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## Combined digestion of insect frass and cow manure for biogas production

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# Combined digestion of insect frass and cow manure for biogas production

Stefan Hol, Hellen Elissen & Rommie van der Weide

Wageningen University & Research

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**Summary:** In an experiment described by Elissen et al. (2019) on anaerobic digestion of black soldier fly (BSF, *Hermetia illucens*) larvae frass, the frass had been previously hygienised due to Dutch law. However, when the frass would stay on the same premises this hygienisation step may not be necessary. A synergistic effect of mixing insect frass with cattle slurry would provide a more positive business case for the insect farmer, since the frass becomes an interesting co-substrate for digesters, resulting in lower disposal costs or even benefits. To determine this synergistic effect, three substrates were tested in lab-scale mesophilic anaerobic digestion tests: Insect frass of BSF larvae grown on agricultural side-streams, either hygienised at 60 °C for 24h or non hygienised and cattle slurry. Also combinations of cattle slurry with insect frass were tested.

Biogas production was determined by measuring gas pressure and analysing gas composition. No synergistic effect of adding insect frass to cattle slurry was found in this experiment, and neither differences between hygienised and non hygienised insect frass were found. In other words, adding insect frass to a cattle slurry digester will not lead to an increase in biogas production different from that expected based on adding up biogas productions of the single substrates. However, the partial replacement of cattle slurry with insect frass will increase the amount of biogas produced per digester volume with a factor 3.5 to 9 for respectively 10 % and 30 % replacement. Therefore, it is still interesting to add insect frass to a cattle slurry digester.

**Keywords:** biogas, methane potential, black soldier fly, frass, cow manure, cattle slurry

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# Preface

In the Netherlands, there are surpluses of manure and digestates/biogas slurries (digested manure and residual flows), which represent a negative value. At the same time, these residual flows contain valuable ingredients for the production of biomass (as raw material for food and feed products), for improving soil quality and for energy production. The number of feasible business cases in which the residual flow is upgraded has so far been limited. This is due both to the efficiency of the technologies used and the legislation and regulations related to the residual flows.

Recent information from research, scientific literature and companies provides new starting points for a biobased valorisation of manure/digestate streams and improving the efficiency of anaerobic digestion. The innovative aspect of our research is the cultivation of new types of biomass on the residual flows and the use of the conversion products to improve anaerobic digestion. This involves the use of separated manure and digestate products for the cultivation of mushrooms/fungi, worms, insects, specific bacteria and aquatic biomass. The resulting biomass can be further refined and marketed as food, feed and bio-based feedstock. There are also processed manure and digestate products that are valuable as fertilizer products for soil and plant growth, as substrate for improvement of anaerobic digestion or for export/use besides in agriculture. This gives a new interpretation to obligatory manure processing.

The aim of this project is to further explore and substantiate/test these ideas on lab and practical scale, leading to a proof of principles for new bio-based upgrading methods for manure and digestate that can be used in conjunction to better close cycles and/or sell outside regular agriculture. Bottlenecks in legislation and regulations are explored and put on the agenda. Key figures are also calculated that are necessary for assessing sustainability (e.g. costs, environmental effects) and for supporting legislation (e.g. minerals, food safety).

The livestock sector gains insight into the possibilities of biobased valorisation and better marketing of their most important residual flows. For the SMEs involved, this research provides proof of principle for their technology and input in their business cases. The combined effects of the technologies provide new knowledge, methods and research directions for science. In a social context, the use and upgrading of manure and digestates in other ways also contributes to the transition to a circular bio-economy with an efficient and sustainable agrifood sector.

More information:

- [http://www.acres.nl/en/projecten\\_acres/biobased-valorization-of-manure-and-digestate/](http://www.acres.nl/en/projecten_acres/biobased-valorization-of-manure-and-digestate/)
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# Summary

In an experiment described by Elissen et al. (2019) on anaerobic digestion of black soldier fly (BSF, *Hermetia illucens*) larvae frass, the frass had been previously hygienised due to Dutch law. However, when the frass would stay on the same premises this hygienisation step may not be necessary. A synergistic effect of mixing insect frass with cattle slurry would provide a more positive business case for the insect farmer, since the frass becomes an interesting co-substrate for digesters, resulting in lower disposal costs or even benefits. To determine this synergistic effect, three substrates were tested in lab-scale mesophilic anaerobic digestion tests: Insect frass of BSF larvae grown on agricultural side-streams, either hygienised at 60 °C for 24h or non hygienised and cattle slurry. Also combinations of cattle slurry with insect frass were tested.

Biogas production was determined by measuring gas pressure and analysing gas composition. No synergistic effect of adding insect frass to cattle slurry was found in this experiment, and neither differences between hygienised and non hygienised insect frass were found. In other words, adding insect frass to a cattle slurry digester will not lead to an increase in biogas production different from that expected based on adding up biogas productions of the single substrates. However, the partial replacement of cattle slurry with insect frass will increase the amount of biogas produced per digester volume with a factor 3.5 to 9 for respectively 10 % and 30 % replacement. Therefore, it is still interesting to add insect frass to a cattle slurry digester.

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# 1 Introduction

In the Netherlands, there are surpluses of animal manure. One way to upgrade the manure is producing biogas from it. Anaerobic digesters can operate on cow manure only (mono-digestion), but there are multiple manure types and residual flows which can be used and combined. Examples are pig manure, organic wastes and insect frass (faeces from insect(s) larvae produced as animal feed). The amount of insect frass produced will probably increase as a result of the global protein transition in the long-term, but in the short-term will be limited. Therefore, mono insect frass digestion will – for the time being – be costly due to its small scale, even though the material has a high biogas potential. According to Bulak et al. (2020) 379.0 nm<sup>3</sup>/ton DM for mealworm frass, 389.5 nm<sup>3</sup>/ton DM for cricket frass and 351.4 nm<sup>3</sup>/ton DM frass for *Hermetia illucens* larva frass (black soldier fly). In previous research a possible synergistic effect on the biogas production was found using a combination of digestate and insect frass from *Hermetia illucens* larvae (Elissen et al., 2019). Xie et al. (2017a) also found a synergistic effect on specific methane yield when mixing primary sludge with food waste. Mixing sewage sludge with food waste led to a surplus in specific methane yield of 33 %-41 % using respectively a ratio of 1:2 and 1:5 food waste to sewage sludge. Increasing the fraction of food waste resulted eventually in a negative, inhibitory effect (Xie et al., 2017b). While Pan et al. (2019) found an increase of methane productivity of a factor 4.59 and enhanced kinetics (11.53 times shorter lag-phase and 3.88 times faster hydrolysis rate) compared to separate mono-digestions of combinations of sludge and food wastes. When mixing primary sludge with paper pulp reject the specific methane yield does not increase much, however the reaction kinetics were enhanced (Xie et al., 2017a). Co-digestion of food waste with cow manure in a ratio 2:1 produces almost three times the amount of methane per organic matter expected (Awosusi et al., 2021). Increasing the ratio food waste : cow manure results in a smaller synergistic effect. According to the results described in Astals et al. (2014) a synergistic effect is mainly based on the ratio of carbohydrates, lipids and protein. In which a high amount of lipids (4:1:1, for lipids:carbohydrates:protein) increase the methane production most. Therefore, methane production can be enhanced by mixing the right substrates together. The question is whether adding *Hermetia illucens* larvae frass leads to an improvement in methane yield. In the experiment described by Elissen et al. (2019) the insect frass was previously hygienised due to Dutch law (at least an hour at 70°C), but when the frass stays on the same premises this does not seem necessary. Within this research the effect of adding hygienised and non-hygienised insect frass to cattle slurry is investigated. A synergistic effect would provide a more positive business case for the insect farmer, since the frass becomes an interesting co-substrate for digesters, resulting in lower disposal costs or even benefits.

## 2 Materials and methods

### 2.1 Input materials/additions

Three substrates were tested in lab-scale mesophilic anaerobic digestion tests.

1. Hygienised insect frass: produced by *H. illucens* larvae on agricultural side-streams, obtained from Bestico (Berkel en Rodenrijs, the Netherlands). Hygienised at 60 °C for 24h.
2. Non hygienised insect frass: produced by *H. illucens* larvae on agricultural side-streams, obtained from Bestico (Berkel en Rodenrijs, the Netherlands). Fresh.
3. Cattle slurry (obtained from the storage of WUR-OT, Lelystad, the Netherlands)

Table 1 shows the basic composition of the input materials. The electric conductivity and pH could only be measured for liquid samples, the insects frasses are too dry and solid for this method.

**Table 1** Composition of pure input materials/additions. C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. N.m. = not measured. DM = dry matter, OM = organic matter

Test substrate	DM %	OM %	EC mS/cm 25°C	pH (approximate)
	ww	DM	(approximate)	
C	8.1	79.0	18.6	7.24
I hy	76.6	89.6	n.m.	n.m.
I	82.8	89.8	n.m.	n.m.

### 2.2 Anaerobic digestion tests

The anaerobic digestion tests were performed by Opure (Ede, the Netherlands). Test substrates were added in triplicate tests according to Table 2.

**Table 2** Test substrates in anaerobic digestion tests (n=3) and test durations C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. 100 = 100 %, 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

Test substrate	Test duration (days)
C 100	56
I hy 100	62
I 100	56
C/I hy 90/10	62
C/I hy 70/30	62
C/I 70/30	56

Pre-treated digestates were used as seed material for the tests. The structure had to be as homogeneous as possible for an equal distribution over the test vessels, with as low biogas formation and as many different bacteria as possible. This was achieved by using three different digestates from low-loaded systems, which were pre-processed (e.g. coarse parts were sieved out). As mentioned, the seed digestates should produce as little gas as possible, otherwise the measurement would be inaccurate (biogas production was measured as the difference between vessels with only seed digestates and vessels with seed digestates plus added test substrate(s), both in triplicate). 200 grams of seed digestates and 0.87-9.39 grams of single and mixed test substrate(s) (Appendix I) were added to 1.2 L vessels. The dosages of the test substrates were based on predetermined loads to obtain the right amount of biogas production and were based on organic matter content of the substrates.

DM and OM were determined in all the substrates prior to the tests. COD could not be determined due to the presence of large particles in the samples. pH values were determined at the start and end of the tests in each vessel for the total mixtures. In each vessel a stirrer was added and they were flushed with nitrogen and closed. The vessels were put in an incubator at 35 °C and stirred only once



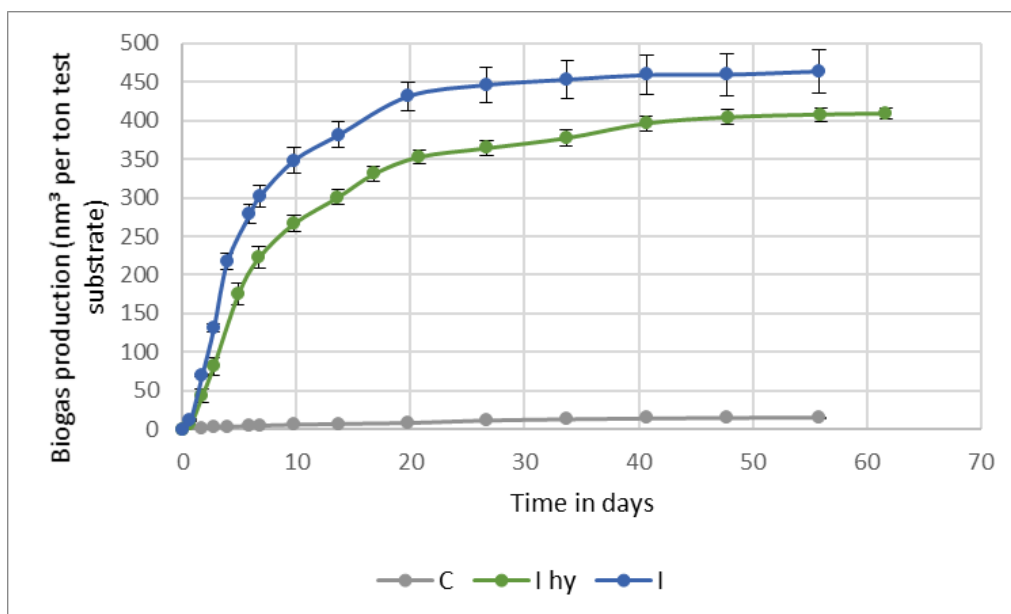
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a week briefly). Biogas production was determined by measuring gas pressure and analysing gas composition, after which results were converted to standard m<sup>3</sup> (nm<sup>3</sup>) biogas (= differences in yield between reference and substrate vessels). Tests were terminated when net biogas formation was zero.

## 3 Results

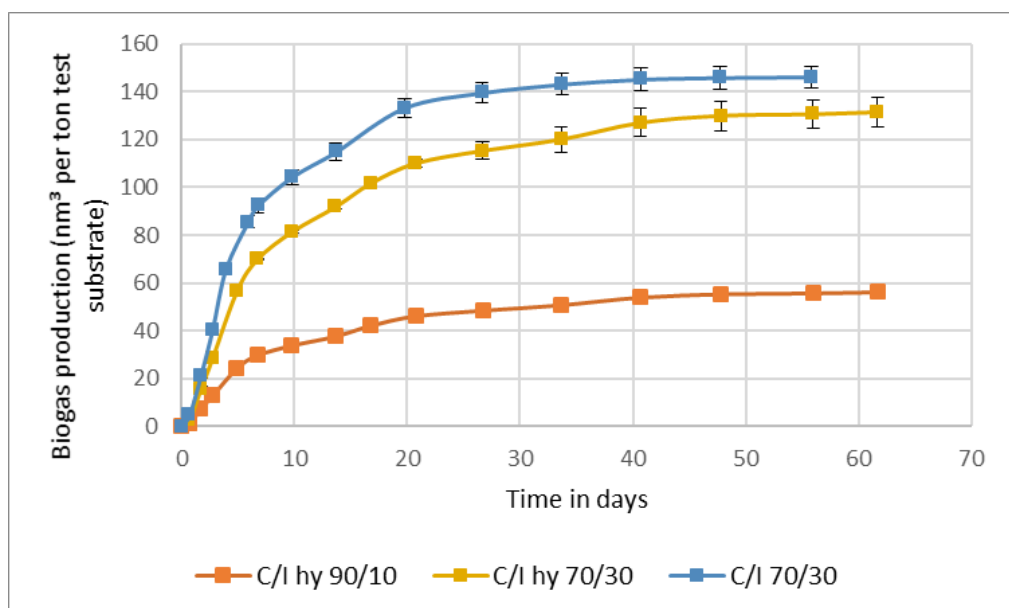
### 3.1 Biogas production and quality

The biogas production in time in the single and mixed substrates per ton (fresh product) are shown in Figure 1 and Figure 2 respectively.



**Figure 1** Biogas production of the single substrates ( $n=3$ ). C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised.

Clearly, insect frass non hygienised had the highest biogas production per ton fresh product, followed by hygienised insect frass. Cattle slurry had a 25-30 fold lower production.



**Figure 2** Biogas production of the mixed substrates ( $n=3$ ). C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

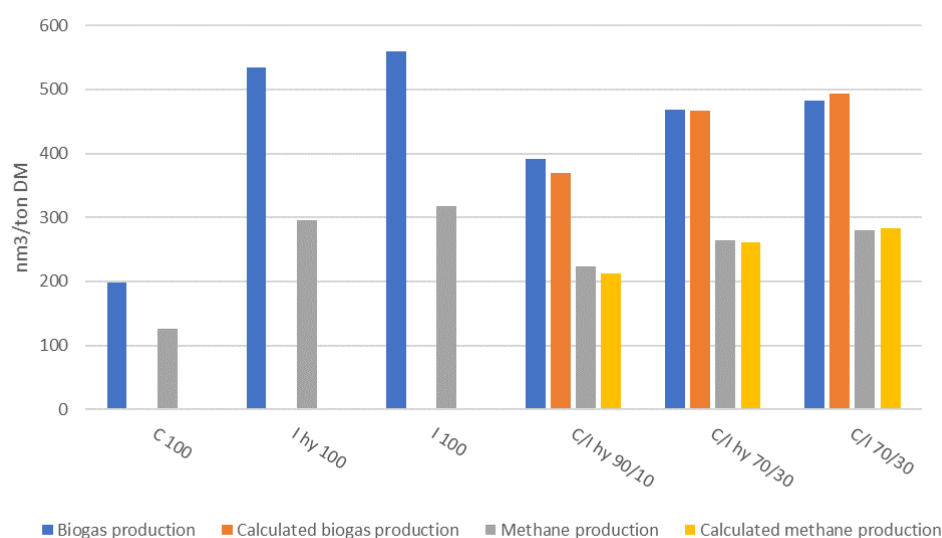
Figure 2 shows that combining cattle slurry and insect frass naturally increases the biogas production in comparison with using only cattle slurry. The higher the proportion of insect frass the higher the biogas production. Using non-hygienised insect frass results in the highest biogas yield for the mixes. Table 3 shows the final values of biogas and methane production in the single and mixed substrates based on fresh product, and the biogas quality, all compared to calculated values based on the results of the single substrates.

**Table 3** Measured biogas and methane productions on fresh product basis and biogas qualities compared to calculated values based on single substrates.

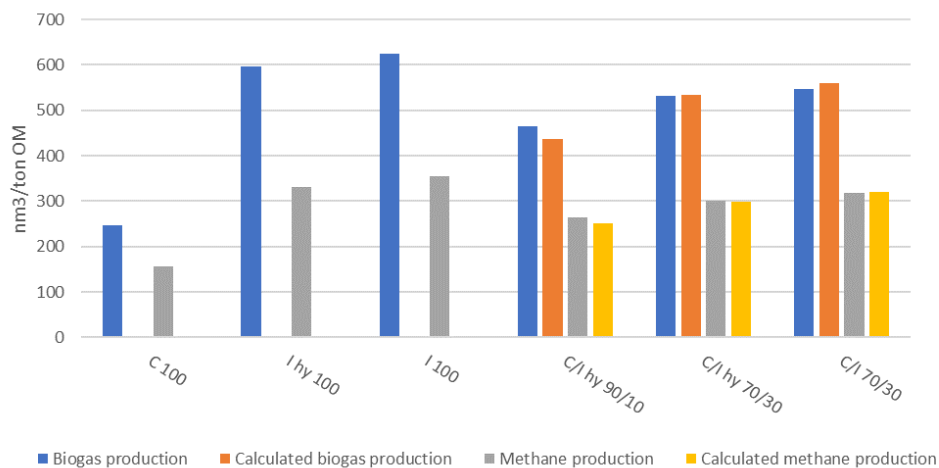
Addition	Biogas (nm <sup>3</sup> /ton prod.)	σ (n=3)	Calc CH <sub>4</sub> (nm <sup>3</sup> /ton prod.)	Calc Biogas quality % CH <sub>4</sub>	σ (n=3)	Calc
C 100	16	0.3	10	64	0.3	
I hy 100	409	7.7	226	55	0.8	
I 100	464	27.7	264	57	0.1	
C/I hy 90/10	56	2.3	55	32	0.2	58
C/I hy 70/30	132	6.2	134	74	0.4	56
C/I 70/30	146	4.5	150	85	0.3	57

Biogas quality ranged from 55-64 % methane and was highest for cattle slurry. In the mixtures biogas quality ranged between 56-58 %. Biogas production was much more variable (as can also be seen in Figure 2) ranging from 16-464 nm<sup>3</sup> biogas/ton product and was by far the highest for non-hygienised insect frass, followed by hygienised insect frass. No differences were found between measured values for the combinations with insect frass and the calculated values for the combinations.

Final biogas and methane productions were also plotted on dry matter (DM) (Figure 3) and organic matter (OM) basis (Figure 4) and compared to calculated values for the mixtures.



**Figure 3** Production of biogas and methane per ton DM compared to calculated data (n=3). C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

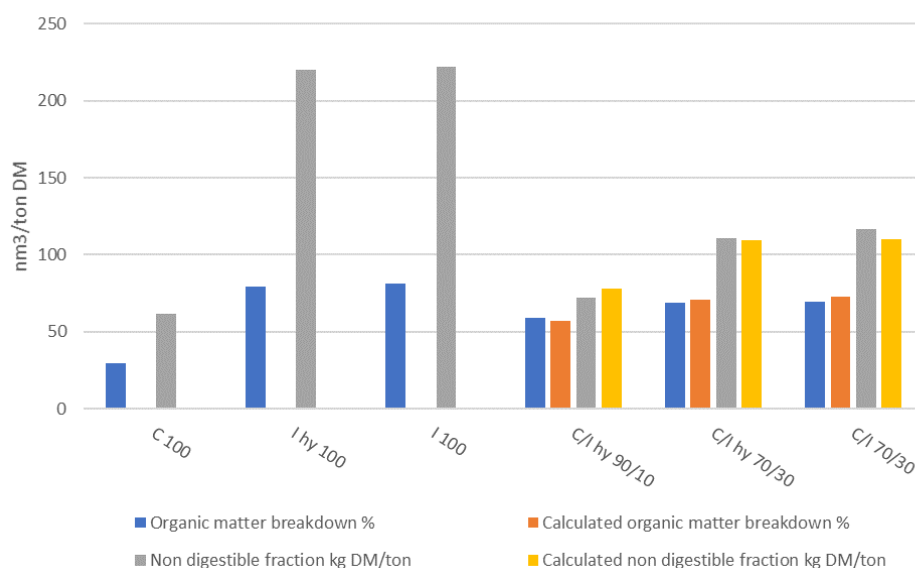


**Figure 4** Production of biogas and methane per ton OM compared to calculated data (n=3). C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

Also, in these figures it can be seen that the highest biogas and methane production per ton dry matter and per ton organic matter was found for non-hygienised insect frass, closely followed by hygienised insect frass. For combinations there was no synergistic effect since calculated values for the mixtures were very comparable to measured values.

### 3.2 Other parameters

In addition to biogas production also the organic matter breakdown in the single and mixed substrates as well as the net residual/non-digestible/non-fermentable material was calculated (Figure 5).



**Figure 5** Organic matter breakdown (%) and net non-digestible fraction of single and mixed substrates compared to calculated data (n=3). C = cattle slurry, I hy = insect frass hygienised, I = insect frass not hygienised. 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

As expected from the biogas data the highest organic matter breakdown was found for the insect frass. The non-digestible fraction of the insect frass was found to be around 220 g DM/kg fresh material, while that of cattle slurry was 62 g DM/kg material. The insect frass consists of both insect

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faeces and (a much smaller proportion of) exuviae (remains of the exoskeleton), the exuviae are assumingly not digestible.



## 4 Discussion and conclusion

No synergistic effect of adding insect frass to cattle slurry was found in this experiment. In other words, adding insect frass to a cattle slurry digester will not lead to an increase in biogas production different from expected on the base of adding up the biogas production of the single substrates. However, the partial replacement of cattle slurry with insect frass will increase the amount of biogas produced per digester volume with a factor 3.5 to 9 for respectively 10 % and 30 % replacement.

Table 4 shows biogas productions from several reference substrates. The biogas production values differ between the measurements of this report and Elissen et al. (2019). The dry matter percentage of the insect frass varies between 63 and 83 %, where the non-hygienised is the highest. The biogas production of hygienised insect frass in our experiments increased 14 percentage points compared to that mentioned by Elissen et al. (2019), which can be due to differences in the feed of the *H. illucens* larvae or storage/processing of the frass. The feed of the larvae influences the composition of the frass resulting in a different biogas production (Gärttling & Schulz, 2019; Poveda et al., 2019).

**Table 4** Measured biogas and methane productions on fresh product basis and biogas qualities compared to calculated values based on single substrates, extended from Elissen et al. (2019).

Substrate	DM %	Biogas quality (% CH <sub>4</sub> )	Biogas per ton product	Biogas per ton DM	Reference
Sewage sludge	5	65	15	300	SGC (2012)
Fish waste	42	71	537	1279	SGC (2012)
Straw	78	70	207	265	SGC (2012)
Food waste	33	63	204	618	SGC (2012)
Cattle slurry	9	65	22	244	SGC (2012)
Potato haulm	15	56	68	453	SGC (2012)
Slaughter waste	16	63	92	575	SGC (2012)
Pig slurry	8	65	26	325	SGC (2012)
Cow manure			56		Biogas-E (2017)
Pig manure			55		Biogas-E (2017)
Poultry manure			48		Biogas-E (2017)
Aerobic sludge			13		Biogas-E (2017)
Grass silage			172		Biogas-E (2017)
Cow manure	8		20	250	Dijk, van and Durks (2014)
Corn silage	33		200	600	Dijk, van and Durks (2014)
Insect frass (hygienised)	63	58	285	454	Elissen et al. (2019)
Vermicompost	32	38	10	32	Elissen et al. (2019)
Champost	37	48	51	139	Elissen et al. (2019)
Digestate 'old'	9	60	11	117	Elissen et al. (2019)
Cow manure	33	54	61	184	Elissen et al. (2021)
Cattle slurry	8	64	16	198	This report
Insect frass (hygienised)	77	55	409	534	This report
Insect frass (non-hygienised)	83	57	464	560	This report

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## 5 Recommendations

Although no synergistic effect between cattle slurry and *H. illucens* larvae frass was found, the addition of a small amount of insect frass will increase the biogas production of a digester per volume unit greatly. As such, the business case of such digesters could become more positive. This should be evaluated.

In Elissen et al. (2019) digestate instead of cattle slurry was used. Digestate is already partly treated with microbes while Opure uses a different type of microbes (based on sewage sludge). The effect of the microbes within the digester can have altered the material in such a way that the Opure microbes can process the substrate more efficiently. This specific synergistic reaction was not the scope of this project, however it can be an explanation for the results found by Elissen et al. (2019). Also, the observed synergistic effect can be explained by a slightly different dosage then provided in the protocol. Extra tests can be performed to determine this possible synergistic effect by comparing mixtures of digestate and cattle slurry with insect frass.

Currently, the frass has to be hygienised in order to leave the premises for further processing. The hygienisation requires heat and results in a lower biogas yield, making it in two ways very unattractive. Besides, after digestion another hygienisation step is required. Therefore, it is wise to seek dialogue with policy makers to legislate the transport of non-hygienised insect frass to an offsite digester.

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# Annex 1      pH values and substrate dosages at start and end of anaerobic digestion tests

C =Cattle slurry, I = Insect frass, I hy = hygienised insect frass. 90/10 = 90 %/ 10 %, 70/30 = 70 %/ 30 %.

	pH start	pH end		
<b>Test substrates</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>3</b>
<b>C</b>	7.72	7.63	7.62	7.62
<b>I hy</b>	7.74	7.55	7.56	7.55
<b>I</b>	7.74	7.50	7.50	7.52
<b>C/I hy 90/10</b>	7.72	7.61	7.61	7.60
<b>C/I hy 70/30</b>	7.74	6.57	7.57	7.58
<b>C/I 70/30</b>	7.74	7.55	7.54	7.54

D = digestate

S = substrate

		1	2	3
<b>Test substrates</b>	<b>g</b>	<b>g</b>	<b>g</b>	<b>g</b>
<b>C</b>	<b>D</b>	199.77	199.73	200.24
	<b>S</b>	9.37	9.37	9.39
<b>I hy</b>	<b>D</b>	199.58	200.09	199.79
	<b>S</b>	0.87	0.87	0.87
<b>I</b>	<b>D</b>	200.10	200.39	199.71
	<b>S</b>	0.81	0.81	0.81
<b>C/I hy 90/10</b>	<b>D</b>	199.55	199.97	200.43
	<b>S</b>	4.96	4.96	4.96
<b>C/I hy 70/30</b>	<b>D</b>	199.74	199.55	200.18
	<b>S</b>	2.43	2.43	2.43
<b>C/I 70/30</b>	<b>D</b>	200.78	200.35	200.57
	<b>S</b>	2.24	2.24	2.24

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The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.





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# Combined digestion of insect frass and cow manure for biogas production

Stefan Hol, Hellen Elissen & Rommie van der Weide

Wageningen University & Research

This study was carried out by the Wageningen Research Foundation (WR), Business Unit Field Crops and was commissioned and financed by the PPP Biobased valorisation of manure and digestate AF-17052b. This project is financially supported by the Dutch Topsector Agri & Food. Within the Topsector, private industry, knowledge institutes and the government are working together on innovations for safe and healthy food for 9 billion people in a resilient world.

WR is part of Wageningen University & Research, the collaboration of Wageningen University and Wageningen Research Foundation.

Wageningen, June 2022

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