Waste Management 117 (2020) 124–135

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Characterisation of microbial communities for improved management of anaerobic digestion of food waste

Nadieh de Jonge [a], Åsa Davidsson [b], Jes la Cour Jansen [b], Jeppe Lund Nielsen [a,*]

[a] Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, DK-9220, Aalborg E, Denmark
[b] Water and Environmental Engineering at Department of Chemical Engineering, Lund University, Box 124, SE-221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 24 April 2020
Revised 21 July 2020
Accepted 22 July 2020
Available online 18 August 2020

Keywords:
Food waste
Microbial community
Full scale
Anaerobic digestion
Biogas production

ABSTRACT

Anaerobic digestion of food waste is an attractive and increasingly popular technology within waste management and energy recovery. A better understanding of the microbiology associated with anaerobic digestion of food waste will provide new insight into the operational conditions required for optimizing this process, as well as its potential for utilisation in co-digestion systems. Eighteen full-scale reactors processing varying proportions of food waste under diverse operational configurations were subjected to microbial community analysis by amplicon sequencing of the 16S rRNA and *mcrA* genes to capture the bacterial and methanogenic populations. Statistical correlations between microbial populations, plant design and operating conditions revealed that the microbial communities were shaped by operational parameters such as the primary substrate type and operational temperature, while the methanogenic communities showed a more reactor specific distribution. The distribution of microbes based on the waste processed in the surveyed digesters was explored, as well as the presence of specialist populations such as syntrophs and methanogens. Food waste digester communities were not associated with a strong microbial fingerprint compared to other waste types (wastewater and manure) but contained greater abundance and unique syntrophic acetate oxidising populations, suggesting that co-digestion with food waste may improve the functional diversity of anaerobic digesters.

1. Introduction

Food waste (FW) is the organic fraction of municipal solid waste (OFMSW). In 2016, 2.01 billion tons of municipal solid waste were produced globally, with an organic fraction ranging from 32 to 50% depending on country, and this is expected to increase to 3.4 billion tons by 2050 (Kaza et al., 2018). The majority of this waste type is sent to either landfills or incineration plants for disposal. Food waste has a highly biodegradable nature and high nitrogen content. Anaerobic digestion (AD) has therefore been suggested as a viable way to produce energy and stabilise this type of organic waste for further use of the digestate as fertiliser (Uçkun Kiran et al., 2014; Zhang et al., 2014). Countries that are actively promoting and utilising FW for biogas production include Germany, Sweden, Brazil, the United States and China (Bernstad and la Cour Jansen, 2011; De Clercq et al., 2017). FW has a high VS content, favourable carbon to nitrogen ratio (C/N) for anaerobic digestion and high carbohydrate and protein content. However, due to the variable composition of the FW, some variation may be observed in the nutrient balance and overall substrate characteristics (Dhamodharan and Kalamdhad, 2014; Fisgativa et al., 2017). It often has a biomethane potential (BMP) twice as high as biological wastewater sludge and manure (Fisgativa et al., 2017; Sembera et al., 2019). This makes FW very suitable for energy recovery through AD, and it is applicable for both separate digestion, but more often used in co-digestion with other mixed organic wastes due to the high nitrogen content or to enhance the AD process with other less efficient wastes (Yun et al., 2015). FW generally needs little pre-treatment beyond mechanical disintegration and removal of missortings e.g. plastic and glass. However, sorting out unwanted materials is not without technical and practical challenges and residual glass and plastics can have severe consequences for the operation of digesters (Bernstad and la Cour Jansen, 2012; Sembera et al., 2019). Furthermore, processes such as chemical pre-treatment and thermal hydrolysis have also been observed in FW AD, typically for hygienisation purposes (Carlsson et al., 2012; Dhamodharan and Kalamdhad, 2014).

The anaerobic digestion of organic material can be roughly divided into four stages, of which the initial hydrolysis and acido-

* Corresponding author.
E-mail addresses: ndj@bio.aau.dk (N. de Jonge), asa.davidsson@chemeng.lth.se (Å. Davidsson), jes.la_cour_jansen@chemeng.lth.se (J. la Cour Jansen), jln@bio.aau.dk (J.L. Nielsen).

https://doi.org/10.1016/j.wasman.2020.07.047
0956-053X/

genesis involve the majority of the microbial diversity present in an AD reactor (Ali Shah et al., 2014). These initial stages break down the substrate material into their monomeric components including volatile fatty acids (VFA) and alcohols, and operate under a delicate balance with the acetogenesis and methanogenesis. Specialised populations of microbes transform the resulting simple compounds to methane (CH_4), water (H_2O) and carbon dioxide (CO_2) (Ali Shah et al., 2014; Merlin Christy et al., 2014).

Previous studies have suggested that food waste can be used to enhance AD of other (lesser) degradable substrates (Yun et al., 2015; Zhang et al., 2013), and that FW based AD is capable of adaptation to and recovery from various perturbations (Guo et al., 2014; Kim et al., 2006; Sheng et al., 2013). It can therefore be theorised that addition of FW to co-digestion systems could lead to improved functional redundancy and stress resilience, and ultimately a more stable and robust AD system. In addition, a better understanding on how to utilise substrates to manipulate the (diversity of) AD microbial communities will lead to better microbial management and overall better optimised AD systems in the future (Carballa et al., 2015).

The microbiology of food waste AD has been of growing interest. High throughput sequencing approaches using 454 and Illumina platforms have been used to describe FW AD microbiomes (Cheng et al., 2014; Guo et al., 2014; Shi et al., 2018). A high level of microbial diversity has been observed, and bacterial phyla *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Proteobacteria* have generally been observed as the abundant microbial consortia. The majority of these microbial populations are not limited to FW AD, but has also been observed in other ADs processing other substrate types as well as co-digestion systems (Guo et al., 2014; Rui et al., 2015; Zamanzadeh et al., 2016). While the majority of the studies focusing on FW AD microbiology have described the microbial community structure, the drivers affecting the microbiome as well as characteristic features and potential have not been explored. These parameters need to be elucidated in order to utilise knowledge gained from the microbial community composition in practice.

Three of the strongest effectors on the efficiency of the AD processes in general have been reported to be the operational temperature, substrate composition and the feeding regime (Chen et al., 2008). The C/N ratio ties directly into these factors, as optimal availability of nutrients promotes stable anaerobic growth and activity (Chen et al., 2008). The high biodegradability and nitrogen content of food waste can cause an accumulation of ammonia, which is severely inhibitory to various types of microbes involved in AD and in particular the methanogens, and thus have considerable effect on the efficiency of FW AD (Sheng et al., 2013). Thus, the combined effects of pH and NH_4^+ and a high BMP make FW generally more suited for co-digestion with other waste types (Sheng et al., 2013). Limitations relating to AD of FW include the variable nutrient composition, potential accumulation of toxic compounds such as heavy metals and phthalates (Tyagi et al., 2018), as well as potential antimicrobial activity from food waste components (Sahu et al., 2017). These factors may pose a challenge in the process control relating to FW AD. Management of AD to maintain a stable operation has traditionally been carried out based on the empiric knowledge obtained through evaluation of the yield by altered HRT, substrate composition and organic loading rate (OLR). However, with the development of rapid methods for analysis of microbial communities, a more dynamic approach can be achieved by integrating the understanding of how the active microbial community respond to operational change into existing management strategies (Carballa et al., 2015).

Microbial studies in pilot- and full-scale AD systems have primarily focused on descriptions of the overall microbial community structure, often in relation to perturbation of (lab scale) AD systems. One of the populations of great interest for AD system management are the syntrophs; microorganisms that live in close mutualistic partnerships to exchange intermediate compounds, such as acetogens and methanogens (Sieber et al., 2012). However, many questions in regard to specialised microbes engaging in syntrophy, dynamic microbial interactions and system robustness (e.g. resilience during stress events) during regular operation of full-scale AD processes remain to be studied. One of the most well-described microbial responses in AD systems is a change in metabolic pathway utilisation from predominantly acetoclastic methanogenesis (AM) to predominantly hydrogenotrophic methanogenesis (HM) via syntrophic acetate oxidation (SAO), which has been shown to occur under periods of ammonia stress and other major environmental changes (Schnürer & Nordberg 2008). This suggests that monitoring the syntrophic and methanogenic communities could serve as an important tool for describing the stability and potential for resilience in digester systems. In order to move towards better AD system management, it is imperative that a deeper understanding of the microbial ecosystems in digesters is obtained, and that this knowledge is linked to the operational parameters that control the AD environment, so that effective application may be achieved in practice.

The aim of the present study was to evaluate factors influencing the microbial community compositions in food waste AD by investigating the microbial consortia in a representative set of 18 full-scale biogas reactors processing food waste under diverse operational configurations, in most cases with replications. Characteristic microbial populations for food waste AD were investigated, and correlations between utilisation of food waste, syntrophic and methanogenic population occurrence were explored. Finally, the potential microbial contribution from food waste to digester systems and potential utilisation of the microbial community information are discussed.

2. Materials & methods

2.1. Sample collection

Samples were collected from 18 CSTR reactors performing liquid anaerobic digestion, stemming from 8 anonymously participating full-scale biogas plants in Sweden (Fig. 1). Reactors from the same biogas plant were all sister reactors, and therefore considered as replications. Operational data was provided by plant operators, and all participating digesters were reported as operating under stable conditions, and no major seasonality of substrates was reported. The sampled full-scale digesters represent a diversity of operational designs and strategies, with 6 mesophilic (plant A – F) and 2 thermophilic (plant G and H) plants running at operational temperatures ranging from 37 to 58 °C and hydraulic retention times (HRT) between 10 and 45 days. The amount of food waste (FW) processed per digester ranged from 5 to 80 % in the mesophilic reactors and from 74 to 95 % in the thermophilic reactors. Other major waste types processed by the analysed reactors included wastewater (primary and surplus) sludge (WWS), food industry (FI) and slaughterhouse (SH) waste and grease. One of the sampled plants (plant F) employed a thermal hydrolysis pre-treatment process (CAMBI).

2.2. DNA extraction and amplicon sequencing

Total DNA was extracted from technical triplicates of 50 μL homogenized digestate, as previously described (Kirkegaard et al., 2017). To capture the bacterial communities of the digesters, amplicon sequencing of 16S rRNA gene variable region V1-V3 was performed as described elsewhere (Albertsen et al., 2015). Analysis of the methanogenic communities was performed on a single replicate of each digester through amplification of *mcrA* gene fragments

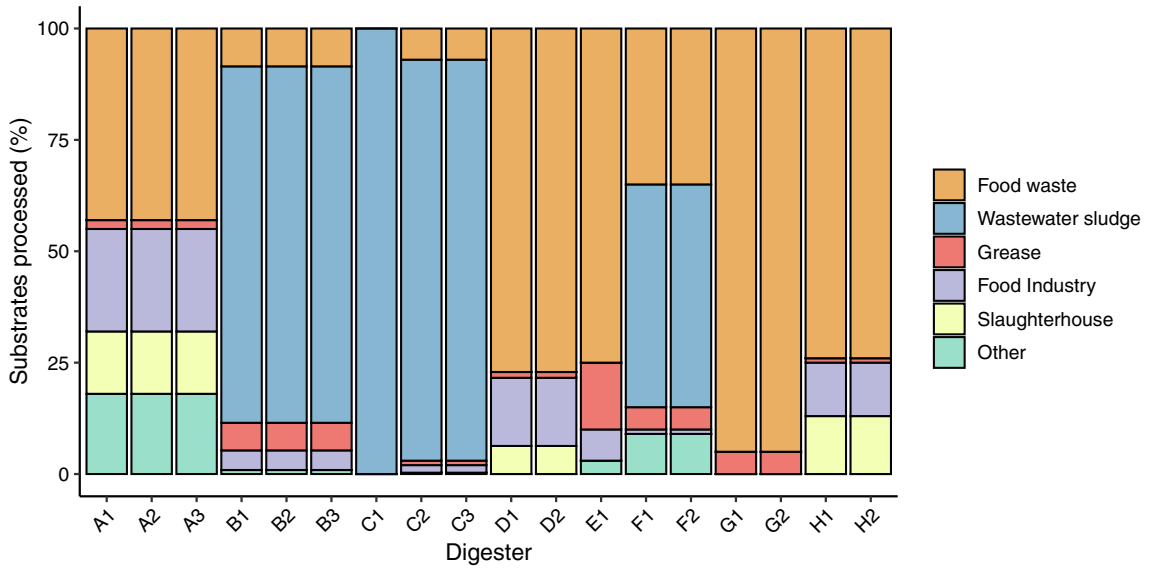

Fig. 1. Substrate composition of 18 full-scale digesters. Waste types processed (%) are displayed as stacked bar plots, per reactor. FW = food waste, WWS = wastewater sludge.

in accordance with previously described protocols (Agneessens et al., 2017) and degenerate primers (Luton et al., 2002), and subsequently barcoded for sequencing using the Nextera XT adapter protocol (Illumina, USA). Equimolar amounts of the respective amplicon pools were sequenced on a MiSeq platform (Illumina, USA), using reagent kit v3 (2 × 300 PE).

2.3. Data analysis

Sequenced libraries were quality checked and trimmed using trimmomatic version 0.32 (Bolger et al., 2014), merged with FLASH version 1.2.7 (Magoč and Salzberg, 2011), screened for PhiX contamination and subsequently formatted for use with UPARSE (Edgar, 2013). Chimeric sequence removal and clustering into Operational Taxonomic Units (OTUs) was performed using USEARCH7 at 97 % and 89 % sequence similarity for bacterial 16S rRNA gene (species level) and *mcrA* gene (genus level) sequences respectively. Taxonomy was assigned to the bacterial OTUs using RDP classifier as implemented in QIIME (Caporaso et al., 2010) using SILVA release S132 (Quast et al., 2013) as the reference database. Taxonomic classification of *mcrA* gene OTUs was assigned with UCLUST as implemented in QIIME using a manually curated custom database of 508 *mcrA* gene reference sequences collected from the FunGene repository (Fish et al., 2013).

Analyses of the acquired sequencing data was performed using R version 3.6.1 (R Development Core Team, 2020) via Rstudio version 1.2.5001 (https://www.rstudio.com), and the data were visualised using the ampvis2 package (Andersen et al., 2018). Microbial community composition was visualised using stacked bar plots and heatmaps. Alpha diversity was measured using the observed OTUs and Shannon-Weaver index (Shannon, 1948). Beta diversity was investigated using principal component analysis (PCA), in combination with the *envfit* function from the vegan package (Oksanen et al., 2016). Correlations between the identified microbes and operational parameters were generated using Spearman's correlations.

2.4. Data availability

Raw sequencing data has been made publicly available via the European Nucleotide Archive (ENA) under project accession number PRJEB37891.

3. Results

The bacterial community analysis yielded a total of 1,096,471 reads across 54 high quality samples with an average 20,305 ± 3,344 reads and a total of 3,653 uniquely identified OTUs. Distributed across 47 phyla and 519 genera, 658 OTUs made up at least 0.1 % of total read abundance in a sample. The methanogenic community analysis yielded a total of 243,610 reads across 18 samples containing 62 unique OTUs and an average 13,534 ± 2,733 reads per sample. Five methanogenic orders and 13 described genera were identified, and 47 of the identified OTUs were abundant (≥ 0.1 % relative abundance in at least one sample). Based on a rarefaction curve for the bacterial community (Fig. S1a) and methanogenic community (Fig. S1b) samples, all samples were deemed to be of sufficient quality for microbial community analysis.

3.1. Food waste AD microbial community diversity and composition

Diversity measurements of the surveyed reactors showed an inverse relationship between reactor temperature (RT) and bacterial community diversity (richness) (Tables 1 and 2). Microbial community richness is a measure for the number of different microorganisms (or OTUs) present in a given digester (Hugerth and Andersson, 2017), this measure has previously been suggested as a measure for stability in microbial ecosystems.

The highest bacterial diversity was measured in plant C (average 1,132 OTUs; RT = 37 °C) and the lowest in plant H (average 151 OTUs; RT = 55 °C). The relative abundance distribution of bacterial community members within each sample (evenness) was determined by scoring on the Shannon-Weaver index (Table 2), and a similar tendency was observed; much less evenly distributed bacterial communities were present in the reactors operated under thermophilic conditions. A notably higher richness and complexity was observed in reactors processing WWS with FW as a supplementary substrate (Plant B and C). A relationship between diversity and temperature was not immediately present in the methanogenic communities, although it is notable that the most diverse communities were observed in the three mesophilic plants processing a high percentage of WWS (Plant B, C and F). Overall diversity of methanogenic communities ranged from 13 to 43 OTUs observed per digester, and community evenness showed great

Table 1
Overview of operational characteristics of 18 full-scale biogas digesters.

Plant	# of reactors sampled	Operational temperature (°C)	Operation type	HRT (days)	pH (effluent)	Food waste processed (%)	$FeCl_2$ addition
A	3	37	Mesophilic	30	R1: 7.7	43	Yes
					R2: 7.7	43	
					R3: 7.8	43	
B	3	37	Mesophilic	21	7.6	8.5	No
C	3	37	Mesophilic	15	7	7	No
D	2	37	Mesophilic	45	7.5	77	Yes
E	1	42	Mesophilic	17	7.6	75	Yes
F	2	40	Mesophilic + CAMBI	R1: 21	7.7	35	No
				R2: 10		35	
G	2	58	Thermophilic	15	8	95	No
H	2	52	Thermophilic	35	8.1	74	Yes

Table 2
Microbial community richness and evenness of 18 full-scale digesters, by plant.

Bacterial community			Methanogenic community		
Plant	Observed OTUs	Shannon index	Plant	Observed OTUs	Shannon index
A (n = 9)	455 ± 91	3.71 ± 0.15	A (n = 3)	14 ± 3	0.27 ± 0.18
B (n = 9)	1090 ± 96	3.91 ± 0.27	B (n = 3)	43 ± 3	1.85 ± 0.16
C (n = 9)	1132 ± 65	5.38 ± 0.05	C (n = 3)	45 ± 1	2.33 ± 0.09
D (n = 6)	570 ± 80	4.29 ± 0.06	D (n = 2)	17 ± 3	1.85 ± 0.02
E (n = 3)	489 ± 18	3.83 ± 0.04	E (n = 1)	15	0.48
F (n = 6)	689 ± 15	4.03 ± 0.06	F (n = 2)	23 ± 0	1.63 ± 0.01
G (n = 6)	312 ± 177	1.96 ± 0.46	G (n = 2)	13 ± 2	0.95 ± 0.29
H (n = 6)	151 ± 5	2.27 ± 0.06	H (n = 2)	13 ± 0	1.18 ± 0.01

variability across sampled reactors (0.27 – 2.33). Samples originating from different reactors from the same plant, as well as their replications, were generally highly similar in observed richness and evenness.

The observed differences in richness and evenness across sampled reactors are also reflected in the microbial community structure (Fig. 2a).

Major contributing phyla to the mesophilic bacterial communities included *Firmicutes* (9.8 – 65.9 % of total reads), *Bacteroidetes* (0.3 – 37.6 %), *Cloacimonetes* (0.5 – 33.2 %) and *Chloroflexi* (0.6 – 28.4 %), which were observed in all reactors of this type. Organisms observed exclusively under mesophilic conditions included *Fastidiosipila*, *Cloacimonetes* genus W5, *Sedimentibacter*, *Christensenellaceae* R-7 group and *Rikenellaceae* RC9 gut group (Fig. S2). The *Proteobacteria* and *Actinobacteria* also contributed abundantly to mesophilic environments with a high WWS load (Plant B, C and F). In two plants (A and D) processing a high FW load, but no WWS, the phylum *Tenericutes* contributed to the abundant microbial community, and was detected only at very low abundance (< 0.5 %) in the other plants. The sampled thermophilic reactors were primarily colonised by representatives of the phyla *Thermotogae* (25.7 – 69.6 %), *Firmicutes* (27.6 – 61.4 %), and smaller contributions by *Bacteroidetes* (0.3 – 10.2 %) and *Synergistetes* (0.7 – 2.3 %). The abundantly colonising organisms included the *Firmicutes* family MBA03, and the genera *Defluviitoga* and *Anaerobaculum* (Fig. S2).

The largest contributor to the identified methanogenic communities (Fig. 2b) was observed to be affiliated with the family *Methanobacteriaceae*, which was abundantly present in all sampled reactors (7.6 – 98.9 %) and the dominant family in four of the surveyed plants. *Methanosphaera* and *Methanobacterium* were the most abundant genera in five mesophilic reactors (plant A and F) (Fig. S3), while *Methanothermobacter* was very abundant in both thermophilic participants (plant G and H). In plants A, E, G and H, a single methanogenic population was dominant, with the exception of one reactor in plant H, which was made up by two dominant populations (*Methanoculleus* and *Methanomassiliicoccus*). The acetoclastic methanogen *Methanosarcina* was the dominant methanogen (87.9 %) in plant E. Contributions from the family *Methanoregulaceae*, and the genera *Methanothrix*, *Methanospirillum* and "*Candidatus* Methanofastidiosum" were observed in plants B, C, D and F, which represented the most diverse methanogenic communities detected in this study.

3.2. Association of specific microbes to food waste and other substrate types

To investigate the association of specific microorganisms to digesters processing either food waste, wastewater sludge or mixed waste ($\leq$ 60 % of a single substrate), rank abundance curves were generated for the bacteria and methanogen data in order to estimate the abundant fraction of OTUs (the number of OTUs in order of abundance that collectively explain 90 % of total reads) for both datasets (data not shown). A total of 350 and 12 OTUs were extracted from the bacteria and methanogen dataset respectively and visualised as a ternary plot (Fig. 3). Microorganisms where $\geq$ 80 % of total abundance could be associated to a single substrate type were considered of interest; this yielded 58, 114 and 53 OTUs associated to digesters processing food waste, wastewater sludge and mixed waste, respectively.

Organisms associated to food waste digesters included representatives of *Ruminococcaceae*, *Cloacimonadaceae* group W5, *Syntrophomonadaceae*, *Tepidimicrobium* and *Methanothermobacter*, while *Proteiniphilum*, *Methanosphaera*, *Fastidiosipila* and *Tepidanaerobacter* were found to be associated to mixed waste reactors. Individual representatives of *Syntrophaceticus* were found to be associated with digesters processing either primarily food waste or mixed waste. Digesters processing wastewater were associated to representatives of *Anaerolineaceae*, *Smithella, Cloacimonadales* group W27, *Longlinea* and "*Candidatus* Methanofastidiosum".

3.3. Influence of operational parameters on microbial communities

A model generated by Principal Component Analysis (PCA) can be used to visualise differences between the analysed digesters (beta diversity) and the entire microbial community associated

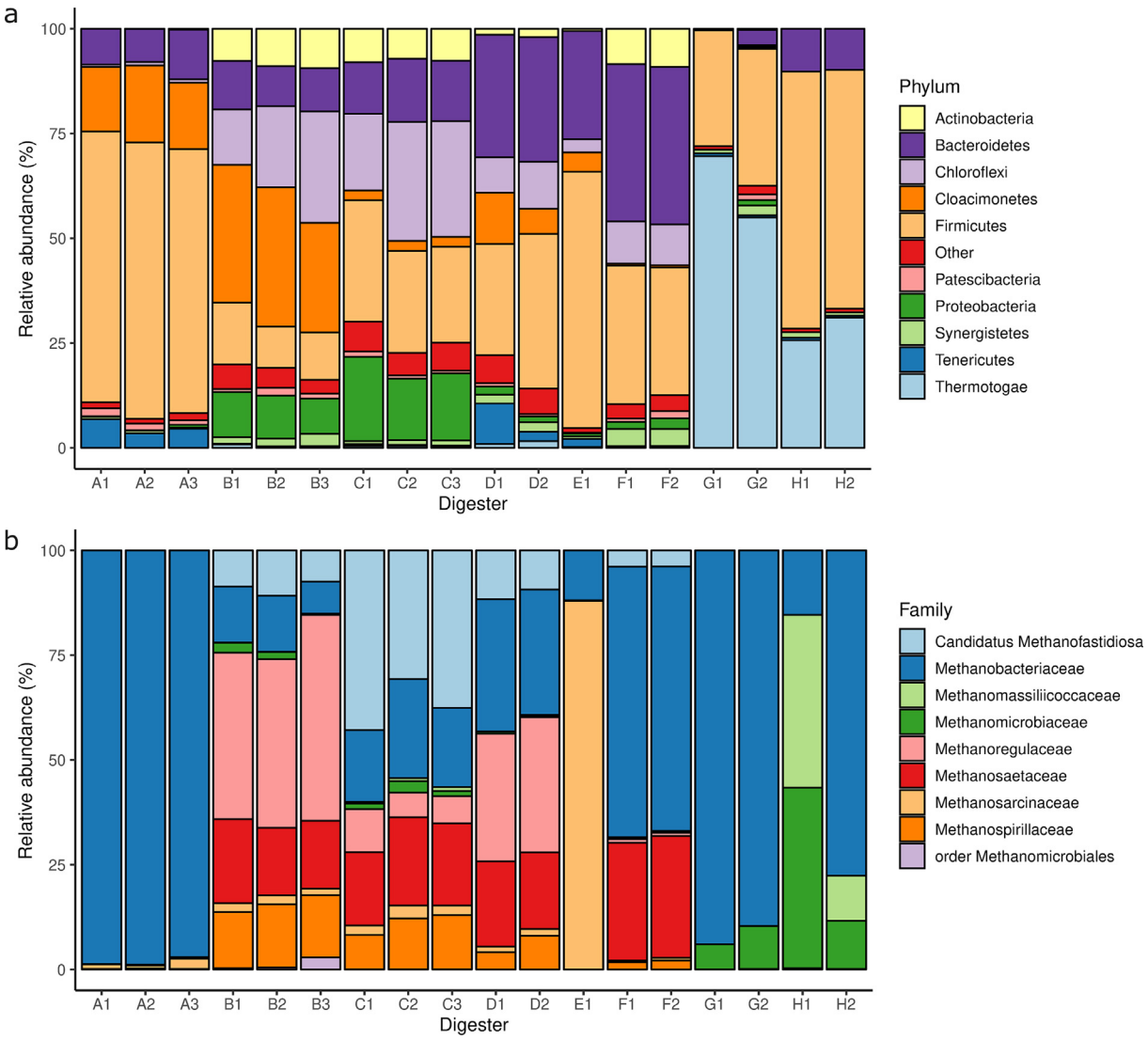

Fig. 2. Microbial community composition. The 10 most abundant phyla in the bacterial community (a) and 9 most abundant methanogenic families (b) observed in the surveyed full-scale reactors are displayed as a stacked bar plot. The remainder of the microbial communities is aggregated under the category "other".

to each reactor by distance. Principal components describe linear gradients of the variation between samples in order of magnitude. The PCA models generated for the ADs investigated in this study showed that many operational parameters such as temperature, fraction of WWS and pH had a strong influence on the composition of the microbial community (Fig. 4). Samples taken from different digesters at the same plant were highly similar in composition, and clustered tightly together.

The temperature had the greatest impact on the bacterial community, explaining up to 29.1 % of total variance in the dataset as a gradient along the horizontal axis (Fig. 4a), while RT was visible as a gradient on the diagonal for the methanogens (Fig. 4b) suggesting the presence of other variables with a significant impact on its composition. The effect size and directionality of different operational variables was tested using PERMANOVA (goodness of fit) and visualised using arrows within the PCA model. A secondary relationship was observed between the bacterial communities in the reactors processing a large amount of WWS relative to those processing FW and other waste types, including SH waste, as their primary substrate. The measured pH was also observed to be higher in the reactors with low amounts or no WWS processed, and in reactors run under thermophilic conditions. Goodness of fit testing of operational data to the PCA model revealed that most of the tested operational data parameters had an influence on the microbial community composition, with RT having a very good fit for both the bacterial and methanogenic communities (p = 0.001, R^2 > 0.89). The bacterial community observed in the sampled reactors also correlated strongly with the percentage FW (p = 0.001, R^2 = 0.74) and WWS (p = 0.001, R^2 = 0.90) processed in the reactors, and pH (p = 0.001, R^2 = 0.77), whereas the methanogenic community showed a strong relationship with the percentage miscellaneous waste types (p = 0.001, R^2 = 0.74) processed.

The presence of individual OTUs in the analysed reactors determine the placement of a digester within the PCA model, and their (Euclidian) distance from the centre of the model determines their effect size. OTUs with a large effect size in both PCA models were examined to assess association to investigated digesters. The thermophilic reactor samples clustered together with OTUs representing *Defluviitoga,* MBA03 and *Methanothermobacter*, while representatives of the genera *Fastidiosipila*, *Syntrophaceticus*, *Tissierella* and *Methanosphaera* were observed to group with mesophilic reactors processing a higher percentage FW and/or mixed

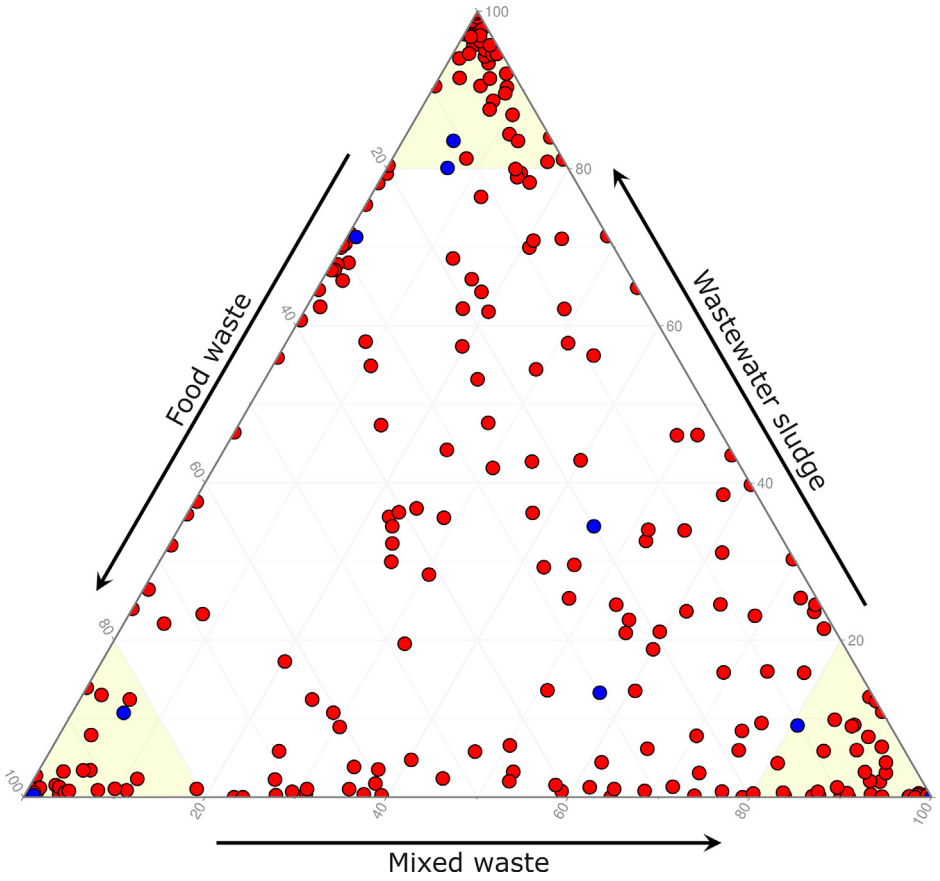

Fig. 3. Ternary plot of the abundant fraction of the bacteria (350 OTUs, red) and methanogens (12 OTUs, blue). Placement of the individual OTUs is displayed as a percentage (%) of total read abundance in each category, and a yellow polygon indicates microorganisms closely associated to a single substrate type.

waste types. Reactors processing primarily WWS grouped together with OTUs representing "*Candidatus* Cloacamonas", *Methanoregulaceae* and "*Candidatus* Methanofastidiosum".

3.4. Correlations between the presence of syntrophic and methanogenic communities in full-scale digesters processing food waste

Spearman's correlation analysis of the abundant known syntrophic and methanogenic microorganisms, as well as important operational parameters, revealed potential association profiles. The six most abundant methanogens and 9 abundant known syntrophs (*Aminobacterium*, *Gelria*, *Smithella*, *Syntrophaceticus*, *Syntrophomonas*, *Syntrophorhabdus* and *Tepidanaerobacter, Syntrophus, Syntrophobacter*) (Mosbaek et al., 2016; Müller et al., 2016; Narihiro et al., 2015) were included in this analysis (Fig. 5).

Highly positive correlation coefficients (Spearman's $\rho > 0.75$) were observed between different syntrophs and methanogenic populations; *Smithella*, *Syntrophobacter, Syntrophus* and *Syntrophorhabdus* associated with "*Candidatus* Methanofastidiosum", *Methanoregulaceae*, *Methanospirillum* and *Methanothrix* (only *Smithella*), and all showed a negative correlation to the presence of food waste. Positive correlations ($\rho > 0.4$) were also observed between organisms belonging to either population such as between *Tepidanaerobacter, Syntrophaceticus*, and *Aminobacterium* to a lesser degree ($\rho = 0.13$), and the presence of food waste as a substrate, while also highly negatively correlated to wastewater sludge. Highly negative correlations (Spearman's $\rho < -0.75$) were observed between *Tepidanaerobacter* and a number of methanogens, as well as to wastewater sludge as a substrate.

4. Discussion

The in-depth microbial community study of eighteen stable full-scale food waste AD systems with diverse operational characteristics examined in this study gives rise to several observations of practical relevance.

4.1. The microbial community of full-scale reactors processing food waste represent high diversity ecosystems with a large number of reactor specific organisms

The microbial communities observed in the surveyed reactors presented high diversity ecosystems with a large number of reactor specific organisms, especially among the identified methanogens. Organisms belonging to the phyla *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Spirochaetae* were abundant under mesophilic conditions and have previously been observed abundantly in studies of full-scale food waste AD under similar conditions (Guo et al., 2014; Li et al., 2015). The phyla *Thermotogae* and *Firmicutes* class *Clostridia* have consistently been reported in other full-scale AD studies in thermophilic FW digesters (Sundberg et al., 2013; Zamanzadeh et al., 2016). Mesophilic and thermophilic AD systems represent very different ecosystems in regards to which microorganisms thrive. Thermophilic temperatures and the associated chemical conditions during AD select for more specialised microbial populations (Weiland, 2010). The microbial communities identified in the reactors processing a high percentage of food waste as substrates are in line with previous studies in similar systems (Li et al., 2015; Zamanzadeh et al., 2016). Differences between proportions of abundant bacterial populations at a low taxonomic level

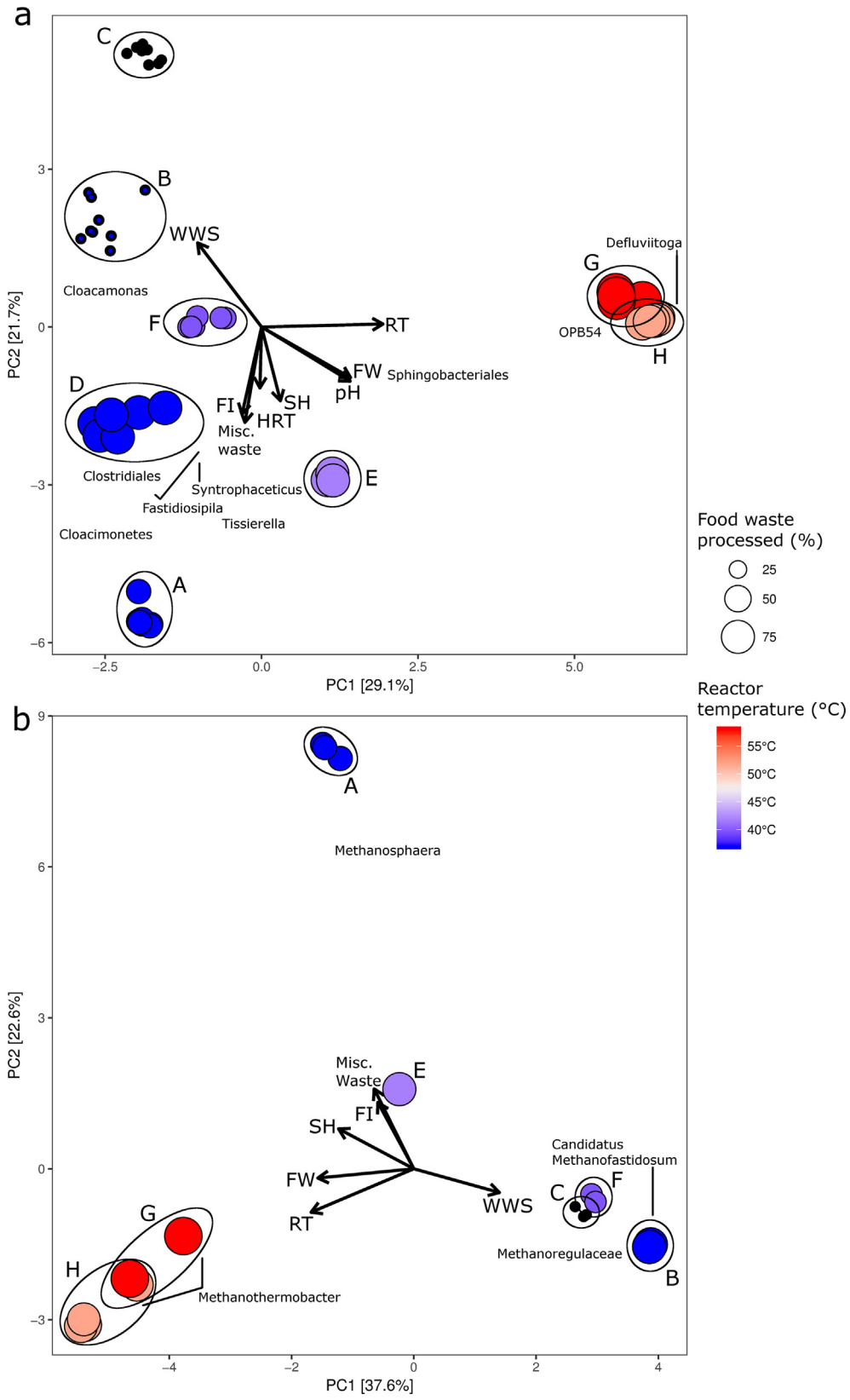

Fig. 4. Impact of operational condition on the microbial communities in food waste AD. Principal component analysis on square root transformed OTU abundances of bacterial (a) and methanogenic (b) communities. Points are coloured by operational temperature, and their size represents the percentage food waste processed. FW = food waste, WWS = wastewater sludge, FI = food industry waste, SH = slaughterhouse waste, RT = reactor temperature, HRT = hydraulic retention time.

appeared to be mostly plant specific and can partially be attributed to the influence of the substrate composition and confirms previous reports on the impact of substrate on the composition of the microbial community (De Vrieze et al., 2015). No apparent effect

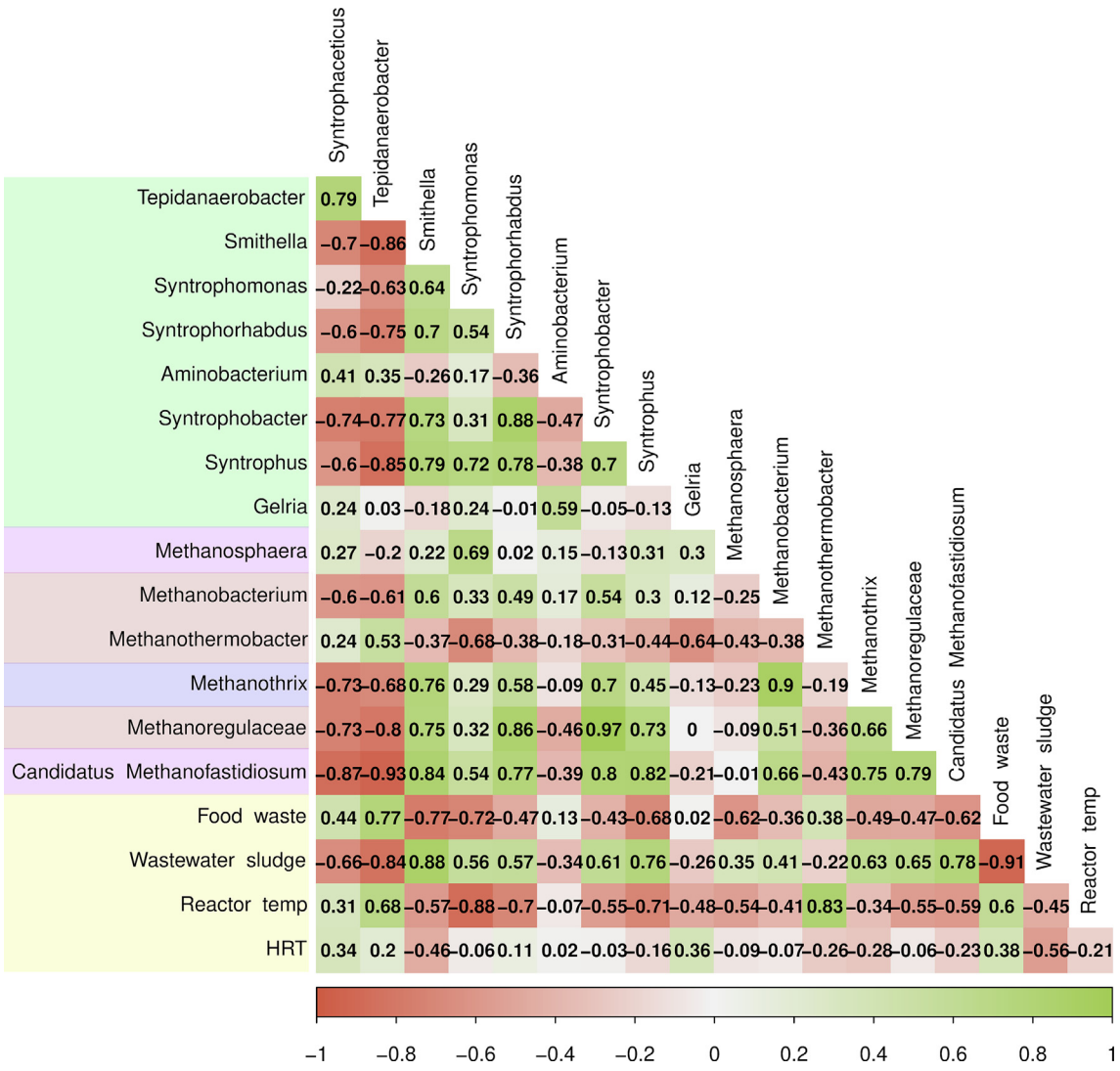

Fig. 5. Spearman's correlation matrix of syntrophic and methanogenic populations in food waste AD. Correlation coefficients are shown on the plot, microorganisms representing synthrophic acetate oxidising bacteria (SAOB) (green), acetoclastic (blue), hydrogenotrophic (red) and methylotrophic (purple) methanogenesis, as well as operational conditions (yellow) were included in the analysis.

of the inoculum source originally used for starting up the ADs studied herein were observed. Therefore the inoculum source was not considered to be an influential factor, which is in accordance with previous studies showing that different AD inocula are able to converge towards a similar microbial community structure when operated under the same conditions over a long period of time (Peces et al., 2018). However, for inoculating a new AD, it can be hypothesized that using an inoculum source that resembles the desired major influential factors on the microbial communities, such as similar substrate feeding regime, reactor temperature and pH will be advantageous. This will allow for an initial methane production without pre-adjustment of the microbial communities to the operational regime governing the new reactors.

The most distinct microbial communities were seen in digesters primarily processing WWS, compared to the other surveyed reactors. Previous studies have also reported distinct microbial community structures between digesters processing WWS and food industry wastewater (Lee et al., 2018), as well as significant influence from the influent primary and surplus sludge (Kirkegaard et al., 2017; Lee et al., 2018), suggesting a large degree of microorganisms simply "passing through", rather than immigrating into the system. This is in line with previous studies where a shared community was observed in activated sludge at wastewater treatment plants (Saunders et al., 2016), as well as digesters processing WWS (De Vrieze et al., 2015). Examination of the overlap between observed populations and their abundance between digesters processing a combination of FW, WWS or mixed waste substrates also revealed an overlap in observed organisms between FW and mixed waste, and WWS and mixed waste, but not FW and WWS, supporting the observation that these two substrates greatly differ in terms of associated microbes. The less distinct microbial communities in the food waste based digesters may suggest that this substrate contains populations with a less specialised substrate preference, partially due to the inherent degradability of food waste.

The diversity of observed methanogenic populations was generally lower for the reactors processing a large percentage of food waste, compared to reactors receiving a complex mixture of substrate types. A low diversity would generally indicate a more sensitive and less stable ecosystem. Most of the abundantly observed organisms in these populations belonged to *Methanobacteriaceae*, a hydrogenotrophic family of methanogens, with the exception of plant E, where *Methanosarcina* (capable of both AM and HM) was observed as the dominant methanogen. This could potentially be

explained by the higher nitrogen content of food waste and the resulting increased production of ammonia, which is less favourable for acetoclastic methanogens (Yenigün and Demirel, 2013). Plant E was characterised by a higher fraction of grease waste processed compared to the other reactors, and may have selected for *Methanosarcina* due to its ability to perform under harsh condition such as high ammonia and VFA concentrations (De Vrieze et al., 2012). The presence of hydrogenotrophic methanogens in the majority of the sampled digesters is supported by the observed increased diversity of known genera containing organisms capable of utilising the SAO pathway, namely *Syntrophaceticus* and *Tepidanaerobacter*, in these digesters. This pathway and related microorganisms have been shown to be upregulated in AD systems with high concentrations of ammonia (Müller et al., 2016), and are known to live in close symbiosis with hydrogenotrophic methanogens. Furthermore, distinct syntrophic populations including known syntrophs such as *Syntrophomonas* and *Aminobacterium* (Narihiro et al., 2015), were observed in the surveyed digesters and found to be affected by the composition of the primary substrate. Syntrophic populations have previously been hypothesised to have a highly important role in the maintenance and recovery of stable AD processes in a number of previous studies (Li et al., 2015; Wu et al., 2016).

A positive relationship was observed between the percentage of WWS in the substrate composition of the digesters and overall microbial richness. This is likely due to the high microbial diversity of WWS (primary and surplus) sludge (Kirkegaard et al., 2017). Previous studies have also reported a higher microbial community richness of WWS based digesters compared to those processing other substrate types, such as manures or co-digestion including food waste (De Vrieze et al., 2015), or food industry wastewater (Lee et al., 2018). Reactors processing substrates with a high proportion of WWS contained a more diverse representation of the phyla *Bacteroidetes* (specifically orders *Sphingobacterales* and *Flavobacteriales*), *Proteobacteria* and *Actinobacteria* compared to the other studied digesters. The recently proposed methanogenic candidate class *Methanofastidiosa* was also primarily observed in reactors receiving WWS. This could be indicative of a higher concentration of sulfur compounds in the reactor, as this methanogen is proposed to exclusively use methylated thiol reduction to achieve methanogenesis (Nobu et al., 2016).

A secondary correlation was apparent between co-digestion and overall evenness of the microbial community. More even distribution of abundant microorganisms in a mixed substrate digester could indicate that the greater diversity of biomasses and therefore microorganisms increases the number of functionally specialised organisms present in the system. Based on the observations that mixed substrate systems exhibit greater microbial diversity, it can be suggested that co-digestion of multiple substrates yields a more robust ecosystem compared to systems digesting individual substrates. Furthermore, it has also been suggested that selection of substrates for co-digestion allows for manipulation of the microbial diversity and composition, thereby affecting the stability and performance of the digester (Divya et al., 2015).

4.2. Temperature and fraction of WWS are the main drivers of the microbial communities in food waste AD

The primary parameters influencing the bacterial and methanogenic communities of the surveyed reactors included firstly RT, as well as the substrate types FW and fraction of WWS. Other substrates including slaughterhouse waste also influenced the microbial community, but to a lesser degree. Strict separation was observed between bacterial communities of the mesophilic and thermophilic reactors. Microbial AD ecosystems are primarily shaped by their operating temperature and nutrient availability, which in this study is represented by the substrate composition used to feed the reactors. These results are in line with those obtained by previous studies in full-scale AD systems (De Vrieze et al., 2015; Sundberg et al., 2013). The methanogenic community showed a strong correlation to most of the tested operational parameters and substrate type, and is likely influenced by environmental characteristics specific to the individual digesters (De Vrieze et al., 2015). Previous studies have also reported a complex combination of operational factors to have an influence on the methanogenic community structure (Lee et al., 2017). However, methanogenic populations associated specifically to municipal wastewater sludge digesters have previously been reported, suggesting that some substrates may select for specific methanogens more so than others (Kirkegaard et al., 2017).

4.3. Food waste promotes presence of diverse syntrophic populations

Spearman's correlation analysis of the abundant known syntrophic and methanogenic populations observed in the surveyed digesters revealed positive correlations in and between the two functional groups, based on primary substrate component. A strong positive correlation was observed between two described SAO populations (*Syntrophaceticus* and *Tepidanaerobacter*), and food waste as a primary substrate component. The results support the findings of Fig. 3, which showed specific presence of several organisms associated with syntrophy in digesters processing food waste, and to a lesser degree mixed waste, including *Syntrophaceticus*, *Tepidanaerobacter*, *Tepidimicrobium* and a representative of *Syntrophomonadaceae*. However, few positive associations were identified between hydrogenotrophic methanogens and SOAB. This could be explained by the limited number of described SAOB populations, and the exclusion of SAO representative *Clostridium ultunense*, which cannot be identified with conventional 16S rRNA gene amplicon sequencing due to insufficient classification by taxonomic databases. Instead, a number of positive associations between other syntrophic organisms and methanogens were present in the surveyed digesters. Furthermore, the drivers of methanogenic communities, their syntrophic partners and which pathways are utilised under which conditions are not yet well described. The presence of the genus *Smithella* was positively correlated to the presence of a number of methanogenic genera; this genus has been linked to syntrophic propionate degradation to acetate (Nelson et al., 2011), which can subsequently be utilised by acetoclastic methanogens such as *Methanothrix*.

Addition of food waste in co-digestion systems has previously been linked to increased robustness in AD when processed together with WWS, manures and other waste types (Tyagi et al., 2018), and it is likely that syntrophic populations immigrating from the FW significantly contribute to this effect. This is supported by previous studies where syntrophic populations have repeatedly been linked to process recovery from different disturbances, as well as general stability in food waste AD (Li et al., 2018). However, as food waste can also contain high concentrations of ammonia and be susceptible to acidification (Sheng et al., 2013; Tyagi et al., 2018), addition of food waste to existing AD systems needs to be carefully optimised to the co-digested substrates in order to obtain improved performance.

4.4. Strategy for utilisation of microbial community data for management of anaerobic digester systems processing food waste

This study presents information regarding the composition, drivers and presence of specialised microbial populations in digester systems processing food waste. These results add to our knowledge regarding the microbiome of food waste in AD, but in order to apply this data in the management of AD, observations from the

microbial ecosystem need to be translated into indicators and information that can be used in practice.

One clear example is that the PCA-model analysing the beta diversity of the digesters showed that it is possible to separate the microbial communities of individual reactors based on a range of operational parameters, including temperature, primary substrate component and pH (and therefore likely VFA and ammonia concentration as well). These strong relationships suggest that it is possible to use such models to investigate a given digester's microbial community similarity to an existing reference, for example to investigate adaptation to a change in substrate composition, or recovery from perturbation. The digesters included in the present study were all reported as operating under stable conditions, but previous studies have shown that digester systems that are disturbed by e.g. overloading or feed interruptions are able to (partially) recover their original composition after disruption (de Jonge et al., 2017; Regueiro et al., 2015). The results of the present study are supported by previous works which also showed that the microbial community of digester systems can cluster reactors based on e.g. temperature and ammonia (De Vrieze et al., 2015), and feedstock composition (Calusinska et al., 2018). These types of models could potentially be extended to assess a given digester's efficiency, and lead to a better understanding of how performance, operation and microbial ecosystem are interconnected on a more detailed level.

Due to the sample size of the present study, statistical strength of the analyses is relatively low, although the comparability of replications and similarity between digesters from the same plant did demonstrate a degree of robustness. Increased sampling of digesters as well as temporal studies of full-scale AD systems processing food waste are needed in order to develop highly robust models and expand the possibilities for the implementation of microbial management into operational procedures. However, the systematic survey of 18 digesters of commonly used operational strategies and substrate compositions revealed strong correlations and indicators that clearly demonstrate not only the potential, but also the applicability of microbial community data in AD system operation. Furthermore, as the sampled digesters were all reported to have been operated under stable conditions and performance over a long period of time, it can be assumed that the microbial communities present herein were representative of long term well-adapted AD microbiomes, thus strengthening the link between the microbial data and their ecological relevance despite a smaller sample size.

In summary, we recommend that the general strategies for application of microbial community data in FW AD management are useful. Firstly, microbial community data can serve as an additional performance parameter in parallel with those already in use, providing the digester is sampled regularly. Secondly, this type of monitoring can be used to establish a base line microbial community composition associated to stable operation with good performance, which can then be used to assess the presence of (un) wanted microorganisms and potentially an early warning system for disturbances based on microbial community change. Finally, monitoring of many different AD systems processing food waste will make it possible to generate models to reveal microbes and operational conditions that result in optimal AD system performance.

5. Conclusion

The present study aimed to characterise the composition of microbial community of 18 digesters processing food waste under diverse conditions. The observations were used to predict the drivers of the composition and to discuss how these can be used to improve the management of anaerobic digestion of food waste in the future.

Temperature was shown to be one of the primary drivers of the bacterial and methanogenic communities. The bacterial community composition was also strongly influenced by the presence of wastewater sludge in the substrate, while the methanogenic communities also had strong relationships with many other operational conditions. This suggests that methanogenic communities in digesters are controlled by combinations of parameters, and their composition is not as straightforward to predict. However, differences in methanogenic representation were observed between digesters processing mostly food waste, and those that did not. The highest overall microbial diversity was observed in digesters processing a large proportion of wastewater sludge, but a more diverse presence of operationally important syntrophic microorganisms was associated to the food waste and mixed waste reactors.

The results of the present study add to our knowledge of the microbiology associated with food waste AD, and also demonstrated the potential of using microbial community data to supplement operational monitoring by cataloguing differences in community structure based on operational conditions. Furthermore, the increased diversity of syntrophs in reactors processing mostly food waste or mixed waste suggests that amendment of food waste has potential for improving the robustness of existing AD systems as a supplementary substrate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the Danish Council for Strategic Research under the project Nomigas (grant number: 1305-00018B). The authors wish to thank Sara Johansson and Marc Lund Nielsen for their assistance in sample collection and laboratory work.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2020.07.047.

References

Agneessens, L.M., Ottosen, L.D.M., Voigt, N.V., Nielsen, J.L., de Jonge, N., Fischer, C.H., Kofoed, M.V.W., 2017. In-situ biogas upgrading with pulse H2 additions: The relevance of methanogen adaption and inorganic carbon level. Bioresour. Technol. 233, 256–263. https://doi.org/10.1016/j.biortech.2017.02.016.

Albertsen, M., Karst, S.M., Ziegler, A.S., Kirkegaard, R.H., Nielsen, P.H., 2015. Back to basics – The influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One 10,. https://doi.org/10.1371/journal.pone.0132783 e0132783.

Ali Shah, F., Mahmood, Q., Maroof Shah, M., Pervez, A., Ahmad Asad, S., 2014. Microbial ecology of anaerobic digesters: The key players of anaerobiosis 183752 Sci. World J., 1–21 https://doi.org/10.1155/2014/183752.

Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. Ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. bioRxiv 299537. 10.1101/299537

Bernstad, A., la Cour Jansen, J., 2012. Separate collection of household food waste for anaerobic degradation – Comparison of different techniques from a systems perspective. Waste Manage. 32, 806–815. https://doi.org/10.1016/j.wasman.2012.01.008.

Bernstad, A., la Cour Jansen, J., 2011. A life cycle approach to the management of household food waste - A Swedish full-scale case study. Waste Manage. 31, 1879–1896. https://doi.org/10.1016/j.wasman.2011.02.026.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Calusinska, M., Goux, X., Fossépré, M., Muller, E.E.L., Wilmes, P., Delfosse, P., 2018. A year of monitoring 20 mesophilic full-scale bioreactors reveals the existence of stable but different core microbiomes in bio-waste and wastewater anaerobic digestion systems. Biotechnol. Biofuels 11, 1–19. https://doi.org/10.1186/s13068-018-1195-8.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

Carballa, M., Regueiro, L., Lema, J.M., 2015. Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr. Opin. Biotechnol. 33, 103–111.

Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F., 2012. The effects of substrate pre-treatment on anaerobic digestion systems: A review. Waste Manage. 32, 1634–1650. https://doi.org/10.1016/j.wasman.2012.04.016.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.

Cheng, W., Chen, H., Yan, S.H., Su, J., 2014. Illumina sequencing-based analyses of bacterial communities during short-chain fatty-acid production from food waste and sewage sludge fermentation at different pH values. World J. Microbiol. Biotechnol. 30, 2387–2395. https://doi.org/10.1007/s11274-014-1664-6.

De Clercq, D., Wen, Z., Gottfried, O., Schmidt, F., Fei, F., 2017. A review of global strategies promoting the conversion of food waste to bioenergy via anaerobic digestion. Renew. Sustain. Energy Rev. 79, 204–221. https://doi.org/10.1016/j.rser.2017.05.047.

de Jonge, N., Moset, V., Møller, H.B., Nielsen, J.L., 2017. Microbial population dynamics in continuous anaerobic digester systems during start up, stable conditions and recovery after starvation. Bioresour. Technol. 232, 313–320. https://doi.org/10.1016/j.biortech.2017.02.036.

De Vrieze, J., Hennebel, T., Boon, N., Verstraete, W., 2012. Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. Bioresour. Technol. 112, 1–9. https://doi.org/10.1016/j.biortech.2012.02.079.

De Vrieze, J., Saunders, A.M., He, Y., Fang, J., Nielsen, P.H., Verstraete, W., Boon, N., 2015. Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome. Water Res. 75, 312–323. https://doi.org/10.1016/j.watres.2015.02.025.

Dhamodharan, K., Kalamdhad, A.S., 2014. Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. J. Environ. Chem. Eng. 2, 1821–1830. https://doi.org/10.1016/j.jece.2014.07.024.

Divya, D., Gopinath, L.R., Merlin Christy, P., 2015. A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. Renew. Sustain. Energy Rev. 42, 690–699. https://doi.org/10.1016/j.rser.2014.10.055.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Fisgativa, H., Tremier, A., Le Roux, S., Bureau, C., Dabert, P., 2017. Understanding the anaerobic biodegradability of food waste: Relationship between the typological, biochemical and microbial characteristics. J. Environ. Manage. 188, 95–107. https://doi.org/10.1016/j.jenvman.2016.11.058.

Fish, J.A., Chai, B., Wang, Q., Sun, Y., Brown, C.T., Tiedje, J.M., Cole, J.R., 2013. FunGene: The functional gene pipeline and repository. Front. Microbiol. 4, 1–14. https://doi.org/10.3389/fmicb.2013.00291.

Guo, X., Wang, C., Sun, F., Zhu, W., Wu, W., 2014. A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. Bioresour. Technol. 152, 420–428. https://doi.org/10.1016/j.biortech.2013.11.012.

Hugerth, L.W., Andersson, A.F., 2017. Analysing microbial community composition through amplicon sequencing: From sampling to hypothesis testing. Front. Microbiol. 8, 1–22. https://doi.org/10.3389/fmicb.2017.01561.

Kaza, S., Yao, L., Perinaz, B.-T., Van Woerden, F., 2018. What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050. Urban Development Series. World Bank, Washington, DC. 10.1596/978-1-4648 -1329-0

Kim, J.K., Oh, B.R., Chun, Y.N., Kim, S.W., 2006. Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. J. Biosci. Bioeng. 102, 328–332. https://doi.org/10.1263/jbb.102.328.

Kirkegaard, R.H., McIlroy, S.J., Kristensen, J.M., Nierychlo, M., Karst, S.M., Dueholm, M.S., Albertsen, M., Nielsen, P.H., 2017. The impact of immigration on microbial community composition in full-scale anaerobic digesters. Sci. Rep. 7, 9343. https://doi.org/10.1038/s41598-017-09303-0.

Lee, J., Kim, E., Han, G., Tongco, J.V., Shin, S.G., Hwang, S., 2018. Microbial communities underpinning mesophilic anaerobic digesters treating food wastewater or sewage sludge: A full-scale study. Bioresour. Technol. 259, 388–397. https://doi.org/10.1016/j.biortech.2018.03.052.

Lee, J., Shin, S.G., Han, G., Koo, T., Hwang, S., 2017. Bacteria and archaea communities in full-scale thermophilic and mesophilic anaerobic digesters treating food wastewater: Key process parameters and microbial indicators of process instability. Bioresour. Technol. 245, 689–697. https://doi.org/10.1016/j.biortech.2017.09.015.

Li, L., He, Q., Ma, Y., Wang, X., Peng, X., 2015. Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: Relationship between community structure and process stability. Bioresour. Technol. 189, 113–120. https://doi.org/10.1016/j.biortech.2015.04.015.

Li, L., Peng, X., Wang, X., Wu, D., 2018. Anaerobic digestion of food waste: A review focusing on process stability. Bioresour. Technol. 248, 20–28. https://doi.org/10.1016/j.biortech.2017.07.012.

Luton, P.E., Wayne, J.M., Sharp, R.J., Riley, P.W., 2002. The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148, 3521–3530. https://doi.org/10.1099/00221287-148-11-3521.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

Merlin Christy, P., Gopinath, L.R., Divya, D., 2014. A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renew. Sustain. Energy Rev. 34, 167–173. https://doi.org/10.1016/j.rser.2014.03.010.

Mosbaek, F., Kjeldal, H., Mulat, D.G., Albertsen, M., Ward, A.J., Feilberg, A., Nielsen, J. L., 2016. Identification of syntrophic acetate-oxidizing bacteria in anaerobic digesters by combined protein-based stable isotope probing and metagenomics. ISME J. 10, 2405–2418.

Müller, B., Sun, L., Westerholm, M., Schnürer, A., 2016. Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria. Biotechnol. Biofuels 9, 48. https://doi.org/10.1186/s13068-016-0454-9.

Narihiro, T., Nobu, M.K., Kim, N.K., Kamagata, Y., Liu, W.T., 2015. The nexus of syntrophy-associated microbiota in anaerobic digestion revealed by long-term enrichment and community survey. Environ. Microbiol. 17, 1707–1720. https://doi.org/10.1111/1462-2920.12616.

Nelson, M.C., Morrison, M., Yu, Z., 2011. A meta-analysis of the microbial diversity observed in anaerobic digesters. Bioresour. Technol. 102, 3730–3739. https://doi.org/10.1016/j.biortech.2010.11.119.

Nobu, M.K., Narihiro, T., Kuroda, K., Mei, R., Liu, W., Masaru Konishi Nobu, Takashi Narihiro, Kyohei Kuroda, R.M., W.-T.L., 2016. Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. ISME J. 10, 2478–2487. 10.1038/ismej.2016.33

Oksanen, A.J., Blanchet, F.G., Kindt, R., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Soly-, P., Stevens, M.H.H., Wagner, H., 2016. vegan: Community ecology package. R package version 2.3-3.

Peces, M., Astals, S., Jensen, P.D., Clarke, W.P., 2018. Deterministic mechanisms define the long-term anaerobic digestion microbiome and its functionality regardless of the initial microbial community. Water Res. 141, 366–376. https://doi.org/10.1016/j.watres.2018.05.028.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Development Core Team, R., 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Regueiro, L., Lema, J.M., Carballa, M., 2015. Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. Bioresour. Technol. 197, 208–216. https://doi.org/10.1016/j.biortech.2015.08.076.

Rui, J., Li, J., Zhang, S., Yan, X., Wang, Y., Li, X., 2015. The core populations and co-occurrence patterns of prokaryotic communities in household biogas digesters. Biotechnol. Biofuels 8, 158. https://doi.org/10.1186/s13068-015-0339-3.

Sahu, N., Sharma, A., Mishra, P., Chandrashekhar, B., Sharma, G., Kapley, A., Pandey, R.A., 2017. Evaluation of biogas production potential of kitchen waste in the presence of spices. Waste Manage. 70, 236–246. https://doi.org/10.1016/j.wasman.2017.08.045.

Saunders, A.M., Albertsen, M., Vollertsen, J., Nielsen, P.H., 2016. The activated sludge ecosystem contains a core community of abundant organisms. ISME J. 10, 11–20. https://doi.org/10.1038/ismej.2015.117.

Schnürer, A., Nordberg, Å., 2008. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci. Technol. 57, 735–740. https://doi.org/10.2166/wst.2008.097.

Sembera, C., Macintosh, C., Astals, S., Koch, K., 2019. Benefits and drawbacks of food and dairy waste co-digestion at a high organic loading rate: A Moosburg WWTP case study. Waste Manage. 95, 217–226. https://doi.org/10.1016/j.wasman.2019.06.008.

Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1145/584091.584093.

Sheng, K., Chen, X., Pan, J., Kloss, R., Wei, Y., Ying, Y., 2013. Effect of ammonia and nitrate on biogas production from food waste via anaerobic digestion. Biosyst. Eng. 116, 205–212. https://doi.org/10.1016/j.biosystemseng.2013.08.005.

Shi, X., Guo, X., Zuo, J., Wang, Y., Zhang, M., 2018. A comparative study of thermophilic and mesophilic anaerobic co-digestion of food waste and wheat straw: Process stability and microbial community structure shifts. Waste Manage. 75, 261–269. https://doi.org/10.1016/j.wasman.2018.02.004.

Sieber, J.R., McInerney, M.J., Gunsalus, R.P., 2012. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Annu. Rev. Microbiol. 66, 429–452. https://doi.org/10.1146/annurev-micro-090110-102844.

Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., Sørensen, S.J., Karlsson, A., 2013. 454 pyrosequencing analyses of bacterial and

archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol. Ecol. 85, 612–626. https://doi.org/10.1111/1574-6941.12148.

Tyagi, V.K., Fdez-Güelfo, L.A., Zhou, Y., Álvarez-Gallego, C.J., Garcia, L.I.R., Ng, W.J., 2018. Anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW): Progress and challenges. Renew. Sustain. Energy Rev. 93, 380–399. https://doi.org/10.1016/j.rser.2018.05.051.

Uçkun Kiran, E., Trzcinski, A.P., Ng, W.J., Liu, Y., 2014. Bioconversion of food waste to energy: A review. Fuel 134, 389–399. https://doi.org/10.1016/j.fuel.2014.05.074.

Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849–860. https://doi.org/10.1007/s00253-009-2246-7.

Wu, B., Wang, X., Deng, Y.Y., He, X.L., Li, Z.W., Li, Q., Qin, H., Chen, J.T., He, M.X., Zhang, M., Hu, G.Q., Yin, X.B., 2016. Adaption of microbial community during the start-up stage of a thermophilic anaerobic digester treating food waste. Biosci. Biotechnol. Biochem. 80, 2025–2032. https://doi.org/10.1080/09168451.2016.1191326.

Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochem. 48, 901–911. https://doi.org/10.1016/j.procbio.2013.04.012.

Yun, Y.-M., Cho, S.-K., Kim, H.-W., Jung, K.-W., Shin, H.-S., Kim, D.-H., 2015. Elucidating a synergistic effect of food waste addition on the enhanced anaerobic digestion of waste activated sludge. Korean J. Chem. Eng. 32, 1542–1546. https://doi.org/10.1007/s11814-014-0271-4.

Zamanzadeh, M., Hagen, L.H., Svensson, K., Linjordet, R., Horn, S.J., 2016. Anaerobic digestion of food waste - Effect of recirculation and temperature on performance and microbiology. Water Res. 96, 246–254. https://doi.org/10.1016/j.watres.2016.03.058.

Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sustain. Energy Rev. 38, 383–392. https://doi.org/10.1016/j.rser.2014.05.038.

Zhang, C., Xiao, G., Peng, L., Su, H., Tan, T., 2013. The anaerobic co-digestion of food waste and cattle manure. Bioresour. Technol. 129, 170–176. https://doi.org/10.1016/j.biortech.2012.10.138.