table 10**.

Table 10 *Results of Experiment 3. TSS I does not contain tarra, the other three substrates do, and chalk is added to those three to balance the pH. Pe= peat, Ta= Tarra, BD= Barley Dust, SSA= Secondary food industry Sludge (Avebe), SFIS= Secondary Food Industry Sludge (Holland Malt).*

Sample code	Worm weight, end (g)	Worm weight compared to start (%)	Substrate wet weight decrease (%)	Reproduction (# eggs)
TSS I (Pe, BD, SFIS)	1030	20.6	13.2	-
TARRA2 II (Ta, BD, SFIS)	1600	32.0	23.0	-
TARRA III (Ta, BD, SSA)	4570	91.4	11.7	6
TARRA40 IV (Pe, Ta)	4460	89.2	17.3	23

Substrates TSS I and TARRA2 II were not suitable for the worms in terms of survival. Issues with the decreasing worm population are hypothesized to be the result of the secondary food industry sludge from Holland Malt, since this stream seemed unstable as a result of not further defined decomposition processes. Thus, in TSS I and TARRA 2 II, worms did not work through the substrate and only ate a limited amount of it. To avoid this problem, after 7 and 19 days 10L of peat was added to TSS I and TARRA 2 II. As a result, the worms stayed above ground but only ate from the peat and they didn't produce any eggs. Worms feeding on TARRA III and TARRA40 IV developed much better, with a survival rate of 91.4 and 89.2 % respectively. The worms looked healthy and reproduced during the duration of the experiment. It is assumed that the worm biomass could have increased by providing them with more substrate since in both TARRA III and TARRA40 IV, substrates were depleted before the end of the experiment.

Figure 7 *Visible eggs in substrate TARRA40 IV.*

Worms and vermicompost were sent in for analyses. Results of the fertilizer analyses are shown in Annex 1. Nutritional values of the (dried) worms are shown in

Table *11* below. Values are shown on dry matter basis. The composition of the substrates and their corresponding sample codes can be found in **Table 4** .

Table 11 *Nutritional values and heavy metals contents of the worms on different substrates in Experiment 3, based on dry matter.*

Sample code	Moisture (% total weight)**	Crude ash (%)	Crude protein (%)	Crude fat, total (%)	Crude fibre (%)	N-free substances (%)	Lead Pb* (mg/kg)	Cadmium Cd* (mg/kg)	Mercury Hg* (mg/kg)	Arsenic As* (mg/kg)
TSS I	6.1	14.5	61.6	9.1	0.9	14.1	4.6	1.6	0.04	4.9
TARRA2 II	5.7	21.0	57.1	7.8	0.8	13.3	6.4	1.4	0.04	5.0
TARRA III	6.6	7.0	68.3	9.0	0.5	15.2	1.4	1.4	0.04	6.4
TARRA40 IV	6.9	8.2	67.3	8.9	0.4	15.1	1.7	1.7	0.03	9.0

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

**worms had been dried prior to the analyses, as such the moisture percentage is not for live worms

The worms performed best in TARRA III and TARRA40 IV, which also seemed to result in a somewhat higher protein content in those worms, respectively 68.3 and 67.3%. In comparison, the protein content of the (left over) worms was 61.6% for TSS I and 57.1% for TARRA2 II. The percentage of protein content found in the worms of between 57 and 68 % is in range with literature (Gunya et al., 2021; Parolini et al., 2020; Sogbesan & Ugwumba, 2008). The fat content of worm meal ranges between 5 to 20 % of dry matter. This is in line with the observed results (fat content ranging from 7.8 to 9.1%). A difference can be observed between the ash content of TARRA III and TARRA40 IV, where 7.0 and 8.2% DM are observed, as opposed to 14.5% DM in TSS I and 21.0% DM in TARRA2 II. A possible explanation for the observed ash content in TSS I and TARRA2 II is the biomass decrease of the worms themselves, because the substrates weren't suitable for growth. The result is a percentual higher ash content. The heavy metal levels are below those in guideline 2002/32/EG (Europees Parlement en de Raad, 2002) with the exception of Arsenic (As). The highest level is measured in TARRA 40 IV, which mainly consists of tarra. Most likely it is accumulation by the worm because As content in TARRA40 IV was 1.5 mg/kg, which is lower than As levels in the worms themselves (see Annex 2). The same is observed for Cadmium (Cd). One other possibility could be the used chalk to increase pH of the substrate, but this was not analysed for heavy metals. Arsenic can be found in natural resources, e.g. peatlands. They often contain higher arsenic concentrations. TARRA40IV contained both tarra and peat, it is likely that the arsenic content originates from the peat (Planer-Friedrich et al., 2021).This needs further investigation. The tarra used in the experiments was obtained at the end of the potato campaign, resulting in a different consistency compared to fresh tarra. Fresh tarra was drier and overall looked like a more suitable substrate. It is hypothesized that the use of fresh tarra would result in better worm growth and a better end product. It is known that earthworms are capable of accumulating heavy metals from the substrate they are feeding on (Singh & Kumar Bhartiya, 2012; Žaltauskaitė et al., 2022). This is however dependent on several factors, e.g. organic matter content of the substrate they feed on. Besides current legislation, the high levels of As in the biomass would exclude worms from use as a feedstuff, if As levels continue to exceed the limits after process optimization.

The vermicomposts of Experiment 3 were analyzed by Eurofins (Wageningen, the Netherlands) on their organic matter, N, P and K content and the presence of heavy metals. The results of the analyses can be found in **Table A 1** and **Table A 2**.

Major differences between the organic matter and the NPK-content are observed between TSS I, TARRA2 II and TARRA III and TARRA40 IV vermicomposts. The organic matter percentages were 61.2 % in TSS, 35.6% in TARRA2 II, 6.7% in TARRA III and 7.1% in TARRA40 IV. The amount of plant available phosphate in the first two was respectively 2237.2 mg/kg and 807.7 mg/kg of dried vermicompost, whereas TARRA III and TARRA40 IV contained only 11.2 and 8.3 mg plant available phosphate/kg dried vermicompost. The amount of K present in TSS I and TARRA2 II was between 5 to 15 times higher compared to the amounts in TARRA III and TARRA40 IV. This trend is also visible in the total amount of N present in the substrates. A less nutrient and organic matter content rich product is expected after vermicomposting, e.g. the vermicompost obtained from TARRA III and TARRA40 IV. Secondly, as stated previously, the worms only ate a limited amount of substrate in TSS I and TARRA2 II. Worms died early in the test and no good growth was observed as opposed to TARRA III and TARRA40 IV. Consequently, the worms did not eat through the substrate and the end product cannot be regarded as vermicompost. Therefore it is concluded that only the end products of TARRA III and TARRA40 IV substrates are considered vermicomposts.

Experiment 4 continued upon findings from Experiment 3. Since the SFIS wasn't of great quality for the worms, it was decided not to continue with this substrate in Experiment 4. Additional waste streams were added to see if the worms grew well. See **Table 12**.

Table 12 *Results of Experiment 4 for the six different substrate combinations. Ta= tarra, BD= Barley Dust, SSA = Secondary food industry Sludge (Avebe), PP= Potato Peels, Bok= bokashi, VFG1 = VFG compost originating from van der Wal, VFG2= VFG compost originating from Ogar*

Substrate code	Worm biomass compared to start (%)	Reproduction (# of eggs)
Rack 1 (Ta, BD, SSA, PP, Bok)	180	28
Rack 2 (Ta, SSA, Bok)	120	8
Rack 3 (Ta, BD, SSA)	128	0
Rack 4 (Ta, BD, SSA, VFG1)	100	0
Rack 5 (Ta, BD, SSA, VFG2)	100	0
Rack 6 (Ta, BD, SSA, PP, VFG2)	100	0

Based on these results and visual observations throughout the experiment the substrates in racks 1, 2 and 3 were best for growing worms. Racks 3, 4, 5 and 6 showed no worm reproduction but rack 1 and 2 did. Racks 4, 5 and 6 all contained VFG compost and we hypothesize that this stream was not optimal for worm growth. The worms didn't go into this substrate and ate minimally from it. The substrate mixture of rack 1 was most successful: worms grew and reproduced.

The final experiment of this report was the bed experiment, Experiment 5. Here, it is discussed which substrate mixtures look most promising from visual observations of the worm beds.

Table 13 *Results of Experiment 5. Ta = tarra; BD = Barley Dust; SSA = Secondary food industry Sludge (Avebe); PP = Potato Peels; VFG2 = VFG from Ogar; Ap = Apple pulp; CPP = Carrot and Pumpkin Peels; Cham = Champost.*

#	Residual substrate (L)	Remarks
Bed 1 (Ta, SSA, PP, VFG2)	2	Looks pretty good, worth to continue with
Bed 2 (BD, SSA, PP, Ap, CPP)	4	Okay results
Bed 3 (Ta, SSA, PP, Ap, CPP)	Finished	The worms are feeding well, worth to continue with
Bed 4 (Ta, SSA, PP, Cham)	Finished	The worms are feeding well, worth to continue with
Bed 5 (Ta, SSA, Ap, Cham)	Finished	The worms are eating well, worth to continue with

The mixed substrates on beds 3, 4 and 5 had been completely eaten by the worms at the end of the experiment. These substrates would be interesting to continue with. Bed 3 contains a relatively high amount of Ta and SSA compared to the other substrates mentioned. Mixed with the apple pulp, potato, carrot and pumpkin peels, this seems to be an interesting substrate for further research.

Throughout the five experiments, a lot of different substrates and mixtures have been tested on their performance for worm cultivation. Similar substrates have been tested previously, Hanc *et al.*, (2020) researched the feasibility of vermicomposting (with *Eisenia andrei)* of malting sludge in combination with straw pellets. The worms only grew when the malting sludge was mixed with at least 50% of pelleted straw (Hanc et al., 2020). This study found that it is possible to rear worms on malting sludge, as opposed to the results in this report. It is hypothesized that the malting sludge was of a different composition or decomposition of the malting sludge led to undesired components for worm growth, possibly in combination with a negative effect of the flocculant present in the malting sludge. The decomposition of the substrate could also be the problem with the Germinated Barley. A different storage method and/or a pretreatment might solve these issues regarding decomposition and decrease of the effect of the flocculant. As such, more research is necessary.

Tarra is a waste stream originating from the potato industry. As Tarra is residual soil attached to the potatoes after harvest it is possible that it contains harmful nematodes, which were present in the potato fields (Nederlandse Voedsel en Warenautoriteit, 2021). There is some literature on the survival of harmful nematodes during vermicomposting. In this report, this specific subject is not further researched, although it is worth to mention the findings of previous studies about nematode survival and the management of nematodes by use of vermicompost as a fertilizer to benefit crop growth. Boyer et al. (2013) studied the interaction between earthworms and plant parasitic nematodes. They found a decrease in nematode populations, possibly by direct ingestion and digestion by the earthworms. The transit of nematode cysts through the gut of the earthworms decreased the emergence of juvenile nematodes. However, the passage of nematode in the gut alone had no effect on the juveniles, yet the exposure through the gut in combination with its soil content reduced the ability to produce females and cysts. Boyer et al. (2013) suggest that this is an indirect effect of enzymes present in the digestive tract of the earthworms. Much more research is necessary to understand these mechanisms (Boyer et al., 2013). Arancon et al. (2002), studied the effect of vermicompost, produced from cattle manure, food and recycled paper on the growth of tomatoes, bell peppers, strawberries and grapes in field plots. Vermicompost, compost and inorganic fertilizer were incorporated in the field beds of the crops. The nematode populations were assessed in each plot. They found that the populations of plant parasitic nematodes declined in all plots where vermicompost was applied, when compared to plots with applied inorganic fertilizer or compost (Arancon et al., 2002). This conclusion was backed by Mondal et al. (2021) who studied the effect of vermicompost on nematodes in rice fields. They observed a decrease in nematode population build up with increased doses of vermicompost. The addition of vermicompost did not lead to direct mortality or less infectivity, but reduced nematode reproduction (Mondal et al., 2021).

4 Conclusion

From the five experiments described in this report it was concluded that some waste streams originating from potato processing and malting industries can be used to cultivate worms. As compared to growth rates in conventional worm breeding businesses the growth rates on the substrates described in this report are relatively slow. Substrates TARRA III (containing tarra, barley dust and secondary food industry sludge (Avebe)) and TARRA40 IV (containing tarra and peat) in Experiment 3 are promising. The challenge is to exclude peat, which is currently used to get a more loose growth medium for the worms. A potential substitute is ground straw. Bed 5 from Experiment 5, containing tarra, Secondary food industry Sludge from Avebe, Apple pulp and Champost, seems very promising and does not contain peat, which is the substrate of choice in the conventional worm breeding business. The disadvantage is that mixing in the additional components decreases the share of tarra, as a result of which production costs will increase (because not all of these products have a negative price) and also logistics will become more complex.

The vermicompost produced by the worms in the five experiments has a good structural consistency. Some of the vermicomposts in Experiment 3 contain high amounts of nutrients (N, P, K) and organic matter. The vermicomposts are suitable for use as a fertilizer or soil improver, although it should be applied with care to the fields, due to this high fertilizer value.
The observations on the substrates lead to the conclusion that tarra is acidic and needs to be mixed with other substrates, in order to be suitable for the worms to feed on. Fresh tarra is hypothesized to be better suitable as a substrate and this should be further investigated. Barley Dust is a good addition to make the substrate drier, however there might be cheaper solutions to create a desired consistency in the substrate. Secondary Food Industry Sludge and Germinated Barley from Holland Malt, started to degrade already during storage and transport. The partly degraded substrates were less suitable for the compost worms to feed on. In general, it is of importance that waste streams are stable and pH level needs to be neutral.

Compost worms contain a very high amount of protein, around 60% of the dry matter, which makes them very suitable as a protein source in animal feed for example, under the condition that the insects are a legalized animal feed for that specific animal and the substrate used for feeding the worms is acknowledged as an animal feed source. In addition, the worm biomass should be screened for unwanted components.

In conclusion, waste streams originating from Avebe are suitable as a substrate for worm breeding. Additions of other substrates make the tarra and secondary food industry sludge from Avebe even more suitable for the worms. The challenge is to minimize these additions. Probably, addition of peat will not be necessary, making the waste streams of Avebe a sustainable alternative substrate for compost worms. Future legislative developments will determine if worms can be used as animal feed. Future research regarding the arsenic content of the worms and it's source is essential. It is probable that fresh tarra is less acidic, leading to a limited need for chalk addition. This might result in a lower arsenic content. Moreover, the risk of using tarra includes the presence of nematodes and other (potato) diseases, which can be redistributed onto the fields as vermicompost. Future research can determine if passing through the worm gut is an effective way to reduce the nematode population. The waste streams originating from Holland Malt decayed quickly and a different storage technique might increase the shelf life of germinated barley and secondary food industry sludge. This is also something to investigate further.

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Annex 1 Compost analyses of Experiment 3

Table A 1 PAE= plant available, AL= soil available, KZK= Carbonated lime, OM = organic matter
All results are presented on a dry matter base.

Compost code	DM (%)	pH	N tot (g/kg)	P-PAE (mg/kg)	P-AL (mg P2O5/100 g)	K (mg/kg)	S-total (mg/kg)	S-PAE (mg/kg)	Mg (mg/kg)	Na (mg/kg)	B (µg/kg)	Cu-PAE (µg/kg)	Mn (mg/kg)	Co-PAE (µg/kg)	Zn-PAE (mg/kg)	KZK (%)	OM (%)	C/N	Lutum (%)	Sand (%)
TSS I	47.1	5.4	25.6	2237.2	907	5394	3855	685.7	1115	694	1202	139	9.1	24	5.0	1.5	61.2	12.0	3	30
TARRA2 II	63.1	5.5	14.75	807.7	418	2826	2130	327.2	732	395	839	107	8.2	14	1.8	1.1	35.6	12.1	3	49
TARRA III	86.7	6.1	3.92	11.2	74	594	505	45.8	198	93	298	53	5.1	8.4	4.1	0.2	6.7	8.5	1	81
TARRA40 IV	83.8	6.0	3.85	8.3	64	363	585	39.8	224	70	235	37	8.2	7.3	0.6	0.2	7.1	9.2	1	81

Table A 2 *Heavy metals. ND = Not determined.*

Substrate code	CD (mg/kg DM)	Chr (mg/kg DM)	Cu (mg/kg DM)	Hg (mg/kg DM)	Ni (mg/kg DM)	Pb (mg/kg DM)	Zn (mg/kg DM)	As (mg/kg DM)	Se (µg/kg DM)	Si (mg/kg DM)	Mo (µg/kg DM)	Fe (µg/kg DM)
TSS I	ND	ND	ND	ND	ND	ND	ND	ND	4.3	102.7	4	23.4
TARRA2 II	0.18	7.1	11	0.03	3.4	9.4	57	1.7	3.2	59.2	4	12.1
TARRA III	ND	ND	ND	ND	ND	ND	ND	ND	2.1	24.5	8	4
TARRA40 IV	0.1	6.9	7.3	0.03	2.6	9.1	22	1.5	2.1	15.0	4	2.4

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