Volatile fatty acids (VFAs) and methane from food waste and cow slurry: Comparison of biogas and VFA fermentation processes

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Volatile fatty acids (VFAs) and methane from food waste and cow slurry: Comparison of biogas and VFA fermentation processes

Elina A. Tampio[1,2] | **Lucia Blasco**[1] | **Markku M. Vainio**[1] | **Minna M. Kahala**[1] | **Saija E. Rasi**[3]

[1]Production Systems, Natural Resources Institute Finland (Luke), Jokioinen, Finland

[2]Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

[3]Production Systems, Natural Resources Institute Finland (Luke), Jyväskylä, Finland

Correspondence
Elina A. Tampio, Production Systems, Natural Resources Institute Finland (Luke), Jokioinen, Finland.
Email: elina.tampio@luke.fi

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Abstract

The potential of various biomasses for the production of green chemicals is currently one of the key topics in the field of the circular economy. Volatile fatty acids (VFAs) are intermediates in the methane formation pathway of anaerobic digestion and they can be produced in similar reactors as biogas to increase the productivity of a digestion plant, as VFAs have more varying end uses compared to biogas and methane. In this study, the aim was to assess the biogas and VFA production of food waste (FW) and cow slurry (CS) using the anaerobic biogas plant inoculum treating the corresponding substrates. The biogas and VFA production of both biomasses were studied in identical batch scale laboratory conditions while the process performance was assessed with chemical and microbial analyses. As a result, FW and CS were shown to have different chemical performances and microbial dynamics in both VFA and biogas processes. FW as a substrate showed higher yields in both processes (435 ml CH_4/g VS_{fed} and 434 mg VFA/g VS_{fed}) due to its characteristics (pH, organic composition, microbial communities), and thus, the vast volume of CS makes it also a relevant substrate for VFA and biogas production. In this study, VFA profiles were highly dependent on the substrate and inoculum characteristics, while orders Clostridiales and Lactobacillales were connected with high VFA and butyric acid production with FW as a substrate. In conclusion, anaerobic digestion supports the implementation of the waste management hierarchy as it enables the production of renewable green chemicals from both urban and rural waste materials.

KEYWORDS

anaerobic digestion, biogas, cow slurry, food waste, methane, microbial diversity, volatile fatty acid, volatile fatty acid fermentation

1 | INTRODUCTION

Anaerobic digestion is a biological method for the treatment of organic waste from different sectors, for example, agriculture, industry, and municipalities. Anaerobic digestion generates renewable energy in the form of biogas also allowing the recycling of nutrients through the application

of digestion residues in crop production. The mixed microbial consortium within a biogas plant is constantly producing other energy carrier compounds, such as volatile fatty acids (VFAs) and hydrogen (H_2), as intermediates in the methane formation pathway (Merlin Christy, Gopinath, & Divya, 2014). The interest in these intermediate compounds, especially VFAs, has recently increased, as they are acknowledged to have more varying and high-value possibilities for their end uses compared to methane. VFAs can be utilized as green and renewable chemical commodities in different sectors and feedstocks in production, for example, of bioplastics and biofuels (Kleerebezem, Joosse, Rozendal, & Loosdrecht, 2015).

Anaerobic digestion is a well-known valorization method for complex waste materials and, for example, across Europe there are over 17,000 biogas plants (situation in the end of the year 2016; EBA, 2018). VFAs, for example, acetic acid, butyric acid, or lactic acid, are produced industrially with certain bacterial strains or pure cultures (Cavinato, Frison, et al., 2017), and the substrates suitable for these processes are usually simple molecules, for example, C5 and C6 sugars (Baumann & Westermann, 2016). The production of VFAs through mixed consortia fermentation can utilize more complex substrates in nonsterile conditions (Jankowska, Chwiałkowska, Stodolny, & Oleskowicz-Popiel, 2015; Jankowska, Duber, Chwialkowska, Stodolny, & Oleskowicz-Popiel, 2018). However, to date, the VFA production process through anaerobic digestion with mixed consortia is still in the upscaling phase (Esteban-Gutiérrez, Garcia-Aguirre, Irizar, & Aymerich, 2018) and the full-scale production has been tested with very narrow spectrum of utilizable biomasses, for example, with sewage sludge (Liu et al., 2018). Therefore, there is currently a need to evaluate the potential of various biomasses as substrates for mixed consortia VFA fermentation to boost the production of green chemicals in the circular economy. VFA fermentation can be applied using the existing infrastructure of a biogas plant (the reactor, heating systems, and gas collection), and the process start-up is possible with the mixed microbial consortia from the anaerobic digestion process after the inactivation of methanogens (Kleerebezem et al., 2015). In addition, VFA fermentation and methane production processes could be implemented in series to maximize the utilization of biomass carbon for the production of both methane and VFAs (Cavinato, Da Ros, Pavan, & Bolzonella, 2017), similarly as in the two-stage H_2 and CH_4 production concept (Dareioti, Vavouraki, & Kornaros, 2014). In the near future, the production of VFAs instead of methane in biogas plants could be an interesting option to increase revenues due to decreasing energy prices and feed-in tariffs (Appel, Ostermeyer-Wiethaup, & Balmann, 2016; Pablo-Romero, Sánchez-Braza, Salvador-Ponce, & Sánchez-Labrador, 2017).

So far, it is known that both the microbial inoculum and the characteristics of the substrate have an effect on both the biogas and the VFA fermentation process. Both processes are dependent on the process conditions (e.g., loading rate and pH) and the composition of microbial consortia present (van Aarle et al, 2015). Previous studies have mainly focused on the VFA fermentation of food waste (FW; Cavinato, Frison, et al., 2017; Shen et al., 2017; Yin, Yu, Wang, & Shen, 2016) and sewage sludge (Jankowska et al., 2015; Liu et al., 2018; Peces, Astals, Clarke, & Jensen, 2016), while a few studies report the use of rural biomasses such as cattle or swine manure (Cavinato, Da Ros, et al., 2017; Huang et al., 2016). Food waste is a major waste fraction in urban areas and it has potential for the production of various energy carriers in a centralized urban context, while manure and slurry are produced in vast quantities in areas of intense animal production and have potential mostly in decentralized farm systems. However, both materials are promising substrates to study the effect of shifting process outputs from methane to VFAs to increase the product-based resilience of the biogas/VFA plants in the renewable energy and chemical markets. Currently, there is a gap in the literature on the comparison of biogas and VFA production with these kinds of urban and rural materials in identical reactor and experimental setups. This may be related to the different composition of these materials as well as gate fees which affect the interest of industry toward the substrates. However, the comparative results on microbial community dynamics and other process parameters of different materials are valuable information for co-digestion plants, which can utilize different substrates.

In this study, the aim was to assess the biogas and VFA production potential of two abundant biomasses from urban and rural contexts, FW and cow slurry (CS), along with their corresponding anaerobic biogas plant inocula. Two substrates were compared considering their chemical characteristics as well as microbial communities and taxonomical structure, which were reflected according to the performance of the production of methane and acids during operation in both biogas and VFA modes.

2 | MATERIALS AND METHODS

2.1 | Origin and pretreatment of materials

In this study, FW and CS were digested using anaerobic inocula. The FW consisted of separately collected biodegradable municipal waste from households and services from the Forssa area, in Finland. Samples were collected from a local waste treatment facility (Envor Biotech Ltd, Forssa, Finland). Cow slurry was obtained from a

slurry tank of a cowhouse (Luke, Jokioinen, Finland). In the experiments, the FW was digested by inoculum from a full-scale biogas plant treating municipal and industrial biowastes (Envor Biotech) and the CS by inoculum from a farm-scale biogas plant treating cattle slurry (Luke Maaninka, Kuopio, Finland). Both inocula originated from mesophilic (37°C) anaerobic digestion processes.

The FW was macerated in a Retsch Grindomix GM300 knife mill (Retsch Gmbh, Germany) into a paste-like form. Prior to maceration, plastic bags and other harmful materials (e.g., bones) were sorted out. The mixed materials were stored in a freezer (−20°C, 6 months), thawed, and stored in a fridge (4°C) for 3 days prior to use. Cow slurry, after collection, was stored in a fridge (4°C) 14 days prior to the experiments.

Both inocula were sieved to remove coarse material before further treatment. Thermal pretreatment was applied to the inocula to prevent the growth of methanogens during batch assays. The mechanism in the pretreatments consisted especially of the disruption of cells of methane-forming archaea, which are more vulnerable to the treatment compared to fermentative bacteria, for example, spore-forming bacteria. The inocula were separately pretreated by boiling at 94–100°C for 30 min (Pakarinen, Lehtomäki, & Rintala, 2008). Each inoculum was boiled in 2–3 L portions, which were combined. Untreated inocula and batches of thermally treated inocula were stored in a fridge (4°C, FW inoculum 7 days, CS inoculum 14 days) and used as inocula in biogas processes as such.

2.2 | Batch experiments

Untreated and thermally treated inocula from anaerobic digesters were used in BMP (biochemical methane potential) assays (Figure 2). The assays were performed in mesophilic (37°C) conditions using automated testing equipment (Bioprocess Control Ltd, Sweden) and mechanically mixed (84 rpm) for one minute per hour. Tests were done in 500 ml bottles, with a liquid volume of 400 ml. The substrate/inoculum VS:VS ratio was 0.5 (Ghimire et al., 2015; Kuruti, Nakkasunchi, Begum, Juntupally, & Arelli, 2017) with inoculum volumes from 214 to 300 g per bottle (Table 1). Distilled water was added to achieve the desired liquid volume. All bottles were flushed with N_2 to obtain anaerobic conditions. From the biogas, CO_2 was fixed with a 3 M sodium hydroxide solution and the volume of methane was determined by water displacement. The VFA content and microbial communities during the experiment were analyzed from the BMP bottles using destructive sampling by terminating three parallel test bottles after 1, 3, 6, and 10 days from each treatment (Figure 1). A 10-day experiment duration was also proposed by Jankowska et al. (2015) and Kuruti et al. (2017).

2.3 | Chemical analyses

The pH was determined using a VWR pH100 pH analyzer (VWR International). The total and volatile solids (TS and VS) were analyzed according to SFS 3008 (SFS, 1990). For the analysis of the soluble chemical oxygen demand (SCOD), samples were diluted 1:10 with distilled water and centrifuged twice as described in Tampio et al. (2014) and analyzed according to SFS 5504 (SFS, 2002). VFAs (C2-C6; acetic, propionic, isobutyric, n-butyric, isovaleric, valeric, and caproic acids) were analyzed using an HP 6,890 gas chromatograph (Tampio et al., 2014).

2.4 | Microbial analyses

To observe differences in microbial communities, duplicate (days 1, 3, and 6) or triplicate (day 10) samples during the process from both experiments were collected for DNA extraction. DNA was extracted following the method described previously (Blasco et al., 2014) from the propidium monoazide (PMA)-treated samples. PMA was used as a DNA-binding agent to differentiate viable and dead bacterial cells. The PMA treatment was done according to supplier instructions (Biotium). Next-generation sequencing

TABLE 1 Substrate and inocula characteristics

	TS (%)	VS (%)	VS/TS (%)	g FM/bottle (g VS/bottle)	VFA (g/L)	SCOD (g/L)	pH
Substrates							
Food waste (FW)	30.4	28.1	92.5	17.7 (5.0)	2.5	120.4	4.2
Cow slurry (CS)	5.9	4.7	78.9	87.8 (5.2)	6.9	19.8	7.0
Inocula							
Inoculum from FW digester[a]	6.0	3.8	62.1	300.0 (10.0)	0.1	4.6	7.7
Thermally treated FW digester inoculum[b]	7.6	4.6	61.0	225.9 (10.0)	0.2	11.2	9.3
Inoculum from CS digester[a]	4.0	2.7	68.5	265.0 (11.9)	0.2	7.3	7.8
Thermally treated CS digester inoculum[b]	5.3	3.6	68.6	214.3 (11.9)	0.2	13.5	9.6

[a]Control inoculum (biogas process). [b]VFA fermentation inoculum.

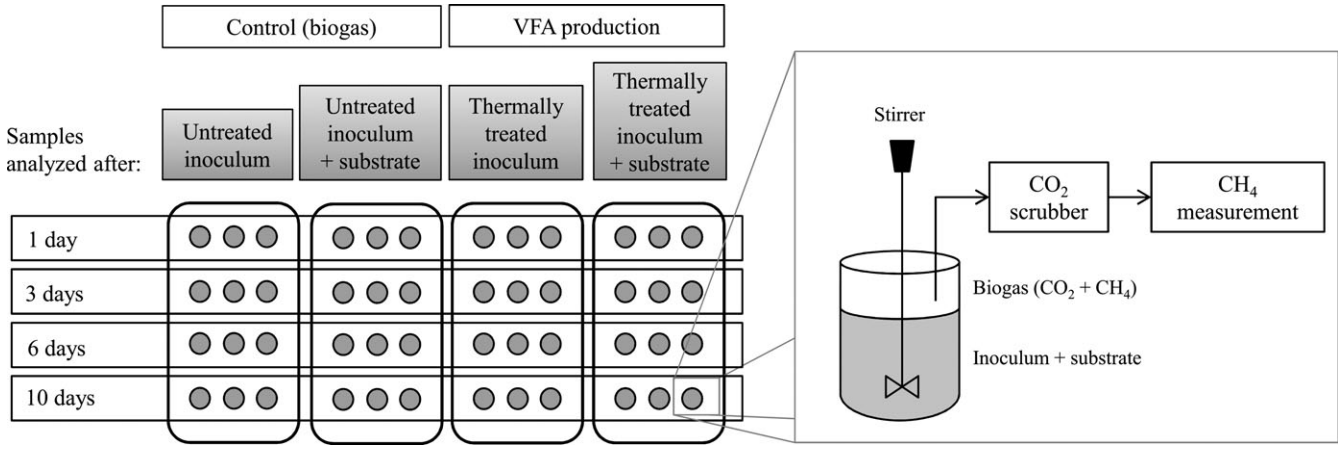

FIGURE 1 A schematic diagram of the experimental setup and the sampling method

was performed at the Finnish Functional Genomics Centre (FFGC, in Turku, Finland).

The extracted DNA was amplified using the 341F/805R set of primers for bacteria and 349F/806R set for archaea targeting the V3-V4 region. Pyrosequencing was conducted using Illumina MiSeq platform. Sequences were processed using the MOTHUR 1.39.5 software package (Schloss et al., 2009). Raw sequences were processed by trimming primers, making contigs, and removing low quality (Q score <30) and sequences shorter than 350 bp. Those sequences with an average length of 415 to 470 bp were aligned to the Greengenes 13_8 database (DeSantis et al., 2006) and used for further analyses. A principal coordinates analyses (PCoA) and a canonical correspondence analysis (CCA) were conducted using the PAST software package (Hammer, Harper, & Ryan, 2001).

2.5 | Calculations

Methane yields in the BMP assays were converted to STP conditions (0°C, 100 kPa) according to the ideal gas law. Methane yields (ml/g VS_{fed}) in the batch assays were calculated by dividing the cumulative methane production (ml) by the VS of the added substrate (or inoculum) (in g). The methane production of the inoculum was subtracted from the results containing both substrate and inoculum to determine the methane or VFA production of the substrate. In the case of pretreated inoculum, the gas production of the pretreated inoculum was used. VFA yield (mg/g VS_{fed}) was calculated per gram of VS fed by first calculating the VFA production of substrate and then dividing the VFA production of the substrate (mg/L) with the VS content of the substrate in the batch bottle (g/kg).

The theoretical COD equivalences 1.066, 1.512, 1.816, 2.036, and 2.204 g COD/g were used for acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid, respectively (Lim et al., 2008). The solubilization and acidification during the experiments were calculated using the initial and final (day 10) VS, SCOD, and VFAtot concentrations according to Peces et al. (2016) and Cavinato, Da Ros, et al. (2017) by dividing the difference between final and initial SCOD (g/L) or VFA (g COD/L) with the initial VS fed (g/kg). The initial concentrations were calculated by taking into account the volumes of inoculum and substrate in the test bottles and the total liquid volume (400 ml).

3 | RESULTS

3.1 | Substrate and inoculum characteristics

Both the inocula and substrates used in this study showed variation in their characteristics (Table 1). The FW had high TS and VS values (30% and 28%, respectively) compared to the CS (TS 6% and VS 5%). The FW contained over 100 g/L of SCOD, of which around 2% consisted of VFAs. In the CS, the initial SCOD concentration was around 20 g/L, of which 35% were VFAs. The pH in the FW was acidic (4.2) and neutral (7.0) in the CS. The inocula characteristics were typical to the digesters the materials originated from and showed stable digestion process qualities (VFA concentrations were under 0.2 g/L and the pH was around 7.7–7.8). The thermal pretreatment of the inocula to inactivate the methanogens and enable VFA production increased the inoculum TS, VS, and SCOD concentrations and the pH value (Table 1) due to water evaporation and material solubilization.

3.2 | Batch fermentation performance

During the 10 days of the experiment, control inocula and substrates acted as conventional biogas processes, where the pH balanced to levels of around 7.7 and the SCOD decreased as the readily solubilized material was converted

into biogas (Table 2). With the CS, an increase in the SCOD on day 10 was observed less degradable biomass was further degraded, which was not seen with FW, where the initial SCOD was higher compared to the CS (see Table 1). On the first day of the experiment, the VFA content was high (1.5 g/L for CS and 2.9 g/L for FW), but decreased close to 0 g/L as VFAs were converted into methane. Overall, the control biogas production tests displayed similar sorts of performance and characteristics for both FW and CS, where the differences were mainly related to different initial SCOD concentrations and SCOD solubilization. Cow slurry as substrate showed low initial SCOD concentrations (20 g/L, Table 1) which led to solubilization during the tests, while with FW, the organic matter was initially solubilized and there was no further solubilization of SCOD (Table 3).

The digestion with thermally treated inoculum had different acid fermentation performances compared to the biogas control. The pH decreased from the initial 9 to around 6 with the FW substrate and from 9 to 7 with CS. The decrease in pH during the test indicates the formation of acids, which was confirmed by increasing VFA concentrations after day 1 (Table 2). The total concentration of VFAs (including VFAs from both inoculum and substrate) increased from the initial 0.2 to 8.2 g/L for the FW, while for the CS, the increase was more moderate, from 1.6 to 3.7 g/L. The SCOD concentration remained at >10 g/L with both materials during the tests, and positive SCOD solubilization was observed with both materials (Table 3). Although the SCOD solubilization was more restricted for the FW (32 g SCOD/kg VS_{fed}) compared to the CS (183 g SCOD/kg VS_{fed}), the acidification was high with FW indicating the higher VFA fermentation potential of FW as a substrate (284 g COD_{VFAtot}/kg VS_{fed} with FW and 87 g COD_{VFAtot}/kg VS_{fed} with slurry).

3.3 | VFA profiles

During the control biogas tests with untreated inocula, the VFA profiles for both substrates had a relatively similar shape and the VFA on day 1 constituted mainly of acetic acid and had lower concentrations of propionic acid (Figure 2a,c). With the FW substrate, the VFA production decreased rapidly after day 1, and only traces of VFAs were observed on day 3. With the CS substrate, the VFAs

TABLE 3 The solubilization and acidification of food waste (FW) and cow slurry (CS) on day 10

		SCOD solubilization (g SCOD/kg VS_{fed})	Acidification (g COD_{VFAtot}/kg VS_{fed})
Control (biogas)	FW	−140.9	−4.2
	CS	62.5	−64.7
VFA process	FW	32.0	283.6
	CS	182.9	87.4

TABLE 2 The pH, SCOD, and volatile fatty acid (VFA) contents in batch bottles during the experiment

	Days	0	1	3	6	10
pH						
Control (biogas)	FW	7.7	7.3 ± 0.01	7.7 ± 0.03	7.6 ± 0.03	7.7 ± 0.01
	CS	7.7	7.6 ± 0.03	7.7 ± 0.01	7.7 ± 0.01	7.7 ± 0.02
VFA process	FW	9.3	6.5 ± 0.12	5.8 ± 0.02	5.7 ± 0.01	6.0 ± 0.13
	CS	8.9	8.2 ± 0.02	7.9 ± 0.02	7.3 ± 0.02	7.3 ± 0.03
SCOD (g/L)						
Control (biogas)	FW	8.4[a]	5.8 ± 0.12	3.6 ± 0.07	3.1 ± 0.05	3.1 ± 0.17
	CS	9.8[a]	13.6 ± 0.10	6.6 ± 0.27	7.3 ± 0.36	11.8 ± 0.62
VFA process	FW	11.3[a]	8.5 ± 1.34	11.5 ± 1.69	11.9 ± 0.86	12.5 ± 1.16
	CS	12.0[a]	15.8 ± 0.33	12.9 ± 0.13	12.1 ± 0.92	17.5 ± 2.57
VFAs (g/L)						
Control (biogas)	FW	0.2[a]	2.9 ± 0.61	0.00 ± 0.00	0.1 ± 0.06	0 ± 0.06
	CS	1.7[a]	1.5 ± 0.00	0.4 ± 0.06	0.10 ± 0.00	0.1 ± 0.00
VFA process	FW	0.2[a]	2.6 ± 0.51	7.0 ± 0.17	8.2 ± 0.70	8.2 ± 0.67
	CS	1.6[a]	2.5 + 0.06	2.9 + 0.12	3.6 + 0.06	3.7 + 0.12

Note. The results include the total production of both inoculum and substrate within the batch bottles. Averages and standard deviations from triplicates. Day 0 is the experiment start, and day 1 is the first day of sampling.

CS: cow slurry; FW: food waste.

[a]Calculated from the initial concentrations in inoculum and substrate.

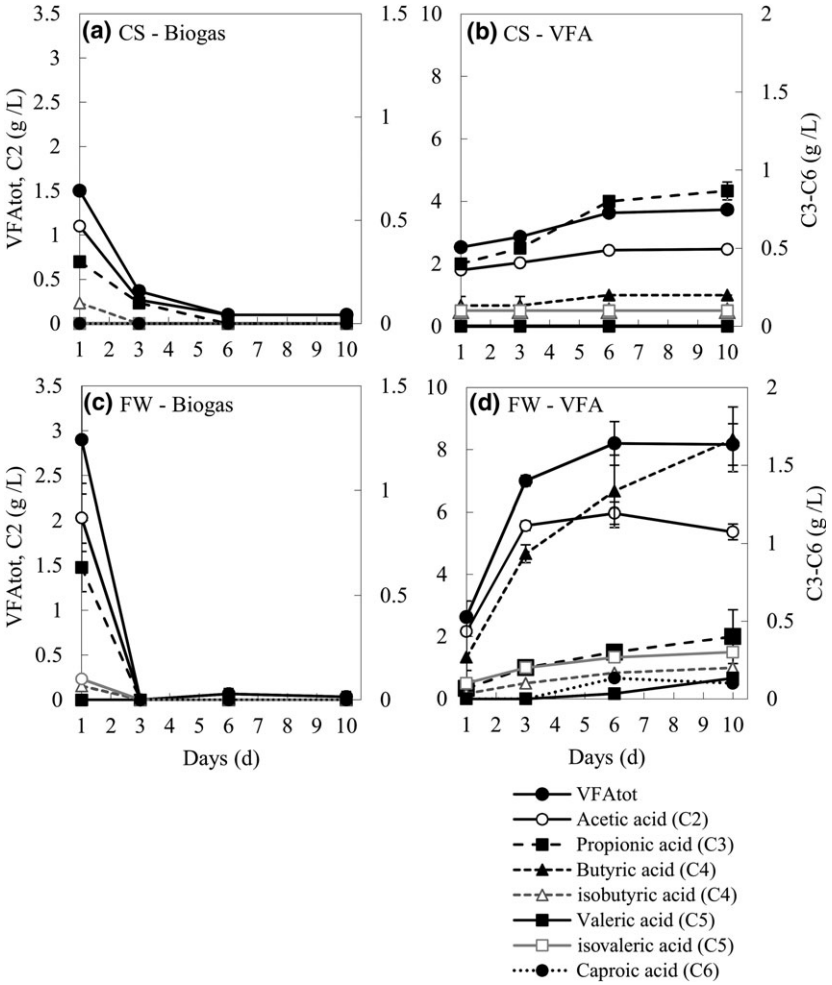

FIGURE 2 Evolution of different volatile fatty acids (VFAs) during batch tests. Note the separate *y*-axis for total VFAs, acetate, and other acids (C3-C6) as well as a different *y*-axis for food waste (FW) and cow slurry (CS)

displayed a decreasing trend after day 1, but concentrations of around 0.4 g/L of total VFAs were still detected on day 3.

The VFA profiles from the VFA fermentation with thermally treated inocula showed differences not only in both VFA components but also in VFA production kinetics. The FW produced VFAs rapidly during the first days of the test reaching maximum concentrations on day 6 (around 8 g/L, Figure 2d). After day 6, the acetic acid concentration started to decrease (from a maximum of 80% to 66% of the VFAtot), while butyric acid concentration was slightly increasing (from 3% of VFAtot on day 3% to 20% of VFAtot on day 10). The concentration of propionic acid was from 3% to 5% and valeric acid around 3.5% of VFAtot in BW. Both isovaleric and caproic acids were present in low concentrations and were around 1.5% of the VFAtot.

With CS, the VFA production was more moderate and increased more evenly until day 6, after which the production remained stable until day 10 (Figure 2b). The acetic acid concentration decreased from 71% of the VFAtot on day 3% to 66% on day 10, while the concentration of propionic acid increased from 17% to 23%. The butyric acid corresponded to around 5% isobutyric and isovaleric acids 3% of the VFAtot with the CS substrate.

3.4 | Methane production

Methane production in the biogas tests with untreated inoculum (control) increased rapidly, and during the first day, FW produced almost four times the amount of methane (170 ml/g VS_{fed}) compared to CS (<50 ml/g VS_{fed}, Figure 3a,c). During the 10 days of the experiment, the methane production was around 435 ml/g VS_{fed} for FW and 160 ml/g VS_{fed} for CS. However, it seemed that the daily gas production rate of FW started to decrease after day 3, while with CS, the gas production was still increasing on day 10. The methane production of the substrates was consistent with the production of VFAs, which showed a decreasing trend along with increasing methane production (Figure 4).

The VFA fermentation of FW and CS with thermally treated inocula either showed very low (around 15 ml/g VS_{fed} with FW) or totally inhibited methane production (with CS) during the first 3 days of the VFA fermentation

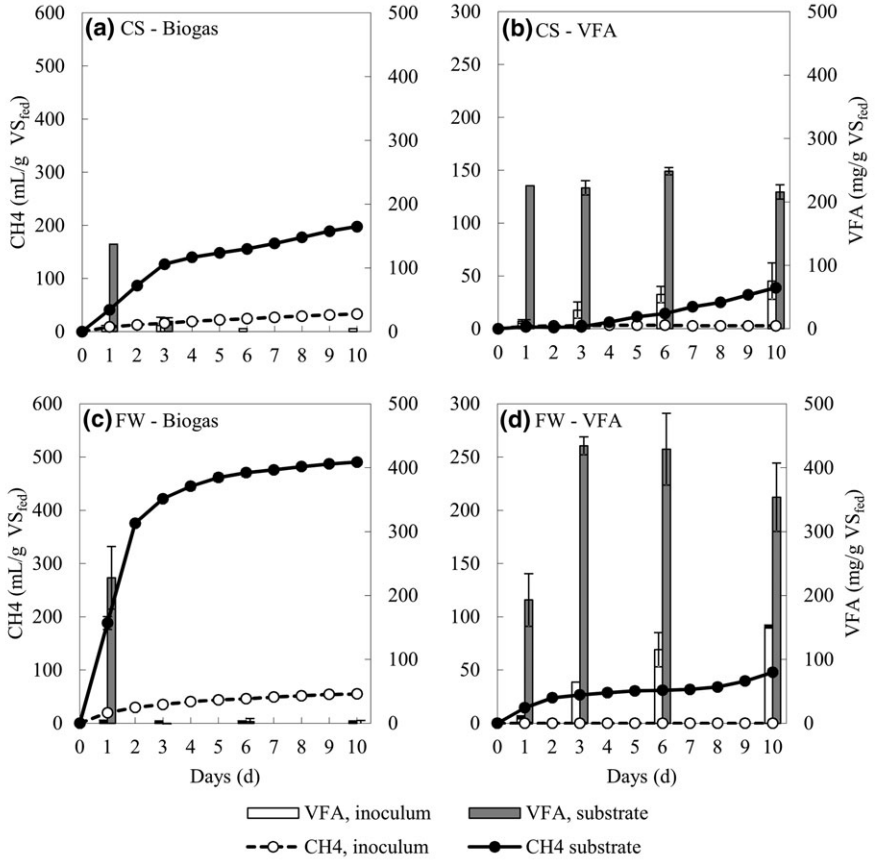

FIGURE 3 Methane and volatile fatty acid (VFA) yields of both substrates (food waste, FW; cow slurry, CS) and respective inocula. The results from the substrates show the share of the substrate only, as the methane/VFA production of the inoculum was subtracted from the results

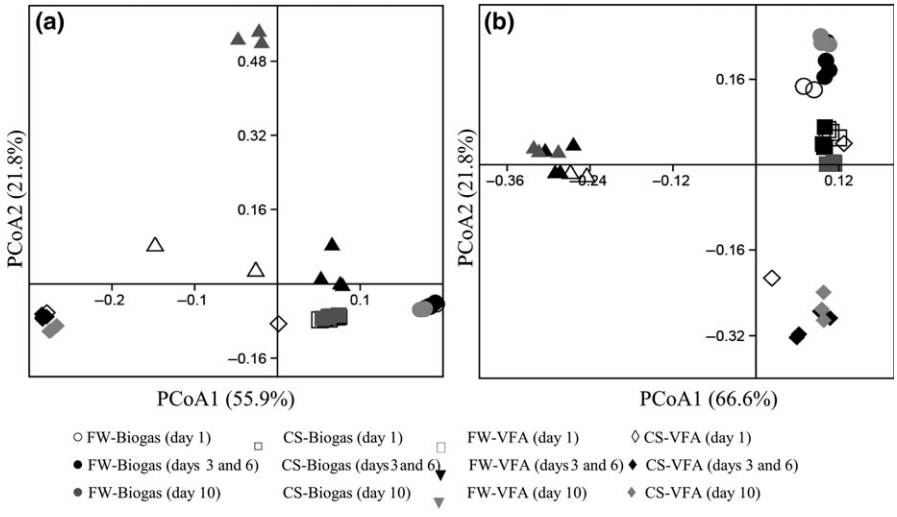

FIGURE 4 Principle coordinate analysis (PCoA) of the samples based on the taxonomic composition of archaea at the family level (a) and bacteria at the order level (b)

(Figure 3b,d). Highest VFA yield (434 mg/g VS_{fed}) was observed on day 3 with FW and on day 6 (248 mg/g VS_{fed}) with CS. By day 10, the production of VFAs with both substrates declined 14%–18%, which is in line with the increasing methane production during that time (increase from 15 to 49 ml/g VS_{fed} with FW and from 2 to 39 ml/g VS_{fed} with CS, Figure 3a,c).

Concerning VFA fermentation with thermally treated inocula only, the VFA production was still increasing on day 10 and was higher than the VFA production of FW and CS substrates (Figure 3c,d). On day 10, VFA production of FW inoculum reached yield of 151 mg/g VS_{fed} and CS inoculum 75 mg/g VS_{fed}. Unlike VFA production, the methane production from the inoculum only balanced

during 10 days of the biogas experiment being 55 ml/g VS_{fed} for FW and 33 ml/g VS_{fed} for the CS inoculum (Figure 3a,c). The thermal treatment of the inoculum most likely degraded some otherwise easily undegradable inoculum components and enabled prolonged VFA production.

3.5 | Microbial diversity

Differences in microbial diversity were seen within the experiments (biogas production and VFA production) and between both feed substrates (FW and CS). The microbial compositions of the control experiments (biogas) for FW and CS were more stable during the experiment than those of VFA experiments. A PCoA based on the Bray–Curtis distance was used to visualize the distances and variations between the samples (Figure 4). A comparison of the taxonomical patterns between the samples showed differences within biogas and VFA experiments as well as between both substrates. The microbial compositions during biogas experiments for both substrates remained more constant during the 10-day experiment than those of VFA experiments (Figure 4). VFA production experiment with a FW substrate showed a higher dispersion than with CS, meaning a more dynamic population evolving during the experiment.

A CCA was performed to examine potential correlations between the microbial composition and performance parameters, as well as methane production (ml/g VS_{fed}) and VFAs concentrations (g/L), on each sampling days (1, 3, 6, and 10). The results provided evidence of a correlation between some of the archaeal and bacterial groups present in the communities, as well methane and C3-C6 VFA production (propionic, butyric, valeric, and caproic acids, Figure 5). The CCA model explained 53% and 56% of the total variance of the taxonomy of archaeal and bacterial communities.

It could be observed that certain families were responsible for the sample distribution. In archaea, A4 (Methanomicrobia) was the most abundant (72%–73%) on day 10 of VFA experiment with FW. A8 (Methanosaetaceae) was strongly associated with methane production and was the most abundant during biogas production for both FW and CS (Figure 5a). The families associated with VFA production with CS were mainly A9 (Methanosarcinaceae) and A10 (Methanomassiliicoccaceae), with A9 reaching a maximum relative abundance of 37% on day 10 and A10 24% on day one. Regarding orders of bacteria, B8 (Lactobacillales) and B10 (Clostridiales) were mainly responsible for the grouping of FW samples during VFA production and were shown to be related to VFA production (Figure 5b). B14 (Firmicutes_unclassified) was found to be responsible for the positioning of the CS samples during VFA production.

4 | DISCUSSION

4.1 | Substrates and inocula

The substrate and inocula characteristics reflected the VFA and biogas yields obtained in this study. The relatively low initial VFA concentration and high SCOD with FW led to higher acidification and VFA production during fermentation. In contrast, higher initial VFA and low SCOD for the CS substrate reduced the acidification during fermentation, as CS is an organic material already anaerobically fermented in cow rumen. Previously, CS has been studied to contain varying levels of VFAs depending on the TS content, storage time, and conditions as well as the individual acids analyzed. Stored CS has been reported to contain around 3 g/L of VFAs (Page et al., 2014) and stored solid manure 0.7 g VFAs/L (Kafle & Chen, 2016) and 0.25 g COD/kg (Cavinato, Da Ros, et al., 2017). The CS studied

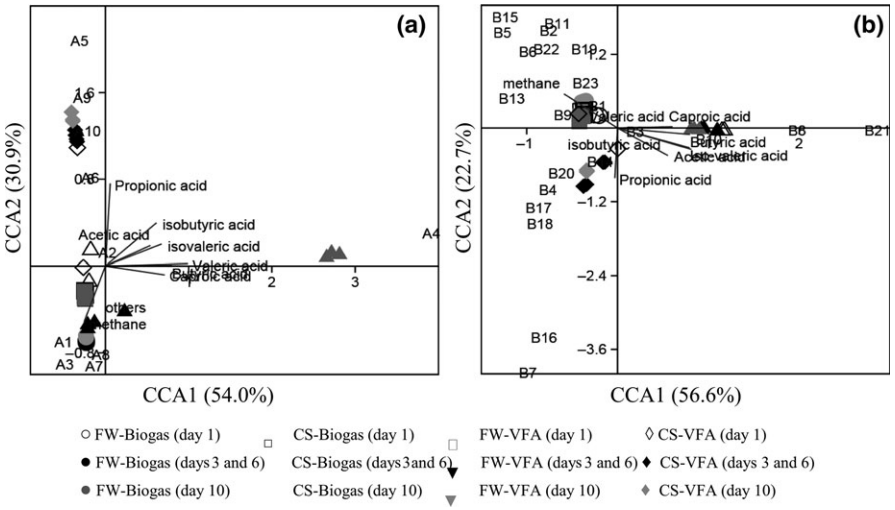

FIGURE 5 Canonical correspondence analysis (CCA) ordination diagrams showing the correlation between the relative abundance of archaea at the family level (a) and bacteria at the order level (b) and the performance variables (methane: cumulative daily methane yield in ml/g VS_{fed}, Volatile fatty acids [VFAs]: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid in g/L). The letters A1 to A12 and B1 to B23 represent all the archaeal families and bacterial orders for the binary matrices

in the present study contained almost 7 g/L (8.6 g COD/kg) of VFAs, which was most likely due to the low TS content of the slurry (6%) as well as the longer storage times at the farm and one week of storage in a fridge before the analyses, which led to CS degradation and VFA formation.

4.2 | VFA fermentation

In this study, the 10-day biogas process and acid fermentation of FW and CS were compared. Previously, FW has been reported to produce methane at around 400 ml/g VS_{fed} (Tampio et al., 2014), which was in accordance with the present results (435 ml/g VS_{fed}). Correspondingly, for CS, a similar degree of methane production was obtained in this study (200 ml/g VS_{fed}) as has been reported before (170 ml/g VS_{fed}, Kafle & Chen, 2016) after 10 days of batch digestion. During biogas runs, VFAs were produced only at the beginning of the experiment, after which they were degraded into biogas components (CH_4, CO_2). On day 1, the higher VFA concentration during the biogas process of FW (1.7 g/L) compared to CS (0.2 g/L) was most likely related to the fast hydrolysis and acidification of the substrate and slower start of methanogenesis. Similar increased VFA concentrations at the beginning of biogas processes have also been observed with, for example, FW substrates (Tampio et al., 2014).

Volatile fatty acid fermentation with the thermally treated inoculum yielded a maximum of around 434 mg/g VS_{fed} of VFAs from FW and 248 mg/g VS_{fed} VFAs from CS (VFAs from inoculum were subtracted). Higher yields from FW were expected, as it contained more soluble COD compared to CS (Table 1), which was already degraded to some extent in the rumen. The VFA concentrations obtained for the FW were in the lower range of VFA yields obtained for similar food-based wastes in the literature (5–40 g/L, Table 4). For the CS, the obtained VFA concentration in the fermentation experiment was higher than the values previously reported (<2 g/L, <15 mg COD/L, Table 4), which was most likely due to the chosen fermentation conditions and the use of external inoculum. However, a direct comparison to the literature values is challenging due to the discrepancy in VFA yield units as well as the inaccuracy in the reported methods, where it is not known whether the inoculum production was included or subtracted from the result. Varying VFAs yields compared to the literature are related to the applied $VS_{substrate}/VS_{inoculum}$ ratio (or food to micro-organisms, F/M, ratio), which is known to affect the degradation pathways (Pakarinen et al., 2008). However, the ratio does not exactly define the amount of readily available organic matter available for the microbes, as both substrate and inoculum contain both available and undegradable components within the VS (Argun & Dao, 2017). The present batch assays were performed in substrate-limited conditions ($VS_{substrate}/VS_{inoculum} = 0.5$) to avoid organic overloading of the FW assays.

Acetate is often the major component of the VFAtot, which was also observed in the present study, where acetate comprised 66%–80% of the total VFAs. In the VFA fermentation with FW, butyrate was the second largest VFA component, while propionic acid was dominant in CS fermentation (Figure 2). The present results are consistent with previous studies, which observed the prevalence of butyrate in FW fermentation (Liu, Wang, Jiang, & Zhang, 2017). The share of propionic acid in the present study (17%–23% of VFAs) was similar to results obtained previously for dry acid fermentation of swine manure (Huang et al., 2016). Accordingly, the macromolecule composition (carbohydrates, proteins, lipids) of the substrate affects VFA formation (Bengtsson, Werker, Christensson, & Welander, 2008) and is related to the degradation pathways of different molecules. For example, the degradation of lipids has been connected to the formation of especially propionic (Liu et al., 2017; Yin et al., 2016) and valeric acid, starch and glucose has been linked to the formation of butyrate (Yin et al., 2016). The degradation of proteins and amino acids has been reported to form both propionic (Yin et al., 2016) and butyric acids (Parawira, Murto, Read, & Mattiasson, 2004) but also favor longer chain acids (Cavinato, Da Ros, et al., 2017), for example, valeric acid (Yuan et al., 2006). Food waste is known to contain around 10%–20% TS lipids and same amount of fats (Davidsson, Gruvberger, Christensen, Hansen, & Jansen, 2007; Tampio, Ervasti, & Rintala, 2015), while cow manure, with TS content of around 10%–20%, contains 8%–25%TS proteins and 1%–8% lipids (Kafle & Chen, 2016; Triolo, Sommer, Møller, Weisbjerg, & Jiang, 2011). The composition of the substrates used in the present study does not fully explain the VFA profiles attained, which can be also affected by the process pH. Throughout the VFA fermentation experiment, the pH remained high (7.7) for CS, while fell to 5.7 with FW (Table 2) mainly due to the acidification of the initially acidic substrate. Previously, it has been reported that lowering the process pH from neutral to acidic decreases propionic acid ratios and increases butyric (Dareioti et al., 2014; Ghimire et al., 2015) and valeric acid (Albuquerque, Eiroa, Torres, Nunes, & Reis, 2007) ratios, which explains the VFA profile in the present study. Additionally, the process pH is also known to affect the hydrolysis of substrates (Jankowska et al., 2015), where the acidic environment of the FW fermentation at a pH of around 6 most likely favored hydrolyzing enzyme production, which led to higher VFA yields with FW compared to CS fermentation (pH >7). In addition, the pH is known to affect the reaction speed due to the dissociation of VFAs

TABLE 4 Volatile fatty acids (VFAs) fermentation results with food waste and cow slurry in mesophilic conditions (30–37°C) with varying F/M (food to microorganism) ratio in literature vs. present study

Substrate	Inoculum treatment	Type	F/M ratio	pH control	Maximum VFA yield	Reference
Food wastes						
Food waste	BESA[a]	Batch	0.5 (VS)	No	5.5 g/L	Ghimire et al. (2015)
Food waste	No	Batch	20 (FM)	No	41 g/L	Liu et al. (2017)
Tofu	No	Batch	5 (VS)	No	7.28 g/L	Shen et al. (2017)
Egg white	No	Batch	5 (VS)	No	15.23 g/L	Shen et al. (2017)
Sterilized food waste	No	Batch	85:15 (TS)	No	36.7% SCOD	Tang, Wang, Hu, Zhang, and Li (2016)
Sterilized food waste	No	Batch	85:15 (TS)	pH 6	54.2% SCOD	
Food waste (88%) + dewatered sludge	No	Semi-continuous	–	pH 9	25.9 g COD/L	Chen, Meng, Nie, and Zhang (2013)
Food waste	No	Semi-continuous	–	pH 5.0, 5.5, 6.0	20–30 g/L	Lim et al. (2008)
Food waste	No	Batch	0.5 (VS)	No	0.03 g/L (0.04 g COD/L)[b]	Present study
Food waste	Thermal	Batch	0.5 (VS)	No	8.2 g/L (10.8 g COD/L)[b]	Present study
Manures, slurries						
Cattle manure	Acidogenic culture	Batch	0.4–1 (VS)	No	0.4–3 kg/kg $VS_{reduced}$	Kuruti et al. (2017)
Cattle manure	Acidogenic culture	Batch	90:10 (FM)	pH 4.5, 5.0, 5.5, 6.0	1.2–1.7 g/L	Kuruti et al. (2017)
Swine manure	No inoculum	Batch	–	No	12.6 mg COD/g VS	Huang et al. (2016)
40% Cow manure (TS basis) + maize silage	No inoculum	Continuous	–	No	6.7–14.7 mg COD/L	Cavinato, Da Ros, et al. (2017) and Cavinato, Frison, et al. (2017)
Cow slurry	No	Batch	0.5 (VS)	No	0.1 g/L (0.1 g COD/L)[b]	Present study
Cow slurry	Thermal	Batch	0.5 (VS)	No	3.7 g/L (4.7 g COD/L)[b]	Present study

[a]Bromoethanesulfonic acid. [b]Inoculum included. Without inoculum VFA yields 5.4 g/L (434 mg/g VS_{fed}) with food waste and 2.5 g/L (248 mg/g VS_{fed}) with cow slurry.

and the permeability through cell membranes (Jankowska et al., 2015). Overall, it can be concluded that the acidic characteristics of the FW substrate had the most influence on the distribution of VFAs compared to the ultimate composition of the substrates.

4.3 | Microbial communities

Clostridiales and Lactobacillales were present in all the samples but were found to be more abundant during the VFA fermentation of FW. These orders have been widely related to VFA production, and mixtures of acetic, n-butyric, caproic, and lactic acids have been shown to be characteristic of clostridial fermentation (Sträuber, Schröder, & Kleinsteuber, 2012). Clostridiales have been shown to contribute especially to butyrate production (Ma et al., 2017) while Lactobacillales have been shown to positively associate with acetate, butyrate, and propionate (Yun & Cho, 2016). The main reason for the presence of clostridia in the thermal treatments is due to their sporulating capacity. The other bacteria are killed during the heat treatment of the inoculum and the spore-forming species become the most abundant bacteria. Regarding the archaea population, the most abundant families associated with methane production were Methanosaetaceae and Methanosarcinaceae (André et al., 2016; Smith & Ingram-Smith, 2007).

4.4 | Biomasses and products

Although FW is able to produce twice the yield of methane or VFAs compared to CS, the potential of CS in the production of renewable energy and green chemicals can still be

high, depending on the biomass volumes available. For example, in Finland, the volume of CS is around 6.8 Mt per year, while the potential volume of FW from households and industry is around 0.8 Mt (Marttinen, Venelampi, & Iho, 2018), which highlights the importance of the regionally available biomass volumes, not only the VFA production potential. For farm-scale biogas plants, the production of VFAs could even be more profitable solutions than biogas as energy prices or energy, especially heat, utilization is low. The value of the product could be also further increased with the implementation of biogas processes for the utilization of fermentation residues, while the market value of VFAs varies depending on the purity and share of different acids (Kleerebezem et al., 2015). However, the obstacle in the scale-up of VFA production is the separation of VFAs from the digestate, which increases costs and infrastructure needed compared to biogas production. A pretreatment step for the VFA separation is often acquired and it can be, for example, solid–liquid separation with a decanter centrifuge, which is widely used digestate treatment technology in biogas plants (Drosg, Fuchs, Seadi, Madsen, & Linke, 2015). The separated VFA containing liquid could be used as such, for example, as a carbon source in wastewater treatment or further processed to recover VFAs (Huang et al., 2016). Technologies for VFA recovery are, for example, membrane separation and liquid–liquid extraction (Kleerebezem et al., 2015; Zacharof & Lovitt, 2013).

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ORCID

Elina A. Tampio http://orcid.org/0000-0002-8080-4492

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