

Article

Biogas Production and Microbial Communities of Mesophilic and Thermophilic Anaerobic Co-Digestion of Animal Manures and Food Wastes in Costa Rica

Mariana Murillo-Roos ¹, Lorena Uribe-Lorío ², Paola Fuentes-Schweizer ³, Daniela Vidaurre-Barahona ², Laura Brenes-Guillén ², Ivannia Jiménez ³, Tatiana Arguedas ¹, Wei Liao ⁴  and Lidieth Uribe ^{1,*}

¹ Centro de Investigaciones Agronómicas (CIA), Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José 11501-2060, Costa Rica; mariana.murillo.roos@uni-jena.de (M.M.-R.); tatiana.arguedas@gmail.com (T.A.)

² Centro de Investigación en Biología Celular y Molecular (CIBCM), Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José 11501-2060, Costa Rica; lorena.uribe@ucr.ac.cr (L.U.-L.); daniela.vidaurre@ucr.ac.cr (D.V.-B.); laura.brenesguillen@ucr.ac.cr (L.B.-G.)

³ Centro de Electroquímica y Energía Química (CELEQ), Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San José 11501-2060, Costa Rica; paola.fuentes@ucr.ac.cr (P.F.-S.); ivannia_jimenez@baxter.com (I.J.)

⁴ Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA; liaow@msu.edu

* Correspondence: lidieth.uribe@ucr.ac.cr



Citation: Murillo-Roos, M.; Uribe-Lorío, L.; Fuentes-Schweizer, P.; Vidaurre-Barahona, D.; Brenes-Guillén, L.; Jiménez, I.; Arguedas, T.; Liao, W.; Uribe, L. Biogas Production and Microbial Communities of Mesophilic and Thermophilic Anaerobic Co-Digestion of Animal Manures and Food Wastes in Costa Rica. *Energies* **2022**, *15*, 3252. <https://doi.org/10.3390/en15093252>

Academic Editor: Attilio Converti

Received: 21 February 2022

Accepted: 5 April 2022

Published: 29 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Biomass generated from agricultural operations in Costa Rica represents an untapped renewable resource for bioenergy generation. This study investigated the effects of two temperatures and three mixture ratios of manures and food wastes on biogas production and microbial community structure. Increasing the amount of fruit and restaurant wastes in the feed mixture significantly enhanced the productivity of the systems (16% increase in the mesophilic systems and 41% in the thermophilic). The methane content of biogas was also favored at higher temperatures. Beta diversity analysis, based on high-throughput sequencing of 16S rRNA gene, showed that microbial communities of the thermophilic digestions were more similar to each other than the mesophilic digestions. Species richness of the thermophilic digestions was significantly greater than the corresponding mesophilic digestions ($F = 40.08$, $p = 0.003$). The mesophilic digesters were dominated by Firmicutes and Bacteroidetes while in thermophilic digesters, the phyla Firmicutes and Chloroflexi accounted for up to 90% of all sequences. Methanosarcina represented the key methanogen and was more abundant in thermophilic digestions. These results demonstrate that increasing digestion temperature and adding food wastes can alleviate the negative impact of low C:N ratios on anaerobic digestion.

Keywords: biogas; chicken litter; manure; fruit and vegetable wastes; restaurant wastes; bacteria; archaea

1. Introduction

Central America contains the largest share of renewable energy sources (approximately 56% of the total energy generation in the region) and the most diverse mixture of renewable energy generation [1]. In Costa Rica, 80% of its electrical energy originates from hydroelectric power generation. However, merely 3% (less than 2.2 MW) of the total electrical energy is produced from biomass, compared to the vast amount of annual biomass production from Costa Rica's agriculture sector (approximately 7-million dry tons per year of organic residues including, manures, crop residues, and food wastes with a potential of 600 MW electricity generation) [2,3]. Development and implementation of environmentally friendly and economically sound technologies to utilize the residues for bioenergy production would help the region diversify its renewable energy profile, and

increase access to affordable clean energy, and reduce negative impacts of the organic wastes. Moreover, upgrading biogas to renewable natural gas could enable biogas as a source for biofuel production [4,5], which is urgently needed by the transportation sector in Central America.

Anaerobic digestion (AD) is a biological conversion process that effectively converts wet organic wastes into biogas capable of producing relatively clean electricity while also alleviating many of the environmental concerns associated with waste disposals such as odor, water pollution, and greenhouse gas emission. The entire AD process involves four key stages of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which is viewed as a metabolic cascade [6,7]. Several groups of microorganisms and enzymes such as cellulolytic, acidogenic bacteria, acetogens, and methanogens are involved in the anaerobic digestion process. Large and complex polymers in organic wastes (i.e., carbohydrates, proteins, and lipids) are first hydrolyzed into monomers (i.e., sugars, amino acids, and fatty acids) that are readily available for other microbes. The monomers from the hydrolysis stage are broken down into volatile fatty acids, ammonia, carbon dioxide, hydrogen sulfide, and other simple components by acidogenic bacteria. The simple molecules are then digested by acetogens to produce acetic acid and hydrogen as well as more carbon dioxide. Finally, methanogens convert acetic acid and hydrogen into methane, carbon dioxide, and water.

Numerous studies have been conducted to understand changes in microbial communities during the process of anaerobic co-digestion. Many factors including feedstock composition, reactor configuration, and operation conditions (temperature, pH, hydraulic retention time, and mixing) influence microbial communities [8,9]. Among them, feedstock composition and temperature are two critical parameters for establishing anaerobic microbial communities to carry out healthy anaerobic digestion. It has been reported that microbial communities from different anaerobic digestion units share several core operational taxonomic units (OTUs) despite different feedstock and digestion conditions [10]. According to several studies, the bacterial phyla Chloroflexi, Proteobacteria, Bacteroidetes, and Firmicutes, and the archaeal phylum Euryarchaeota dominate the digestion process [11–13]. These core microbes with their unique metabolic characteristics are capable of adjusting their abundances to fulfill the metabolic cascade of anaerobic digestion corresponding to feedstock variation [9,13–15]. Temperature also influences the growth rate and community configuration of anaerobic microbes [16–19]. Mesophilic digestion (temperature ranging from 30 to 38 °C) presents a more popular technology compared to thermophilic digestion (temperature ranging from 49 to 57 °C) since it has lower process energy demands (less heat needed) and better microbial stability. Higher temperatures and worse microbial stability significantly increase the cost of implementing thermophilic digestion to treat biomass [20,21]. Nevertheless, thermophilic digestion can increase the growth rate of anaerobic microbes, produce biogas with high methane and low hydrogen sulfide contents, reduce the hydraulic retention time, and suppress the growth of pathogens and weed seeds [22,23].

The objective of this study was to investigate the effects of digestion temperature and mixtures of agricultural residues on co-digestion performance and describe the corresponding responses of microbial communities.

2. Materials and Methods

2.1. Feedstocks

Dairy manure (cow excreta) was collected from the Dairy Facility of the University of Costa Rica located at Cartago (9°54'8.66" N, 83°40'14.62" W). Chicken litter was collected from the Experimental Farm Fabio Baudrit of the University of Costa Rica, located at Alajuela (10°0'26.23" N, 84°15'57.35" W). Fruit/vegetable wastes and post-consumer food wastes were collected from two cafeterias in San Jose, near the University of Costa Rica. The characteristics of each material are presented in Table 1. The feedstocks were mixed at dry matter percentage ratios of 50:50:0:0 (FM1), 45:45:5:5 (FM2), and 40:40:10:10 (FM3) for dairy manure, chicken litter, fruit wastes, and restaurant wastes, respectively (Table 1).

All mixtures were diluted to a final total solids (TS) concentration of 5% to carry out the co-digestion. The mixtures were prepared every two weeks and kept at 4 °C to feed the bench-scale bioreactors.

Table 1. Characterization of the substrates used in the experiment.

Parameter *	Dairy Manure	Chicken Litter	Fruits and Vegetables	Post-Consumer Food Wastes
Total solids (%)	13.2	85	8.3	33.6
C (%TS)	36.9	38.9	42.0	45.7
N (%TS)	2.3	3.9	2.6	3.3
C/N ratio	16.0	9.9	16.2	13.8
Glucan (%TS)	14.7	25.3	18.6	55.9
Xylan (%TS)	12.6	9.4	7.5	2.9
Lignin (%TS)	27.3	6.79	21.8	11.4

* %TS: fiber components expressed as a percentage of total solids.

2.2. Design and Operation of Bioreactors

The bioreactors consisted of glass bottles (1.0 L, working volume 0.5 L). The bottles were sealed with a rubber stopper, which were connected on one side to a water displacement unit that allowed the measurement of biogas volume (Figure 1). Biogas samples were collected using SKC Quality Sample Bags (Valley View Road, PA, USA) for gas composition analysis.

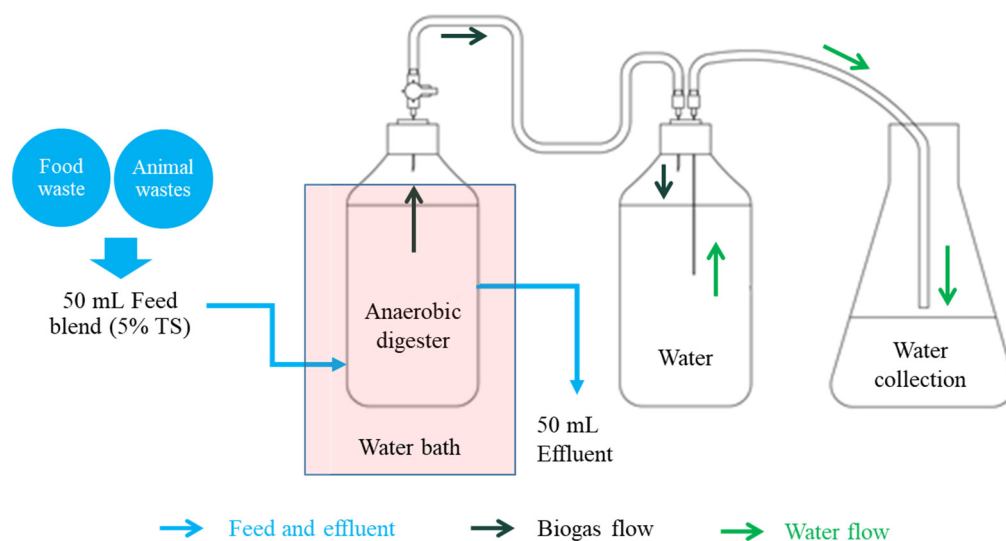


Figure 1. Setup of the bioreactor.

Twelve bioreactors (three mixture ratios with two replicates, each mixture ratio under two temperatures) were placed in two water baths that maintained digestion temperatures of 35 °C and 50 °C, respectively. The bioreactors were fed every other day with 50 mL of the prepared mixture with 5% TS. Prior to the feeding, 50 mL samples of the digestate were removed from individual bioreactors. The pH was adjusted to 6.8 by dosing 20% sodium hydroxide (NaOH) after the feeding. The hydraulic retention time (HRT) was 20 days. The anaerobic co-digestion was run over a period of five HRTs. The operations of sampling, feeding, and pH adjustment were carried out using a Spilfyter “Hands-in-bag” (NPScorp, Green Bay, WI, USA) that was purged with nitrogen gas to create an anaerobic environment.

2.3. Analytical Methods

Volatile fatty acids were measured using a gas chromatograph (GC) (Shimadzu GC-8 A, equipped with an analytical column ECONO—CAP EC-WAX of 30 m × 0.25 mm ID × 0.25 μm and FID detector). The oven temperature was set at 200 °C. The temperatures

of the detector and injector were set at 280 °C. Air pressure was set at 35 kPa. Hydrogen, nitrogen, and auxiliary gases were set at 100 kPa. Fatty acids in the samples were extracted by diethyl ether and then injected into the GC. Hexanoic acid was used as an internal standard.

The biogas composition was quantified using a gas chromatograph (HP 6800) coupled with a Carboxen -1010 PLOT, 30 m × 0.53 µm I.D (25467) column and a thermal conductivity detector (TCD). The chromatograph was operated with a temperature ramp from 100 °C for 4 min and then at 15 °C/min to 200 °C. The injector and detector temperatures were 200 and 230 °C, respectively. Nitrogen gas was used as a carrier gas at a flow rate of 4.0 mL min⁻¹. Certified standard gas (Agilent Technologies, type refinery gas, capacity 1 L, pressure 30 psig at 21 °C, batch number 112PLU1SPC10D) served as a standard.

TS, volatile solids (VS), and fixed solids (FS) were determined weekly according to the standard methods for the examination of water and wastewater [24]. Carbon and nitrogen were quantified via the Dumas dry combustion method [25,26]. The fiber composition of the digestate was analyzed using the protocol from National Renewable Energy Laboratory [27]. This procedure consists of a two-step acid hydrolysis, using H₂SO₄ to convert biomass into simpler compounds. During hydrolysis, monomers remain in the liquid fraction and are quantified by high-performance liquid chromatography (Shimadzu Prominence, Kyoto, Japan) using D-(+) glucose, D-(+) xylose, D-(+) galactose, L-(+) arabinose, and D-(+) mannose standards. The HPLC was equipped with a Biorad Aminex HPX-87H column and a refractive index detector; sulfuric acid (0.005 mol L⁻¹) was used as mobile phase at a flow rate of 0.6 mL/min and the column temperature was set at 65 °C. Lignin was separated into soluble and insoluble material; the former was determined by UV/VIS spectroscopy and, the latter by gravimetric analysis.

2.4. DNA Extraction

Genomic DNA was extracted from the digestate samples under the steady state of the digestion (weeks 12th and 15th) using a DNA extraction kit (Nucleospin Soil II Genomic, Macherey-Nagel). Cell disruption was performed using a mini-bead beater (Biospec Products) for 60 s at 4200 oscillations min⁻¹. The extracted DNA was examined using a 1% agarose gel and quantified using a NanoDrop spectrophotometer (ND-1000 Thermo Scientific). All DNA extracts were stored at -70 °C.

2.5. Microbial Community Analysis

DNA samples were sent to Macrogen Inc. (Seoul, Korea) for sequencing and establishing the 16S rRNA gene NGS libraries. Illumina MiSeq platform was used to perform the sequencing. A forward primer 5'-TCGTCGGCAGCGTCAGATGTGTAT AAGAGACAGCCT ACGGG-NGGCWGCAG-3' and a reverse primer = 5'GTCTCGTGGGCTCGGAGATGTGTAT-AAGAGACAGGACTACHVGGGTATCTAATCC-3' were used as 16S Illumina sequencing primers to amplify the V3-V4 region of 16S rRNA genes. In total, 987,057 tags were obtained. The raw tag-sequences were processed with QIIME (version 1.9.1) [28]. The process is briefly described as follows. Readings were first trimmed and filtered, and then assigned to a corresponding sample. The filtering criteria included homopolymers at least 100 bp in length and an average minimal quality score of 19. A representative sequence was selected for each OTU. To identify chimeras, the data set was processed using usearch61. A taxonomy assignment was delineated using QIIME to search the representative sequences of each OTU against the SILVA 16S/18S rDNA non-redundant reference data set (SSURef 132 NR) [29,30]. Chloroplast sequences and OTUs with small abundance were removed from the output table. OTUs with relative abundance ≥ 0.02% across all samples were used for the following statistical and phylogenetic analyses. To determine the core community in all anaerobic reactors (100% prevalence), we counted the read abundance of all OTUs. Sequences from 55 core communities were submitted to the NCBI GenBank database under accession numbers MK912176-MK912244. For diversity analysis of microbial communities, Bray-Curtis similarity values were calculated from the square-root-transformed data. Chao

and Shannon diversity (H') were estimated with QIIME [28]. For Shade plot analysis, we used PRIMER v.7 software [31]. For the phylogenetic analysis, the sequences were aligned using Muscle [32] in MEGA 6 [33]. Evolutionary distances were calculated with Bayesian inference [34]. Bootstrap was used to evaluate the tree topology on performing 10,000,000 resamplings and was shown for branch nodes supported by more than 50% of the trees. Reference GenBank sequences were used to illustrate the relation of sequences to known taxa.

2.6. Statistical Analysis

Biogas productivity and methane content were compared between treatments via a two-way analysis of variance (ANOVA), followed by Tukey multiple comparisons when significant differences were observed. Statistical analyses of these two variables and the corresponding plots were conducted in R (version 3.4.3, R Core Team, 2020).

3. Results

3.1. Feedstock Composition

The characteristics of feedstocks are shown in Table 1. Among four feedstocks, post-consumer food wastes had the highest glucan content (56%), dairy manure had the highest contents of xylan (13%) and lignin (27%). Regarding the C:N ratio, chicken litter exhibited the lowest value, while dairy manure and fruits and vegetables displayed the highest ratio. It has been widely reported that the C:N ratio plays a critical role in anaerobic digestion [35–37]. Substrates with high C:N ratios cause accumulation of VFA and decrease the pH of the systems, whereas substrates with low C:N ratios lead to carbon deficiency and ammonia accumulation, which consequently inhibits the metabolism of methanogens for methane production [37]. It has also been reported that the digestion of animal wastes does not show significant inhibition with C:N ratios as low as 13 [38]. Therefore, considering the C:N ratio of individual feedstocks, a median number of 13 was selected to formulate three feedstock mixtures for the study (Tables 1 and 2).

Correspondingly, the mixture with a higher percentage of manure (FM1) had a higher lignin content and lower glucan. On the other hand, FM3 had a higher content of glucan and less lignin (Table 2).

3.2. Digestion Performance

Volatile fatty acid (VFA) concentrations of all digesters varied significantly during the first weeks and maintained high levels of approximately 5000 mg/L (Figure 2). During this period, NaOH had to be used to maintain pH of the digestion around 7.0 [39,40].

The methane contents in all reactors were very low during this period (Figure 3b), which coincides with other studies [40,41]. The average values were 31% and 39% for the mesophilic and thermophilic reactors, respectively. After 10 weeks, the total VFA concentrations in all feedstock mixtures under both temperature conditions stabilized, indicating the systems had reached the steady state (Figure 2, Table 2).

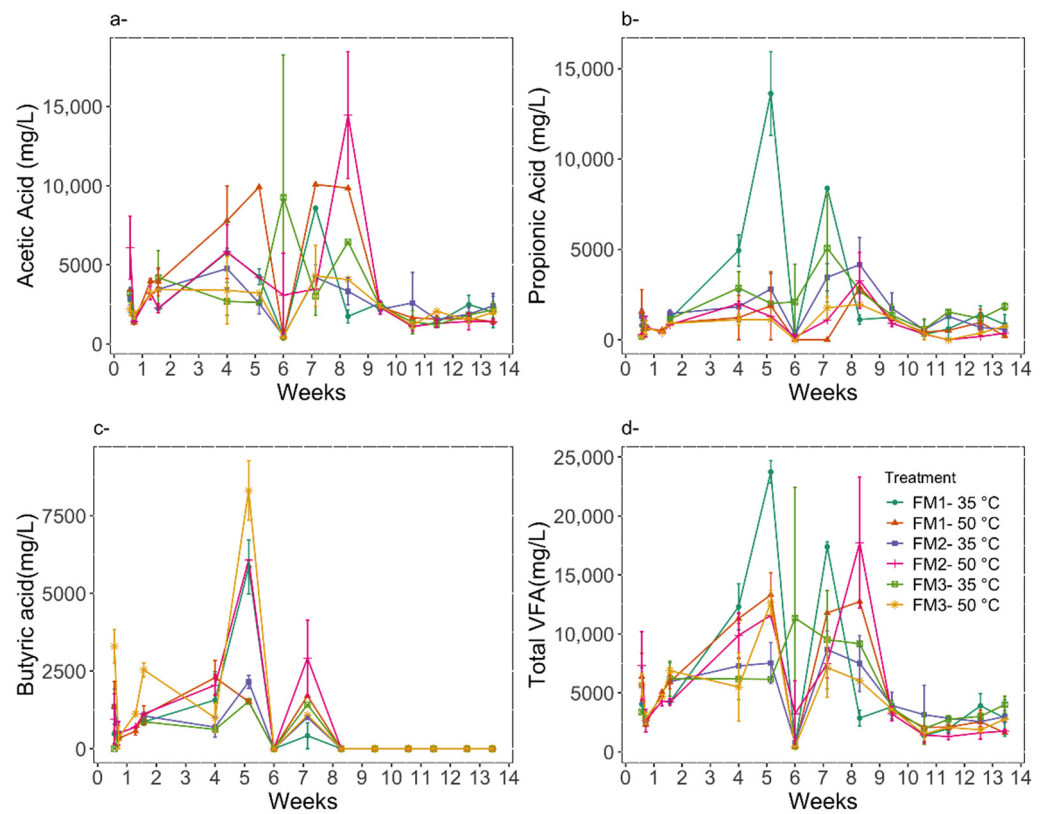


Figure 2. Volatile fatty acid (VFA) concentrations throughout the experiment. (a) Acetic acid. (b) Propanoic acid. (c) Butyric acid. (d) Total VFA. Each data point is the average of two replicates and error bars show the standard error.

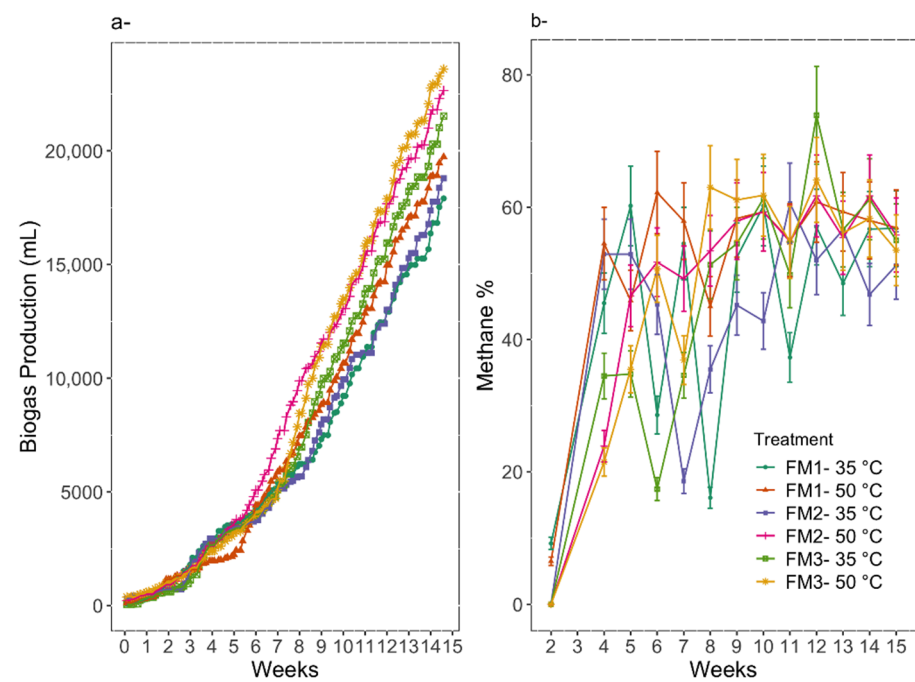


Figure 3. (a) Accumulated biogas production throughout the assay. (b) Methane content in the biogas, determined weekly. Each data point is the average of two replicates. Error bars show the standard error.

Table 2. Average volatile fatty acids during the last five weeks of the assay under the steady state (after ten weeks).

Treatment	Acetic Acid (mg/L)	Propanoic Acid (mg/L)	Butyric Acid (mg/L)	Total VFA (mg/L)
FM1 50 °C	3069 ± 919	1019 ± 284	0	4089 ± 1186
FM2 50 °C	3666 ± 1544	840 ± 391	0	4506 ± 1924
FM3 50 °C	2205 ± 282	768 ± 200	0	2974 ± 465
FM1 35 °C	1879 ± 227	917 ± 170	0	2796 ± 373
FM2 35 °C	2326 ± 331	1500 ± 445	0	3826 ± 680
FM3 35 °C	2568 ± 548	1532 ± 226	0	4099 ± 740

Data displays an average of two replicates with standard error.

The biogas production and methane content significantly increased in all digestions during this stage (Figure 3). The conversion of organic matter into biogas was more efficient in the digestion of FM3 under both temperatures ($p < 0.0001$) (Figures 3a and 4a). In contrast, biogas production in FM1 might have been affected by inhibition of high NH_3 content in poultry manure [42,43]. It has been widely reported that co-digestion can increase digestible organic matters, alleviate inhibition, and balance the C:N ratio, and consequently enhance digestion performance [44–48]. Meanwhile, temperature also impacted the digestion. Thermophilic conditions significantly increased biogas production ($p = 0.0247$), especially in FM2 and FM3 (Figure 4a). Under thermophilic conditions, FM2 and FM3 had average biogas productivities of 461 and 560 m^3 biogas/ton VS/day, respectively, which were significantly higher than those obtained in the mesophilic systems (390 and 472 m^3 biogas/ton VS/day) (Figure 4a). Methane content further confirmed the impact of temperature on digestion. Overall, the methane content was higher in thermophilic systems (58.2%) than in mesophilic conditions (54.6%) ($p = 0.0169$). This trend was clearer in the digesters with none or lower contents of fruit/vegetable wastes and post-consumer food wastes (Figure 4b). This result is consistent with a literature report that found high digestion temperatures could overcome the inhibition of digestion with low C:N ratios [49].

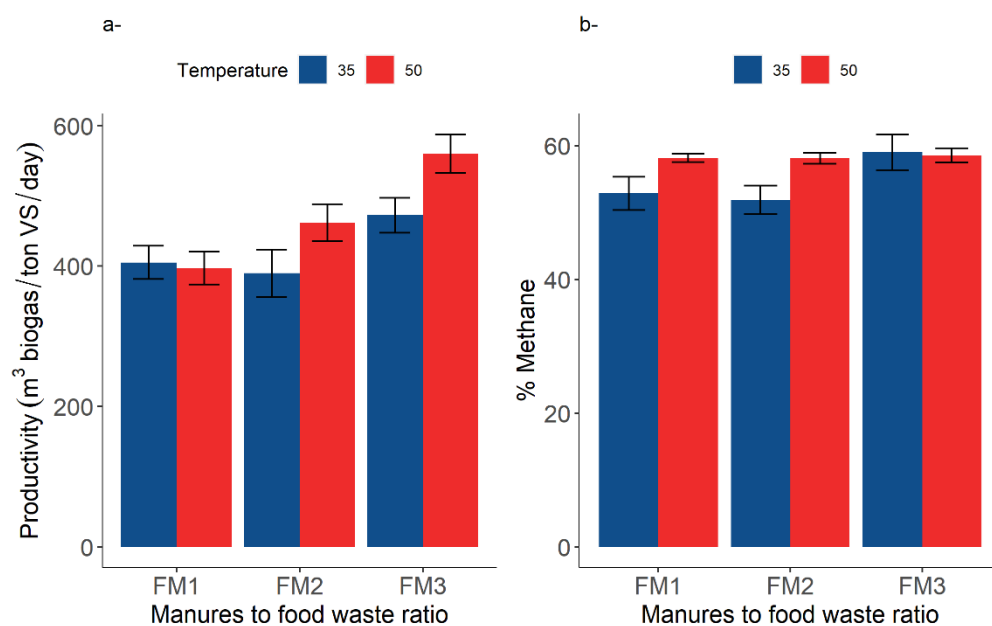


Figure 4. (a) Average biogas productivity (m^3 biogas/ton VS/day) during the last five weeks of the experiment, corresponding to the steady state. (b) Average methane content in the biogas during the steady state.

All digesters showed an average of 50% reduction in total and volatile solids (Tables 2 and 3), similar to other co-digestion studies [44,45]. Compared to the composition of feed mixtures (Table 2), thermophilic co-digestion displayed slightly better performance on VS, glucan, and xylan reduction than mesophilic co-digestion, which is consistent with literature reports [50,51].

Table 3. Fiber composition of the digestate at the end of the experiment.

Treatment	Total Solids (%)	Volatile Solids (%)	Glucan (%TS)	Xylan (%TS)	Lignin (%TS)
FM1 50 °C	2.9 ± 0.04	2.1 ± 0.04	10.7 ± 0.5	9.2 ± 0.2	28.5 ± 0.7
FM2 50 °C	2.6 ± 0.04	1.9 ± 0.04	10.9 ± 0.2	8.7 ± 0.04	27.8 ± 0.5
FM3 50 °C	2.5 ± 0.1	1.8 ± 0.1	14.0 ± 0.5	9.9 ± 0.1	27.1 ± 0.7
FM1 35 °C	2.9 ± 0.04	2.2 ± 0.04	10.8 ± 0.7	9.0 ± 0.5	28.8 ± 0.1
FM2 35 °C	2.5 ± 0.2	1.8 ± 0.2	14.0 ± 0.1	10.3 ± 0.04	26.4 ± 0.1
FM3 35 °C	2.4 ± 0.1	1.7 ± 0.1	14.4 ± 0.6	10.2 ± 0.3	28.0 ± 1.0

Data displays averages with standard errors.

3.3. Prokaryotic Community

Anaerobic digestion is one of the most promising methods for converting waste into energy and other by-products, and the advancement of this technology and its industrial applications are mainly based on the understanding of the relationship between the biodiversity and dynamics of the microbial community, along with the operating conditions and digestion efficiency [10,52,53]. Previous studies on the prokaryotic communities from anaerobic digesters have found a group of phylotypes shared by all systems, and some specific for each digestion conditions, which could be important for AD productivity [10,54,55]. In this study, we highlight a group of OTUs shared by all the treatments that we discuss in the next section, and then a set of phylotypes particular to FM3 samples, which could explain its productivity.

3.3.1. Core Communities

The amplicon metagenomic analysis of the samples from steady-state digestions showed that mesophilic and thermophilic digestions shared a microbial core community composed of bacteria and archaea and had relative abundances greater than 0.02%. The core community of these samples was constituted by fifty-five OTUs belonging to seven phyla: Bacteroidetes, Chloroflexi, Euryarchaeota, Firmicutes, Planctomycetes, Proteobacteria, and Spirochaetes, and these OTUs represented 42% of the total reads in this study. The most abundant classes in the core community were Anaerolineae, Bacteroidia, and Clostridia. To further identify microbes in the digestion samples, taxonomic affiliations were determined using the sequence similarity (Table S4). The most abundant sequences were related to genera *Sedimentibacter*, *Clostridium*, *Bacteroides*, *Acinetobacter*, and *Coprococcus*, which are in class Bacteroidia and families Clostridiaceae and Anaerolineaceae. However, since the metabolism and physiology of these unculturable microbial communities are not well characterized and understood, their detailed metabolic pathways during anaerobic digestion remain unclear. It has been reported that the family Clostridiaceae mainly includes syntrophic bacteria that can degrade volatile fatty acids such as butanoic acid [10] and crystalline carbohydrates into organic acids [7,50]. Hydrogen is also produced and then utilized by hydrogenotrophic methanogens for methane production. Anaerolineaceae (green non-sulfur bacteria) is an obligate anaerobic family of multicellular filamentous photoheterotrophs utilizing carbohydrates and amino acids and can grow under both mesophilic to thermophilic conditions [56–58]. Bacteroides are distributed widely in the environment and are especially common in organic-rich anoxic ecosystems such as animal gut and anaerobic treatment systems [59]. Microbial strains in this group hydrolyze polymer substrates such as polysaccharides, proteins and lipids into volatile fatty acids, CO₂, and hydrogen [60].

3.3.2. Effects of Culture Conditions on Microbial Communities

The most abundant phyla in the samples of this study were Chloroflexi, Firmicutes, and Bacteroidetes, which represented 84.7% of all sequences. However, the relative abundances were remarkably different between the digestion temperatures (Figure 5). Under the mesophilic condition, Bacteroidetes and Firmicutes were the dominant bacterial phyla in all three feedstock mixtures with Chloroflexi contributing only 5% to the abundance. These 3 phyla accounted for 64.3, 76.5 and 79.2% of the sequences in FM1, FM2, and FM3, respectively. Under the thermophilic condition, Chloroflexi and Firmicutes were the two dominant phyla for all three feedstock mixtures, which is consistent with the literature. High temperature is in favor of Chloroflexi growth [57]. FM1 had more Bacteroidetes than FM2 and FM3, which could be due to the fact that FM1 contains more total carbohydrates (glucan and xylan) than FM2 and FM3 (Tables 1 and 2), which requires more hydrolytic microbes for their degradation [7,61,62]. The Bray–Curtis similarity measure shows that bacteria and archaea communities from the thermophilic digestions were more similar to each other (similarity 80%) than microbial communities at 35 °C (50% similarity), regardless of the feedstock mixing ratio. The observed richness (Chao index) and diversity (Shannon’s index) of mesophilic digestions were greater than thermophilic digestions (Figure S1) [63,64]. The results further confirmed that thermophilic conditions led to less diverse and more similar microbial communities in the digestion [55,62,65,66].

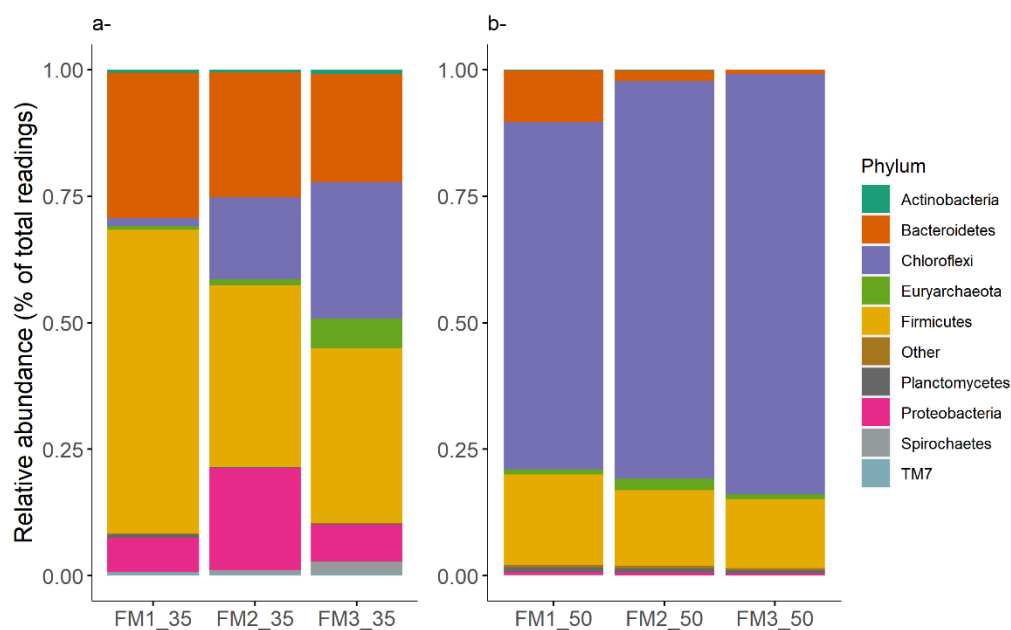


Figure 5. Microbial communities in mesophilic (a) and thermophilic (b) digesters. Relative abundances (% of total readings) of 16S rRNA gene at the phylum level.

For detailed analysis of the communities present in the FM3 samples at 35 and 50 °C, that produced more biogas, we first conducted a Shade plot analysis using Primer v7 software [31], in which the 50 most important genera with similar patterns of abundance across the samples were clustered using the Whitaker index of association [31] (Figure 6). Then, we searched for the OTUs that were significantly more abundant in FM3 samples, which could be associated with their productivity. According to this analysis, the statistically significant microbial community described in sample FM3-35 was highly represented by OTUs similar to species *Synergistes jonesii*, the cellulolytic mesophile *Acetivibrio cellulolyticus*, *Succiniclasticum ruminis*, and the genera *Treponema*, *Syntrophomonas*, and *Prevotella*.

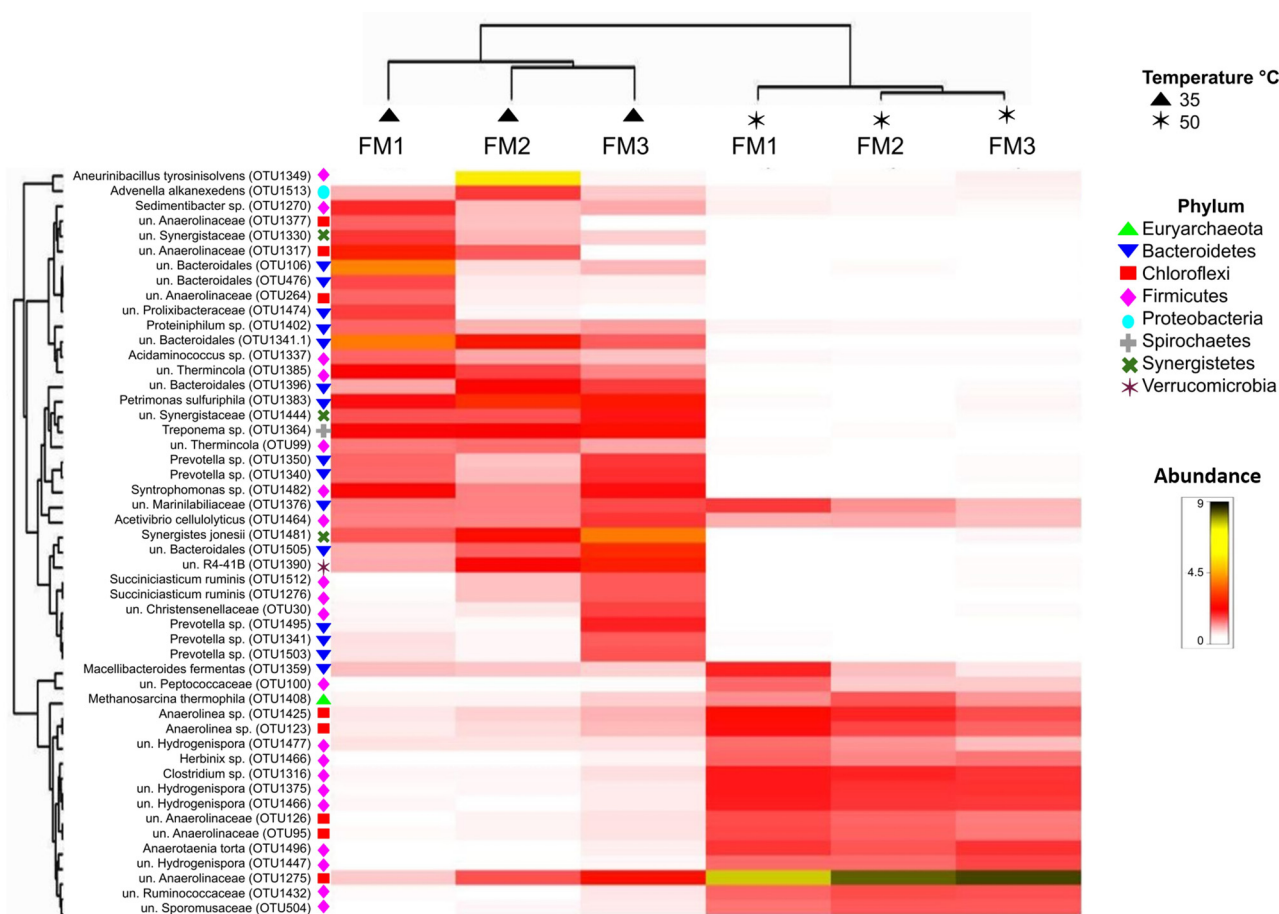


Figure 6. Shade matrix plot (Primer 7) showing relationship among clusters of samples (Bray–Curtis similarity from the standardized and square-root-transformed abundance data). A cluster of 50 most important standardized OTUs was grouped using Whitaker Index of association, independently. Color shading intensity within the matrix indicates the transformed relative abundance of each taxon. Symbols on the sample axis refer to the temperature used in the digestion and on the y-axis indicate the phyla of each genus.

The representatives of the sample FM3 at 35 °C were previously identified in sources showing temperatures from 18 to 40 °C: OTU-1505 in rumen acetogen enrichment cultures (JN196626, not published), OTU-1390 in a sulphate-reducing anaerobic reactor [67], and in ethanol fermentation, as OTU-1444 (un. Synergistaceae) and OTU-1376 (un. Marinilabiliaceae) [68]. Interestingly, this OTU was also similar to a clone from an unpublished study of thermophilic chicken dung–cow slurry fermentation, under similar conditions used in this study.

OTU1481 was significantly more abundant in FM3-35 (10.8% of the total reads in this sample) and exhibited 99.67% similarity to *Synergistes jonesii*. This rumen bacterium protects its host by degrading 3-hydroxy-4(1H)-pyridone (3,4 DHP), a toxic compound produced from mimosine digestion of *Leucaena leucocephala* [69]. *Leucaena* is a nutritionally rich forage legume used in cattle farming. *S. jonesii* appeared to be ubiquitous rather than isolated geographically [70]. The clear dominance of this OTU in sample FM3-35 raises the question of whether this plant was present in the food wastes or if there was another condition that triggered its growth.

Five OTUs in this group were all similar to *Prevotella* sequences isolated from the ruminal content of bovines. OTU1495 and 1341 were reported in a study of the influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers [71]. OTUs 1340 and 1350, were found in an unpublished diversity study of Qinghai yak ruminal bacteria and fungi in China (FJ172841), and OTU 1503 from rumen contents from HEAN

(Hereford × Angus) crossbred beef cattle (HQ399835). These OTUs together represent the second most abundant species in this sample. *Prevotella* is one of the most dominant genera in the rumen fluid. Members of this genus play an important role in breaking down proteins and carbohydrates and some cultured species produce cellulolytic enzymes suggesting they may act synergistically with other cellulolytic organisms involved in fibrolytic activity [72]. In this study, there were 13 OTUs similar to database *Prevotella* sequences, almost all in 35 °C samples, and 73% of them in FM3-35 alone. The high abundance of sequences similar to this genus in FM3-35 sample could have contributed to the higher performance of this mix.

OTU1364 is similar to *Treponema*, which is prevalent in many mesophilic anaerobic reactors [73–76] and could take part in the homoacetogenesis process by the consumption of H₂/CO₂, enhancing the predominance of acetoclastic methanogenesis [77], as well as metabolizing exopolysaccharides produced by the primary cellulolytic bacteria [76]. These results suggested that genus *Treponema* functioned in the processes of hydrolysis, fermentation, and acetogenesis in the reactors seeded with full-scale reactor samples.

The presence of sequences (OTU 1512 and 1276) similar to a *Succiniclasticum ruminis* DSM 9236(T) type strain is interesting, as this is a Gram-negative bacterium that ferments succinate to propionate. It is a common inhabitant of the rumen of cows on diverse diets, but also from subacute rumen acidotic (SARA) conditions which is a consequence of high-grain feeding [78]. Its abundance was higher in FM3 than in samples FM1 and FM2, correlating with the fruit/vegetable and post-consumer food waste contents in the mix, but only at 35 °C, as it was not detected at 50 °C in concordance with the reduced growth at 45 °C that has been reported.

The bacterial community at FM3-50 (based on the Index of association) (Figure 5), showed a clear dominant presence (50.7% of the reads in reactors at 50 °C) of OTU1275, which shows similarity to a sequence of an Anaerolinaceae. Other important OTUs in this mix were representatives of families Hydrogenispora, Ruminococcaceae, Sporomusaceae and species *Anaerotaenia torta*, *Herbinix* sp. and *Methanosarcina thermophila*. All these OTUs were more abundant on FM3-50 samples, and all but two OTUs (504 and 1496), were associated to thermophilic taxa (Figure S2). Interestingly, five OTUs (OTU1275, 1447, 1466, 1375 and 1432), were similar to sequences from two unpublished studies of chicken dung–cow slurry thermophilic fermentation.

OTU1275 is similar to sequence KP150753, an Anaerolinaceae (Chloroflexi). This sequence was the most abundant OTU of this study with 25.9% of the total abundance; 25.1% of that total abundance was detected in 50 °C reactors only. OTU1275 counts increased proportionally to the food waste contents in the samples at 50 °C and its abundance at FM3-50 (53.1% of the reads), could be related to the higher productivity in these systems. It is believed that members of the Anaerolinaceae can provide organic acid to other microorganisms such as acetoclastic methanogens. Furthermore, its filamentous nature may facilitate the degradation of hydrocarbons as this morphology is reported to be conducive to the aggregation of bacteria and substrates [79].

OTU504 is a sequence related to an acetate producing bacteria, 97.17% similar to a *Sporomusa* sp. enrichment culture clone (JQ512036). *Sporomusa* is a Gram-negative anaerobic homoacetogenic bacteria with the ability to grow through decarboxylation of organic acids [80]. These bacteria can also provide acetate to acetoclastic methanogens.

Other significant OTUs present in FM3-50 were OTU1375, 1466 and 1447, similar to an unassigned *Hydrogenispora* from an anaerobic baffled reactor treating acetone–butanol–ethanol fermentation wastewater and similar to clones from thermophilic reactors (HE804890) and thermophilic chicken dung–cow slurry fermentation (KP150669). Members of this order have been detected in fecal samples of diverse origins, but the most studied representative is *Hydrogenispora ethanolica* an anaerobic, ethanol–hydrogen–coproducing bacterium, isolated from an anaerobic sludge [81].

OTU1496 exhibited 97.3% identity to *Anaerotaenia torta*, a Gram-positive, strictly anaerobic, chemo-organotrophic bacteria with fermentative metabolism that use a wide variety

of compounds including xylan [82]. Two sequences similar to cellulolytic bacteria were found, one was OTU1432, 99.67% similar to a Ruminococcaceae (LT718732) isolated from an unpublished study of a thermophilic lab-scale biogas reactor treating increasing poultry manure. Ruminococcaceae are a family of obligate anaerobes with high cellulolytic activity [83]. An important member of this family is *Ruminococcus*, and species within this genus are suggested to play a key role in ruminal cellulose decomposition [72]. The other cellulolytic associated sequence was OTU1466, similar to *Herbinix* sp., a thermophilic cellulose-degrading bacterium isolated from a thermophilic biogas reactor [84].

Abundances of methanogens (0.8–5.9%) were not as high as Chloroflexi and Firmicutes in the digestion for both temperatures (Figure 5). Euryarchaeota was the only phylum detected in the Archaea domain. Its abundances varied among different digestions (Figure 5). According to metagenomic analysis, *Methanosarcina* was the most abundant genus in all digestions, which is consistent with the literature report [85]. This genus can consume a broad spectrum of substrates such as acetate, methanol and hydrogen to grow in a relatively wide temperature range [86,87]. The plasticity of its metabolism and the morphological characteristics of *Methanosarcina* enable it to outcompete other methanogens during anaerobic digestion [88].

A phylogenetic tree of all archaea sequences and their nearest relatives is presented in Figure S2 and Table S3. Three OTUs (OTU1408, OTU920 and OTU1250) from the thermophilic digestions showed 100% similarity to an uncultured *Methanosarcina* sp. clone CL7A-TAD, from the thermophilic anaerobic digestion of thermal-pretreated activated sludge [88] and up to 99.2% to the type strain of *Methanosarcina thermophila* DSM 1825. In the mesophilic digestions, 16% of the sequences had 99% similarity with *Methanosarcina mazei*, isolated from the mesophilic anaerobic digestion system [89]. Genera *Methanomicrobia*, *Methanocorpusculum* and *Methanobacterium* were mainly present in the mesophilic digestions, while genus *Methanoculleus* was present in the thermophilic digestions (Figure 5). OTU1399, OTU 923, and OTU 1042 associated with genus *Methanoculleus*, showed 99% similarity to *Methanoculleus thermophilus*, *Methanoculleus hydrogenitrophicus* [90], and *Methanoculleus thermophilus*, respectively.

4. Conclusions

Feedstock mixtures with high percentages of fruit/vegetable and post-consumer food waste demonstrate a better performance of biogas production than manure and chicken litter feedstocks. The temperature was a main factor affecting the microbial community. Chloroflexi and Firmicutes represented the dominant phyla to enhance the hydrolysis of organic compounds (e.g., cellulose) in thermophilic co-digestion, whereas Firmicutes and Bacteroidetes were more abundant in mesophilic co-digestion to utilize the easily hydrolyzed compounds (e.g., starch and hemicellulose). Genus *Methanosarcina* was the key methanogen present in all treatments but was more abundant in the thermophilic samples. The presence of Anaerolinaceae and Euryarchaeota are critical in acidogenesis and methanogenesis processes since they are responsible for enhancing biogas production. Moreover, our results demonstrate that increasing digestion temperature and adding food waste can alleviate the negative impact of low C:N ratios of animal waste on anaerobic digestion. A co-digestion strategy has been developed to generate biogas from multiple organic wastes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en15093252/s1>; Supplementary tables and figures are provided in the Supplementary File S1 and Supplementary Tables S3 and S4.

Author Contributions: Conceptualization, M.M.-R., W.L. and L.U.; Formal analysis, M.M.-R., L.U.-L., P.F.-S., D.V.-B., L.B.-G. and I.J.; Funding acquisition, W.L. and L.U.; Investigation, M.M.-R., I.J. and T.A.; Methodology, M.M.-R., L.U.-L., P.F.-S., D.V.-B., L.B.-G., I.J. and T.A.; Project administration, W.L. and L.U.; Software, L.B.-G.; Supervision, W.L. and L.U.; Validation, P.F.-S. and I.J.; Writing—original draft, L.U.-L., D.V.-B. and L.B.-G.; Writing—review and editing, M.M.-R., L.U.-L., P.F.-S., W.L. and L.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Costa Rica (Projects VI-733-A1821 and VI-733-B5056) and Michigan AgBioResearch.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Sequences from 55 core communities were submitted to the NCBI GenBank database under accession numbers MK912176-MK912244.

Acknowledgments: The authors thank Michigan AgBioResearch and the University of Costa Rica for funding this work through faculty salaries.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fulbright, N.R. Renewable Energy in Latin America, LLP. Available online: <http://large.stanford.edu/courses/2017/ph240/pinilla2/docs/nrf-feb17.pdf> (accessed on 15 January 2022).
2. Aguilar, R.E.; Bustamante, M.; Kirk, D.; Miranda, J.A.; Baudrit, D.; Aguilar, J.F.; Rodriguez, W.; Reinhold, D.; Liao, W. Technical and economic feasibility of a solar-bio-powered waste utilization and treatment system in Central America. *J. Environ. Manag.* **2016**, *184*, 371–379. [[CrossRef](#)] [[PubMed](#)]
3. Coto, C.O. Uso de los Residuos Agrícolas Orgánicos como Fuente de Energía: Evaluación de la Generación de Residuos Agrícolas Orgánicos (RAO) en Costa Rica e Identificación de Sector Prioritario, FITTACORI, San José, Costa Rica. November 2012. Available online: <https://www.mag.go.cr/proyectos/proy-residuos-agricolas-org/productos/Informe%20RAO%20CR%20Producto%201%20Final.pdf> (accessed on 15 January 2022).
4. Khan, I.U.; Othman, M.H.D.; Hashim, H.; Matsuura, T.; Ismail, A.F.; Rezaei-DashtArzhandi, M.; Azelee, I.W. Biogas as a renewable energy fuel—A review of biogas upgrading, utilisation and storage. *Energy Convers. Manag.* **2017**, *150*, 277–294. [[CrossRef](#)]
5. Piechota, G. Removal of siloxanes from biogas upgraded to biomethane by Cryogenic Temperature Condensation System. *J. Clean. Prod.* **2021**, *308*, 127404. [[CrossRef](#)]
6. Tang, Y.Q.; Ji, P.; Hayashi, J.; Koike, Y.; Wu, X.L.; Kida, K. Characteristic microbial community of a dry thermophilic methanogenic digester: Its long-term stability and change with feeding. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 1447–1461. [[CrossRef](#)] [[PubMed](#)]
7. Yue, Z.; Chen, R.; Yang, F.; MacLellan, J.; Marsh, T.; Liu, Y.; Liao, W. Effects of dairy manure and corn stover co-digestion on anaerobic microbes and corresponding digestion performance. *Bioresour. Technol.* **2013**, *128*, 65–71. [[CrossRef](#)]
8. Wang, S.; Hou, X.; Su, H. Exploration of the relationship between biogas production and microbial community under high salinity conditions. *Sci. Rep.* **2017**, *7*, 1149. [[CrossRef](#)]
9. Mei, R.; Nobu, M.K.; Narihiro, T.; Kuroda, K.; Munoz, S.J.; Wu, Z.; Ye, L.; Lee, P.K.H.; Lee, P.H.; Van Lier, J.B.; et al. Operation-driven heterogeneity and overlooked feed-associated populations in global anaerobic digester microbiome. *Water Res.* **2017**, *124*, 77–84. [[CrossRef](#)]
10. Rivière, D.; Desvignes, V.; Pelletier, E.; Chaussonnerie, S.; Guermazi, S.; Weissenbach, J.; Li, T.; Camacho, P.; Sghir, A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* **2009**, *3*, 700–714. [[CrossRef](#)]
11. Lee, S.H.; Kang, H.-J.; Lee, Y.H.; Lee, T.J.; Han, K.; Choi, Y.; Park, H.-D. Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. *J. Environ. Monit.* **2012**, *14*, 1893–1905. [[CrossRef](#)]
12. St-Pierre, B.; Wright, A.D. Comparative metagenomic analysis of bacterial populations in three full-scale mesophilic anaerobic manure digesters. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2709–2717. [[CrossRef](#)]
13. Freilich, S.; Kreimer, A.; Meilijson, I.; Gophna, U.; Sharan, R.; Ruppin, E. The large-scale organization of the bacterial network of ecological co-occurrence interactions. *Nucleic Acids Res.* **2010**, *38*, 3857–3868. [[CrossRef](#)] [[PubMed](#)]
14. Rui, J.; Li, J.; Zhang, S.; Yan, X.; Wang, Y.; Li, X. The core populations and co-occurrence patterns of prokaryotic communities in household biogas digesters. *Biotechnol. Biofuels* **2015**, *8*, 158. [[CrossRef](#)] [[PubMed](#)]
15. Rezadehbashi, M.; Baldwin, S.A. Core sulphate-reducing microorganisms in metal-removing semi-passive biochemical reactors and the co-occurrence of methanogens. *Microorganisms* **2018**, *6*, 16. [[CrossRef](#)] [[PubMed](#)]
16. Carballa, M.; Regueiro, L.; Lema, J.M. Microbial management of anaerobic digestion: Exploiting the microbiome-functionality nexus. *Curr. Opin. Biotechnol.* **2015**, *33*, 103–111. [[CrossRef](#)]
17. Regueiro, L.; Lema, J.M.; Carballa, M. Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. *Bioresour. Technol.* **2015**, *197*, 208–216. [[CrossRef](#)]

18. Ortseifen, V.; Stolze, Y.; Maus, I.; Sczyrba, A.; Bremges, A.; Albaum, S.P.; Jaenicke, S.; Fracowiak, J.; Pühler, A.; Schlüter, A. An integrated metagenome and -proteome analysis of the microbial community residing in a biogas production plant. *J. Biotechnol.* **2016**, *231*, 268–279. [[CrossRef](#)]
19. Ju, F.; Lau, F.; Zhang, T. Linking microbial community, environmental variables, and methanogenesis in anaerobic biogas digesters of chemically enhanced primary treatment sludge. *Environ. Sci. Technol.* **2017**, *51*, 3982–3992. [[CrossRef](#)]
20. El-Mashad, H.M.; van Loon, W.K.P.; Zeeman, G. A model of solar energy utilisation in the anaerobic digestion of cattle manure. *Biosyst. Eng.* **2003**, *84*, 231–238. [[CrossRef](#)]
21. Ward, A.J.; Hobbs, P.J.; Holliman, P.J.; Jones, D.L. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.* **2008**, *99*, 7928–7940. [[CrossRef](#)]
22. Gebreyessus, G.D.; Jenicek, P. Thermophilic versus mesophilic anaerobic digestion of sewage sludge: A comparative review. *Bioengineering* **2016**, *3*, 15. [[CrossRef](#)]
23. Fernández-Rodríguez, J.; Pérez, M.; Romero, L.I. Comparison of mesophilic and thermophilic dry anaerobic digestion of OFMSW: Kinetic analysis. *Chem. Eng. J.* **2013**, *232*, 59–64. [[CrossRef](#)]
24. APHA. *Standard Methods for the Examination of Water and Wastewater*, 23rd ed.; American Public Health Association (APHA): Washington, DC, USA, 2017.
25. Stewart, B.A.; Porter, L.K.; Beard, W.E. Determination of Total Nitrogen and Carbon in Soils by a Commercial Dumas Apparatus. *Soil Sci. Soc. Am. J.* **1964**, *28*, 366–368. [[CrossRef](#)]
26. Bertsch, F.; Henríquez, C.; Salas, R. *Fertilidad de Suelos: Manual de Laboratorio*, 1st ed.; Asociación Costarricense de la Ciencia del Suelo: San José, Costa Rica, 1995; Volume 39, p. 45.
27. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. *Lab. Anal. Proced.* **2008**, *1617*, 1–16.
28. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Knight, R. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [[CrossRef](#)] [[PubMed](#)]
29. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [[CrossRef](#)] [[PubMed](#)]
30. Yilmaz, P.; Parfrey, L.W.; Yarza, P.; Gerken, J.; Pruesse, E.; Quast, C.; Schweer, T.; Peplies, J.; Ludwig, W.; Glöckner, F. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Res.* **2014**, *42*, D643–D648. [[CrossRef](#)]
31. Clarke, K.R.; Gorley, R.N. *PRIMER v7: User Manual/Tutorial*. PRIMER-E Plymouth; Plymouth Marine Laboratory: Plymouth, UK, 2015.
32. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [[CrossRef](#)]
33. Tamura, K.; Stecher, G.; Peterson, D.; Filipinski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6. *Mol. Biol. Evol.* **2013**, *12*, 2725–2729. [[CrossRef](#)]
34. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. [[CrossRef](#)]
35. Verma, S. Anaerobic Digestion of Biodegradable Organics in Municipal Solid Wastes. Master’s Thesis, Department of Earth & Environmental Engineering, Columbia University, New York, NY, USA, 4 May 2002.
36. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [[CrossRef](#)]
37. Sievers, D.M.; Brune, D.E. Carbon/nitrogen ratio and anaerobic digestion of swine waste. *Trans. ASAE* **1978**, *21*, 537–0541. [[CrossRef](#)]
38. Maclellan, J.; Chen, R.; Kraemer, R.; Zhong, Y.; Liu, Y.; Liao, W. Anaerobic treatment of lignocellulosic material to co-produce methane and digested fiber for ethanol biorefining. *Bioresour. Technol.* **2013**, *130*, 418–423. [[CrossRef](#)] [[PubMed](#)]
39. Shin, S.G.; Yoo, S.; Hwang, K.; Song, M.; Kim, W.; Han, G.; Hwang, S. Dynamics of transitional acidogenic community along with methanogenic population during anaerobic digestion of swine wastewater. *Process. Biochem.* **2011**, *46*, 1607–1613. [[CrossRef](#)]
40. Bouallagui, H.; Touhami, Y.; Cheikh, R.B.; Hamdi, M. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process. Biochem.* **2005**, *40*, 989–995. [[CrossRef](#)]
41. Page, L.H.; Ni, J.Q.; Heber, A.J.; Mosier, N.S.; Liu, X.; Joo, H.S.; Ndegwa, P.M.; Harrison, J.H. Characteristics of volatile fatty acids in stored dairy manure before and after anaerobic digestion. *Biosyst. Eng.* **2014**, *118*, 16–28. [[CrossRef](#)]
42. Callaghan, F.J.; Wase, D.A.J.; Thayanithy, K.; Forster, C.F. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass Bioenergy* **2002**, *22*, 71–77. [[CrossRef](#)]
43. Wang, X.; Yang, G.; Feng, Y.; Ren, G.; Han, X. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresour. Technol.* **2012**, *120*, 78–83. [[CrossRef](#)]
44. Mata-Alvarez, J.; Mace, S.; Llabres, P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresour. Technol.* **2000**, *74*, 3–16. [[CrossRef](#)]
45. Neves, L.; Oliveira, R.; Alves, M.M. Co-digestion of cow manure, food waste and intermittent input of fat. *Bioresour. Technol.* **2009**, *100*, 1957–1962. [[CrossRef](#)]
46. El-Mashad, H.M.; Zhang, R. Biogas production from co-digestion of dairy manure and food waste. *Bioresour. Technol.* **2010**, *101*, 4021–4028. [[CrossRef](#)]

47. Molinuevo-Salces, B.; González-Fernández, C.; Gómez, X.; García-González, M.C.; Morán, A. Vegetable processing wastes addition to improve swine manure anaerobic digestion: Evaluation in terms of methane yield and SEM characterization. *Appl. Energy* **2012**, *91*, 36–42. [[CrossRef](#)]
48. Zhang, C.; Xiao, G.; Peng, L.; Su, H.; Tan, T. The anaerobic co-digestion of food waste and cattle manure. *Bioresour. Technol.* **2013**, *129*, 170–176. [[CrossRef](#)] [[PubMed](#)]
49. Wang, X.; Lu, X.; Li, F.; Yang, G. Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: Focusing on ammonia inhibition. *PLoS ONE* **2014**, *9*, e97265. [[CrossRef](#)] [[PubMed](#)]
50. Lin, J.; Zuo, J.; Gan, L.; Li, P.; Liu, F.; Wang, K.; Chen, L.; Gan, H. Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. *Res. J. Environ. Sci.* **2011**, *23*, 1403–1408. [[CrossRef](#)]
51. Shi, J.; Wang, Z.; Stiverson, J.A.; Yu, Z.; Li, Y. Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions. *Bioresour. Technol.* **2013**, *136*, 574–581. [[CrossRef](#)]
52. Venkiteswaran, K.; Bocher, B.; Maki, J.; Zitomer, D. Relating anaerobic digestion microbial community and process function: Supplementary issue: Water microbiology. *Microbiol. Insights* **2015**, *8*, 37–44. [[CrossRef](#)]
53. Joyce, A.; Ijaz, U.Z.; Nzetue, C.; Vaughan, A.; Shirran, S.L.; Botting, C.H.; Quince, C.; O’Flaherty, V.; Abram, F. Linking microbial community structure and function during the acidified anaerobic digestion of grass. *Front. Microbiol.* **2018**, *9*, 540. [[CrossRef](#)]
54. Lim, J.W.; Park, T.; Tong, Y.W.; Yu, Z. The microbiome driving anaerobic digestion and microbial analysis. *Adv. Bioenergy* **2020**, *5*, 1–61.
55. Shaw, G.T.W.; Liu, A.C.; Weng, C.Y.; Chou, C.-Y.; Wang, D. Inferring microbial interactions in thermophilic and mesophilic anaerobic digestion of hog waste. *PLoS ONE* **2017**, *12*, e0181395. [[CrossRef](#)]
56. Wan, S.; Sun, L.; Douieb, Y.; Sun, J.; Luo, W. Anaerobic digestion of municipal solid waste composed of food waste, wastepaper, and plastic in a single-stage system: Performance and microbial community structure characterization. *Bioresour. Technol.* **2013**, *146*, 619–627. [[CrossRef](#)]
57. Ward, L.M.; Hemp, J.; Shih, P.M.; McGlynn, S.E.; Fischer, W.W. Evolution of phototrophy in the Chloroflexi phylum driven by horizontal gene transfer. *Front. Microbiol.* **2018**, *9*, 260. [[CrossRef](#)] [[PubMed](#)]
58. Overmann, J. Green nonsulfur bacteria. *eLS* **2008**. [[CrossRef](#)]
59. Sun, L.; Toyonaga, M.; Ohashi, A.; Turlousse, D.M.; Matsuura, N.; Meng, X.-Y.; Tamaki, H.; Hanada, S.; Cruz, R.; Yamaguchi, T.; et al. *Lentimicrobium saccharophilum* gen. nov., sp. nov., a strictly anaerobic bacterium representing a new family in the phylum Bacteroidetes, and proposal of Lentimicrobiaceae fam. nov. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 2635–2642. [[CrossRef](#)] [[PubMed](#)]
60. Jabari, L.; Gannoun, H.; Khelifi, E.; Cayol, J.-L.; Godon, J.-J.; Hamdi, M.; Fardeau, M.-L. Bacterial ecology of abattoir wastewater treated by an anaerobic digester. *Braz. J. Microbiol.* **2016**, *47*, 73–84. [[CrossRef](#)]
61. Chen, R.; Murillo, M.; Zhong, Y.; Marsh, T.; Roman, M.B.; Hernández, W.; Uribe, L.; Uribe Lorio, L.; Kirk, D.; Reinhold, D.M.; et al. Responses of anaerobic microorganisms to different culture conditions and corresponding effects on biogas production and solid digestate quality. *Biomass Bioenergy* **2016**, *85*, 84–93. [[CrossRef](#)]
62. Zhong, Y.; Chen, R.; Rojas-Sossa, J.-P.; Isaguirre, C.; Mashburn, A.; Marsh, T.; Liu, Y.; Liao, W. Anaerobic co-digestion of energy crop and agricultural wastes to prepare uniform-format cellulosic feedstock for biorefining. *Renew. Energy* **2020**, *147*, 1358–1370. [[CrossRef](#)]
63. Pagaling, E.; Grant, W.D.; Cowan, D.A.; Jones, B.E.; Ma, Y.; Ventosa, A.; Heaphy, S. Bacterial and archaeal diversity in two hot spring microbial mats from the geothermal region of Tengchong, China. *Extremophiles* **2012**, *16*, 607–618. [[CrossRef](#)]
64. Tank, M.; Thiel, V.; Ward, D.M.; Bryant, D.A. A panoply of phototrophs: An overview of the thermophilic Chlorophototrophs of the microbial mats of alkaline siliceous hot springs in Yellowstone National Park, WY, USA. In *Modern Topics in the Phototrophic Prokaryotes*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 87–137.
65. Lin, Q.; He, G.; Rui, J.; Fang, X.; Tao, Y.; Li, J.; Li, X. Microorganism-regulated mechanisms of temperature effects on the performance of anaerobic digestion. *Microb. Cell Fact.* **2016**, *15*, 96. [[CrossRef](#)]
66. Westerholm, M.; Isaksson, S.; Karlsson Lindsjö, O.; Schnürer, A. Microbial community adaptability to altered temperature conditions determines the potential for process optimisation in biogas production. *Appl. Energy* **2018**, *226*, 838–848. [[CrossRef](#)]
67. Hessler, T.; Harrison, S.T.; Huddy, R.J. Stratification of microbial communities throughout a biological sulphate reducing up-flow anaerobic packed bed reactor, revealed through 16S metagenomics. *Res. Microbiol.* **2018**, *169*, 543–551. [[CrossRef](#)]
68. Zhang, J.; Wei, Y.; Xiao, W.; Zhou, Z.; Yan, X. Performance and spatial community succession of an anaerobic baffled reactor treating-acetone-butanol-ethanol fermentation wastewater. *Bioresour. Technol.* **2011**, *102*, 7407–7414. [[CrossRef](#)] [[PubMed](#)]
69. Allison, M.J.; Mayberry, W.R.; McSweeney, C.R.; Stahl, D.A. *Synergistes jonesii*, gen. nov., sp. nov.: A rumen bacterium that degrades toxic pyridinediols. *Syst. Appl. Microbiol.* **1992**, *15*, 522–529. [[CrossRef](#)]
70. McSweeney, C.S.; Padmanabha, J.; Halliday, M.J.; Hubbard, B.; Dierens, L.; Denman, S.E.; Shelton, H. Detection of *Synergistes jonesii* and genetic variants in ruminants from different geographical locations. *Trop. Grassl.—Forrajes Trop.* **2019**, *7*, 154–163. [[CrossRef](#)]
71. Tajima, K.; Nonaka, I.; Higuchi, K.; Takusari, N.; Kurihara, M.; Takenaka, A.; Mitsumori, M.; Kajikawa, H.; Aminov, R.I. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. *Anaerobe* **2007**, *13*, 57–64. [[CrossRef](#)]
72. Ozbayram, E.G.; Ince, O.; Ince, B.; Harms, H.; Kleinstüber, S. Comparison of rumen and manure microbiomes and implications for the inoculation of anaerobic digesters. *Microorganisms* **2018**, *6*, 15. [[CrossRef](#)]

73. Liu, F.H.; Wang, S.B.; Zhang, J.S.; Zhang, J.; Yan, X.; Zhou, H.K.; Zhao, G.P.; Zhou, Z.H. The structure of the bacterial and archaeal community in a biogas digester as revealed by denaturing gradient gel electrophoresis and 16S rDNA sequencing analysis. *J. Appl. Microbiol.* **2009**, *106*, 952–966. [[CrossRef](#)]
74. Li, L.; He, Q.; Ma, Y.; Wang, X.; Peng, X. Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: Relationship between community structure and process stability. *Bioresour. Technol.* **2015**, *189*, 113–120. [[CrossRef](#)]
75. Li, Y.F.; Nelson, M.C.; Chen, P.H.; Graf, J.; Li, Y.; Yu, Z. Comparison of the microbial communities in solid-state anaerobic digestion (SS-AD) reactors operated at mesophilic and thermophilic temperatures. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 969–980. [[CrossRef](#)]
76. Jia, Y.; Wilkins, D.; Lu, H.; Cai, M.; Lee, P.K. Long-term enrichment on cellulose or xylan causes functional and taxonomic convergence of microbial communities from anaerobic digesters. *Appl. Environ. Microbiol.* **2016**, *82*, 1519–1529. [[CrossRef](#)]
77. Zhang, H.; Banaszak, J.E.; Parameswaran, P.; Alder, J.; Krajmalnik-Brown, R.; Rittmann, B.E. Focused-pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester. *Water Res.* **2009**, *43*, 4517–4526. [[CrossRef](#)]
78. Hook, S.E.; Steele, M.A.; Northwood, K.S.; Dijkstra, J.; France, J.; Wright, A.D.G.; McBride, B.W. Impact of subacute ruminal acidosis (SARA) adaptation and recovery on the density and diversity of bacteria in the rumen of dairy cows. *FEMS Microbiol. Ecol.* **2011**, *78*, 275–284. [[CrossRef](#)] [[PubMed](#)]
79. Liang, B.; Wang, L.Y.; Mbadinga, S.M.; Liu, J.F.; Yang, S.Z.; Gu, J.D.; Mu, B.Z. Anaerolineaceae and Methanosaeta turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term of incubation. *AMB Express* **2015**, *5*, 37. [[CrossRef](#)]
80. Breznak, J.A. The Genus *Sporomusa*. *Prokaryotes* **2006**, *4*, 991–1001.
81. Liu, Y.; Qiao, J.T.; Yuan, X.Z.; Guo, R.B.; Qiu, Y.L. *Hydrogenispora ethanolica* gen. nov., sp. nov., an anaerobic carbohydrate-fermenting bacterium from anaerobic sludge. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 1756–1762. [[CrossRef](#)] [[PubMed](#)]
82. Ueki, A.; Ohtaki, Y.; Kaku, N.; Ueki, K. Descriptions of *Anaerotaenia torta* gen. nov., sp. nov. and *Anaerocolumna cellulositytica* gen. nov., sp. nov. isolated from a methanogenic reactor of cattle waste and reclassification of *Clostridium aminovalericum*, *Clostridium jejuense* and *Clostridium xylanovorans* as *Anaerocolumna* species. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 2936–2943.
83. Wang, L.; Hatem, A.; Catalyurek, U.V.; Morrison, M.; Yu, Z. Metagenomic insights into the carbohydrate-active enzymes carried by the microorganisms adhering to solid digesta in the rumen of cows. *PLoS ONE* **2013**, *8*, e78507. [[CrossRef](#)]
84. Koeck, D.E.; Ludwig, W.; Wanner, G.; Zverlov, V.V.; Liebl, W.; Schwarz, W.H. *Herbinix hemicellulosilytica* gen. nov., sp. nov., a thermophilic cellulose-degrading bacterium isolated from a thermophilic biogas reactor. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 2365–2371. [[CrossRef](#)]
85. Guo, X.; Wang, C.; Sun, F.; Zhu, W.; Wu, W. A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. *Bioresour. Technol.* **2014**, *152*, 420–428. [[CrossRef](#)]
86. De Vrieze, J.; Hennebel, T.; Boon, N.; Verstraete, W. Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresour. Technol.* **2012**, *112*, 1–9. [[CrossRef](#)]
87. Doloman, A.; Soboh, Y.; Walters, A.J.; Sims, R.C.; Miller, C.D. Qualitative analysis of microbial dynamics during anaerobic digestion of microalgal biomass in a UASB reactor. *Int. J. Microbiol.* **2017**, *2017*, 1–12. [[CrossRef](#)]
88. Gagliano, M.; Braguglia, C.M.; Gianico, A.; Mininni, G.; Nakamura, K.; Rossetti, S. Thermophilic anaerobic digestion of thermal pretreated sludge: Role of microbial community structure and correlation with process performances. *Water Res.* **2015**, *68*, 498–509. [[CrossRef](#)] [[PubMed](#)]
89. Kushkevych, I.; Vítězová, M.; Vítěz, T.; Bartoš, M. Production of biogas: Relationship between methanogenic and sulfate-reducing microorganisms. *Open Life Sci.* **2017**, *12*, 82–91. [[CrossRef](#)]
90. Tian, J.; Wang, Y.; Dong, X. *Methanoculleus hydrogenitrophicus* sp. nov., a methanogenic archaeon isolated from wetland soil. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2165–2169. [[CrossRef](#)] [[PubMed](#)]