

Comparison of functional microbial profile between mesophilic and thermophilic anaerobic digestion of vegetable waste

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Research

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Abstract

Background

With increasing accumulation of vegetable waste in China, the valorization of vegetable waste becomes an urgent concern. Based on the characteristics of high moisture content and biodegradable organic matter of vegetable waste, anaerobic digestion as an effective technique was selected to reuse this kind of agriculture waste. The anaerobic digestion performance is highly correlated with the functional microbial community. In this study, mesophilic and thermophilic digestions of vegetable waste were conducted, and dynamics of the microbial community were investigated.

Results

The mesophilic and thermophilic collapsed stages occurred at organic loading rates of 1.5 and 2.0 g volatile solid (VS)/(L·d), respectively, due to severe accumulation of volatile fatty acids. The mesophilic digestion exhibited a higher microbial diversity and richness than the thermophilic digestion. Syntrophic acetate-oxidizing coupled with hydrogenotrophic methanogenesis was the dominant pathway in the thermophilic stable system, and acetoclastic methanogenesis in the mesophilic stable system. The dominant acidogens, syntrophus, and methanogens were *Candidatus_Cloacamonas*, *norank_f_Synergistaceae*, *Methanosaeta*, and *Methanosarcina* in the mesophilic stable stage, and *Anaerobaculum*, *Syntrophaceticus*, *Methanosarcina*, and *Methanothermobacter* in thermophilic stable stage. Spirochaetae and Thermotogae phyla were the characteristic microorganisms in the mesophilic and thermophilic collapsed stages, respectively.

Conclusions

This study unveiled the distribution of the functional microbial consortia at the stable and collapsed periods of the anaerobic digestion of vegetable waste under mesophilic and thermophilic conditions to providing guidelines for the further research of anaerobic digestion of vegetable waste.

1. Introduction

The accumulation of vegetable waste (VW), originally produced from vegetable planting field and secondly collected from wholesale and farmer's markets, has caused severe rural and urban environmental concern. According to China Statistical Abstract 2015, approximately 760×10^6 t of VW was generated in 2014 in China [1]. With high moisture content and biodegradable characteristics, the landfill of VW generates a harmful leachate, which leads to adverse environmental effects. In order to achieve the green-sustainable agriculture development, exploring an effective method to realize the valorization of VW is necessary. VW comprises 75% easily biodegradable organic matter (i.e., sugar, hemicellulose) and 15% recalcitrant organic matter (i.e., cellulose, lignin) [2]. Such types of organic waste

are suitable for anaerobic digestion (AD) to produce biogas for energy generation and organic fertilizer for agricultural application [3]. Li et al. studied the performance and stability of the AD of VW under thermophilic condition, and showed that VW was easily acidified and could only function stably at an organic loading rate (OLR) below 1.5 g volatile solid (VS)/(L • d) [1]. To improve the stability of the AD of VW, two-phase AD for VW was conducted by Zuo et al., which contributed to the improvement of stable OLR threshold from 2.6 to 3 g VS/(L·d) [4]. However, the composition and distribution of the microbial community in the AD of VW, which is the key factor for its stable operation, are not yet understood.

VW undergoes a fast degradation by fermentative and acidogenic bacterial consortium, which generates a large amount of volatile fatty acids (VFAs) that lead to rapid acidification during the AD process [2]. The acidic environment has been found to seriously inhibit the activities of methanogens. Li et al. quoted that the methanogens in an AD reactor would face acidic inhibition when the pH value is below 6.5 [5]. Finally, the collapse of the AD system is occurred. Three types of microbial consortia are involved in an AD system, including fermentative and acidogenic bacterial consortium, syntrophic bacterial consortium, and methanogenic consortium [6]. The stability of the AD process is highly relevant to the balance between the different groups of functional microbial consortia. Therefore, it is significant to unveil the specific functional microbial consortium for a better understanding of the AD system of VW.

Temperature is a key environmental factor that affects the AD performance significantly. Generally, AD conducts at mesophilic (30–40 °C) and thermophilic (50–60 °C) conditions. In fact, temperature was found to be the main cause that causes the apparent shifts of different types of functional microorganisms, which in turn influence the performance of AD. The mesophilic and thermophilic AD of soybean curd residue was conducted by Zhang et al., who systemically analyzed the microbial compositions and dynamics under different temperature conditions [7]. Ryue et al. also compared the differences of the microbial community structures between mesophilic and thermophilic AD for food waste [8]. Nonetheless, little is known about the differences of the functional microbial consortia between the mesophilic and thermophilic AD of VW.

The present study systematically unveiled the distribution of the functional microbial consortia at the stable and collapsed periods of the AD of VW under mesophilic and thermophilic conditions. Two continuous stirred-tank reactors (CSTRs) fed with VW were conducted under anaerobic mesophilic and thermophilic conditions. The parameters of the gaseous and liquid phases were monitored daily to evaluate the performance of the AD for VW. The microbial community shifts at different periods were analyzed via high-throughput sequencing technology.

2. Materials And Methods

2.1 Substrate and inoculum

VW was acquired from Chengdu HIGREEN wholesale vegetable market, and contained cabbage and lettuce leaves among others. Before being fed into the mesophilic and thermophilic reactors, the VW was

chopped into small pieces (4–5 mm) and mixed homogeneously, after which they were stored in a refrigerator at 4 °C. The total solid (TS) and volatile solid (VS) contents of the VW were 105.7 g/kg and 92.1% of TS, respectively. The crude protein, lipid, and fiber of VW were 13.2% of TS, 3.4% of TS, and 11.9% of TS. The C/N ratio of VW were 17.1. The original inoculum was gained from a mesophilic (35 ± 2 °C) anaerobic digester fed with swine manure. The inoculum was introduced into a mesophilic reactor under anaerobic conditions, and acclimated for about 30 days via feeding the VW with an OLR of 0.5 g VS/(L·d) into the reactor daily until the methane content was above 60%. The inoculum in the thermophilic anaerobic reactor was also acclimated for about 30 days by stepwise increasing the temperature (i.e. increase 1 °C per day) from ambient temperature to 55 ± 2 °C while simultaneously feeding the VW with an OLR of 0.5 g VS/(L·d) into the reactor daily until the methane content was above 60%. The TS and VS contents and pH value of the original inoculum were 11.2 g/kg, 72.5% TS, and 7.6, respectively.

2.2 Experimental setup and design

The experimental setup comprised two CSTRs. Each reactor has a total and working volume of 70 L and 55 L, respectively. Furthermore, a set of self-designed online monitor systems was also equipped on the reactors to record the daily changes of biogas production, temperature, and pH. The operational temperatures for the mesophilic and thermophilic reactors were maintained at 35 ± 2 °C and 55 ± 2 °C using heating jackets, respectively. The contents in the reactors were stirred 8 times per day at 40 rpm for 30 min via the impellers, which were equipped with two 200 mm × 40 mm flat blades. The mesophilic AD of the VW was conducted at OLRs of 0.5, 1.0, and 1.5 g VS/(L·d) and a fixed hydrolytic retention time (HRT) of 20 days. The thermophilic AD of the VW was carried out at OLRs of 0.5, 1.0, 1.5, 2.0, and 2.5 g VS/(L·d) and a fixed HTR of 20 days. According to the OLR values, a certain amount of mixture (i.e., VW mixed with water, 2,750 g) was fed into the reactors daily after the same amount of digestate (2,750 g) was discharged from the outlet. The overall running times for the mesophilic and thermophilic experiments were 90 and 129 days, respectively.

2.3 Analytical methods

The TS and VS contents were measured using the standard techniques provided by [9]. The compositions of crude fiber, crude lipid, and crude protein in the VW were measured referring to Chinese standard methods (GB/T 5009 – 2003). A gas flowmeter (Beijing Sevenstar Electronics Co., Ltd, China) was applied to measure the biogas production. The biogas contents (i.e., methane and carbon dioxide) were analyzed via an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). The daily pH value of the liquid digestate in the reactor was determined online. Liquid samples were obtained daily by centrifuging the liquid digestate at 12,000 rpm for 10 min to remove the large insoluble particles, after which the centrifuged liquid samples were filtered with a 0.45-µm membrane filter to analyze the total ammonium nitrogen (TAN) and VFA concentrations. The concentrations of the TAN and VFAs were measured using the methods described by Li, Ran [10].

2.4 Sequence processing and analysis

The digestate samples were extracted from the mesophilic and thermophilic reactors at the stable stages (days 46, 50, and 54; and days 46, 50, and 55, respectively) and the collapsed stages (days 62, 72, 81, and 89; and days 100, 117, and 126, respectively). These stages are defined in Sect. 3. Each of the collected samples (50 ml) were centrifuged at 5,000 rpm for 10 min. The supernatant was then removed and the remaining solid digestate was stored in a freezer at -80 °C for further analysis. Microbial DNA was extracted using E.Z.N.A DNA Extraction Kit for soil (Omega Biotek, Norcross, GA, USA) following the manufacturer's protocols. Furthermore, universal 16S rRNA gene primers of 515F and 806R were used to perform polymerase chain reaction with TransStart FastPfu DNA Polymerase (TransGen Biotech, Beijing, China) [11]. The amplified products were further purified, and the purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

The microbial community structure and dynamics at different reactors and operational stages were analyzed using the Majorbio I-Sanger Cloud Platform (www.i-sanger.com) online tool. The mothur software (version v.1.30.1) was adopted to measure the alpha diversity, while the Bray–Curtis matrix was used to calculate the beta diversity [12]. R Stat was chosen in this study to conduct the statistical analyses to exhibit the significant differences in the relative abundances of microorganisms at difference temperature conditions. All the data from the aforementioned microbial analyses are provided below as the average ± standard deviation of triplicate measurements.

3. Results And Discussion

3.1 Operation and performance of mesophilic and thermophilic anaerobic reactors

As in Fig. 1, the mesophilic experiment was conducted for 90 days in total. Days 31–60 were defined as the mesophilic stable stage (M_{Stable}) and days 61–90 as the mesophilic collapsed stage ($M_{\text{Collapsed}}$). The overall thermophilic experiment was to last for 129 days. The thermophilic stable stage (T_{Stable}) occurred on days 39–64, and the thermophilic collapsed stage ($T_{\text{Collapsed}}$) on days 100–129.

In this study, the performance of the mesophilic and thermophilic reactors was reflected by the fluctuation of gaseous parameters (i.e., the methane content and volumetric methane production rate (VMPR)) and liquid parameters (i.e., VFAs, TAN, and pH value). The VMPR and Total VFA (TVFA) are presented in Fig. 1 while the other parameters are summarized in Table 1. The TVFA sharply increased to 2276.84 and 6476.56 mg/L at $M_{\text{Collapsed}}$ and $T_{\text{Collapsed}}$ stages, respectively, while the VMPR at $M_{\text{Collapsed}}$ and $T_{\text{Collapsed}}$ stages were both decreased to zero. Both the mesophilic and thermophilic anaerobic reactors experienced process failure caused by the severe accumulation of VFAs. The maximum OLRs for the stable mesophilic and thermophilic reactors were both 1.0 g VS/(L·d). The balance between acidogens and methanogens is indispensable for a stable AD system. However, methanogens are more vulnerable to the acid stress than acidogens, which finally led to the disturbance between the acidogens and methanogens

and resulted in severe acidification [13]. In addition, VW is a type of carbohydrate-rich substrate with a weak buffering capacity in the AD process [14]. High temperatures accelerated the degradation of VW; thus, improved the average VMPPR value from 0.16 ± 0.03 L/(L·d) at the mesophilic condition to 0.29 ± 0.02 L/(L·d) at the thermophilic condition when the two reactors operated at a stable OLR of 1.0 g VS/(L·d).

Table 1
Performance of mesophilic and thermophilic anaerobic reactors at different stages

Stage	Mesophilic reactor (35 °C)		Thermophilic reactor (55 °C)	
	M_{Stable}	$M_{\text{Collapsed}}$	T_{Stable}	$T_{\text{Collapsed}}$
CH ₄ (%)	54.31 ± 1.37	48.10 ± 4.17	55.11 ± 1.38	12.02 ± 16.68
Total VFAs (mg/L)	113.38 ± 60.94	741.76 ± 653.07	72.24 ± 36.14	$4,703.56 \pm 1,037.49$
Acetate (mg/L)	89.86 ± 40.88	300.45 ± 152.41	56.78 ± 28.47	$2,083.84 \pm 319.61$
Propionate (mg/L)	15.30 ± 7.10	300.94 ± 360.44	11.28 ± 2.77	450.33 ± 92.40
TAN (mg/L)	371.51 ± 81.13	203.16 ± 61.02	406.45 ± 66.09	264.49 ± 51.76
pH value	6.82 ± 0.11	6.32 ± 0.22	6.91 ± 0.06	4.76 ± 0.64
<i>Notes:</i> M_{Stable} : Mesophilic stable stage, $M_{\text{Collapsed}}$: Mesophilic collapsed stage. T_{Stable} : Thermophilic stable stage, $T_{\text{Collapsed}}$: Thermophilic collapsed stage.				

3.2 Overall microbial diversity

The microbial composition and dynamics of the mesophilic and thermophilic reactors at the stable and collapsed stages were obtained through high-throughput sequencing technology based on the Illumina MiSeq platform. Moreover, the alpha diversity indexes, including the Simpson, Chao, and Simpson even indexes, were analyzed in this study to exhibit the differences in the microbial diversity at different temperatures and stages (Table 2). The Simpson index decreased as the microbial diversity increased, while the Chao and Simpson even indexes increased as microbial richness and evenness increased, respectively [15]. The Simpson index increased slightly from M_{Stable} to $M_{\text{Collapsed}}$, and the Chao index decreased slightly from M_{Stable} to $M_{\text{Collapsed}}$. This result reflects that the microbial diversity and richness decreased at $M_{\text{Collapsed}}$. The accumulation of VFAs at $M_{\text{Collapsed}}$ resulted in the inhibition of microorganisms, which might lead to the reduction of most types of bacteria and methanogens. The decrease of the Simpson even index at $M_{\text{Collapsed}}$ also confirmed the aforementioned speculation that the microbial evenness was less in $M_{\text{Collapsed}}$ compared with that in M_{Stable} . A similar tendency was found for the thermophilic anaerobic system. The accumulation of the total VFAs at $T_{\text{Collapsed}}$ was far more than that at $M_{\text{Collapsed}}$ (Table 1), indicating that the severe acidification resulted in a clear reduction of most types of microorganisms at the thermophilic collapsed condition. The microbial diversity, richness, and evenness at M_{Stable} were slightly higher than those at T_{Stable} . Greses et al. found that the microbial

diversity was higher at the mesophilic condition in comparison with that at the thermophilic condition using *Scenedesmus spp* as the substrate [16]. Vanwonterghem et al. also clarified that the increase of temperature from the mesophilic to thermophilic anaerobic conditions resulted in a limited population of microorganisms [17]. Therefore, temperature is one of the most important factors that influenced the microbial diversity.

Table 2
Summary of alpha diversity indexes

Alpha diversity index			
Stage	Simpson ^a	Chao ^b	Simpson even ^c
M_{Stable}	0.088 ± 0.003	91.548 ± 5.680	0.150 ± 0.015
$M_{\text{Collapsed}}$	0.109 ± 0.046	83.824 ± 8.327	0.132 ± 0.052
T_{Stable}	0.140 ± 0.034	71.675 ± 3.103	0.119 ± 0.035
$T_{\text{Collapsed}}$	0.586 ± 0.196	55.875 ± 12.929	0.041 ± 0.017

Notes: ^aDiversity of the microbial community, ^bRichness of microbial species, ^cDistribution evenness of the microbial community.

The principal coordinate analysis results of the microorganisms in both the mesophilic and thermophilic anaerobic reactors at different stages are exhibited in Fig. 2. Clusters 1, 2, 3, and 4 represent the samples from M_{Stable} , $M_{\text{Collapsed}}$, T_{Stable} , and $T_{\text{Collapsed}}$, respectively. The total microbial community showed a 43.04% variation in principal components 1. Under different temperature conditions, clusters 1 and 2 from the mesophilic condition located in the left side of the plot, and clusters 3 and 4 from the thermophilic condition located in the right side of the plot. The clear separation of the microbial communities under different temperature conditions showed that temperature changes significantly affect the microbial community shifting under the AD of VW. The microbial community showed a 23.83% total variation in principal components 2. Under the mesophilic condition, the samples from the stable and collapsed stages were separated into two clusters (clusters 1 and 2). However, the distance between clusters 1 and 2 was quite close, indicating that the microbial community structure was only slightly shifted under the mesophilic condition. However, all the samples from the stable and collapsed stages were distinctly divided into two clusters (cluster 3 and 4) under the thermophilic condition, demonstrating that the severe acidification caused dramatic shifts of the microbial community structure. The final collapse of the mesophilic and thermophilic AD of VW occurred at an OLR of 1.5 g VS/(L·d) and 2.5 g VS/(L·d), respectively. A high OLR led to a high VFA concentration; thus, inhibits the growth of the bacterial community more severe compared with a low OLR.

3.3 Dynamics of microbial community composition on phylum level

The results of the microbial community analysis that revealed the phylum level are shown in Fig. 3(a). Members of the phyla Bacteroidetes (33.33%), Cloacimonetes (19.70%), Chloroflexi (12.95%), and Euryarchaeota (10.70%) were the most abundant within M_{Stable} , followed by phyla Firmicutes (9.18%), Actinobacteria (6.39%), Synergistetes (3.28%), Atribacteria (1.68%), and Thermotogae (1.42%). Bacteroidetes (43.28%) and Spirochaetae (16.35%) were the most dominant phyla within the $M_{\text{Collapsed}}$, followed by the phyla Euryarchaeota (7.84%), Chloroflexi (6.54%), Firmicutes (6.33%), Cloacimonetes (6.01%), Proteobacteria (4.89%), Actinobacteria (4.00%), Thermotogae (2.34%), and Synergistetes (1.44%). Among these phyla, Euryarchaeota was the only phylum that functioned as methanogens in the mesophilic reactor. Most genus within the phyla of Spirochaetae, Synergistetes, and Atribacteria functioned as syntrophic organic acid oxidation bacteria in the AD system [18–20]. While the remaining phyla were mainly attributed to the fermentation and degradation processes of the AD system. When the mesophilic anaerobic system collapsed, the abundance of Bacteroidetes notably increased, followed by a slight increase in the Thermotogae, indicating that these two phyla were more tolerant to the acidified condition. The Bacteroidetes and Thermotogae phyla play a significant role in the degradation of cellulose and protein under anaerobic conditions [18, 21]. The phyla of Spirochaetae, which existed uniquely in the collapsed stage, could be used as a microbial early warning indicator for the AD instability of VW.

Members of the phyla Firmicutes (46.98%), Euryarchaeota (33.77%), and Synergistetes (12.60%) were the most abundant within T_{Stable} , followed by Bacteroidetes (2.76%) and Atribacteria (2.63%). Meanwhile, Thermotogae (48.93%) and Firmicutes (37.72%) were the most dominant phyla within the $T_{\text{Collapsed}}$, followed by Euryarchaeota (5.48%), Chloroflexi (4.79%), Atribacteria (1.34%), and Bacteroidetes (1.17%). The phyla Firmicutes was dominant in both the thermophilic stable and collapsed stages; however, its abundance in the collapsed stage was lower than it in the stable stage. Thermotogae was the most abundant phyla that existed uniquely in the thermophilic collapsed stage, demonstrating the higher adaptability of Thermotogae to the acidic condition. Thus, Thermotogae might play a key role in relieving the further acidification, which has been reported to degrade various organic substrates under acidic anaerobic conditions [22]. The abundance of the phyla Euryarchaeota and Synergistetes sharply decreased at the collapsed stage, which was consistent with the final failure of AD of VW.

3.4 Dynamics of microbial community composition on genus level

Details regarding the major microbial composition and abundance on the genus level were analyzed to further explore the dynamics of the microbial community (Fig. 3(b)). The most abundant genera from M_{Stable} were *Candidatus_Cloacamonas* (19.7%) and *vadinBC27_wastewater-sludge_group* (11.05%), followed by *unclassified_f__Anaerolineaceae* (8.68%), *Proteiniphilum* (7.78%), *Petrimonas* (6.55%), *Propionimicrobium* (6.39%), *Methanosaeta* (6.10%), *norank_f__Anaerolineaceae* (4.14%), *Methanosarcina* (4.08%), and *Microbacter* (3.43%). In contrast, the most abundant genera from $M_{\text{Collapsed}}$ were *Bacteroides* (15.11%), *vadinBC27_wastewater-sludge_group* (14.01%), and *Sphaerochaeta* (10.15%),

followed by *Candidatus_Cloacamonas* (6.01%), *Methanosaeta* (5.65%), *norank_f__V2072-189E03* (5.61%), *norank_f__Anaerolineaceae* (4.92%), *Aeromonas* (4.09%), *norank_f__Porphyromonadaceae* (4.02%), and *Propionimicrobium* (4.00%). According to previous studies, the specific functions of each major genus from the mesophilic and thermophilic anaerobic reactors are listed in Table 3. At the mesophilic stable stage, the genera *Candidatus_Cloacamonas* and *vadinBC27_wastewater-sludge_group* were classified as syntrophic bacteria. *Candidatus_Cloacamonas* was able to digest cellulose and use hydrolyzing products to produce H₂ and CO₂, and *VadinBC27_wastewater-sludge_group* was reported to degrade amino acids in syntrophic association with hydrogenotrophic methanogens. *Methanosaeta* and *Methanosarcina* were the only two dominant methanogens in the mesophilic stable stage, which functioned as acetoclastic and mixotrophic (i.e., acetoclastic, hydrogenotrophic, and methylotrophic) methanogens, respectively [18, 23]. Thus, only *Methanosarcina* has the ability of syntrophic association with *Candidatus_Cloacamonas* and *vadinBC27_wastewater-sludge_group*, which mainly uses H₂ and CO₂ to produce methane. The genera *unclassified_f__Anaerolineaceae*, *Proteiniphilum*, *Petrimonas*, *Propionimicrobium*, *norank_f__Anaerolineaceae*, and *Microbacter* functioned as fermentative and acidogenic bacteria that degraded glucose to produce acetic and propionic acids. The dominant microbes changed clearly from the mesophilic stable to the collapsed stages. The most abundant bacteria shifted from syntrophic bacteria in the mesophilic stable stage to fermentative, acidogenic, and syntrophic bacteria in the mesophilic collapsed stage. Among the three most dominant genera of *Bacteroides*, *vadinBC27_wastewater-sludge_group*, and *Sphaerochaeta* in the mesophilic collapsed stage, two are fermentative and acidogenic bacteria (i.e., *Bacteroides* and *Sphaerochaeta*). *Bacteroides* are reported to have the ability to hydrolyze a complex insoluble substrate, such as polysaccharides, while *Sphaerochaeta* can ferment carbohydrates to produce acetate, formate, and ethanol (Table 3). *Candidatus_Cloacamonas*, which was the most abundant syntrophic bacteria, decreased dramatically from the mesophilic stable (19.7%) to the collapsed (6.01%) stage. The only methanogen that existed in the top ten genera at the mesophilic collapsed stage was *Methanosaeta*, compared with the two dominant methanogens (i.e., *Methanosaeta* and *Methanosarcina*) at the stable stages. The decrease of methanogens corresponded with the reduction of syntrophic bacteria at the mesophilic collapsed stage. These results reveal that fermentative and acidogenic bacteria were dominant occupants in the mesophilic collapsed stage, compared with syntrophic bacteria in the stable stage. When the performance of the AD of VW changed from the stable to collapsed state, caused by the accumulation of VFAs, the abundance of fermentative and acidogenic bacteria considerably shifted. All the fermentative and acidogenic bacteria of *unclassified_f__Anaerolineaceae*, *Proteiniphilum*, *Petrimonas*, and *Microbacter* were eliminated from the top ten genera at the mesophilic collapsed stage, and instead comprised the genera of *Bacteroides*, *Sphaerochaeta*, *norank_f__V2072-189E03*, *Aeromonas*, and *norank_f__Porphyromonadaceae*. This result indicates that the genera of *Bacteroides*, *Sphaerochaeta*, *norank_f__V2072-189E03*, *Aeromonas*, and *norank_f__Porphyromonadaceae* prefer to thrive under acidic environmental conditions, while the genera of *unclassified_f__Anaerolineaceae*, *Proteiniphilum*, *Petrimonas*, and *Microbacter* are vulnerable to high concentration of VFAs.

Table 3

Functional descriptions of the dominant genera that existed in the mesophilic and thermophilic anaerobic reactors

Genus	Functional description	Reference
<i>VadinBC27_wastewater-sludge_group</i>	Syntrophic bacteria; degrade proteins and carbohydrates in syntrophic association with hydrogenotrophic methanogens under anaerobic condition.	[26]
<i>Candidatus_Cloacamonas</i>	Syntrophic bacteria; digest cellulose, propionate, and amino acid to produce H ₂ and CO ₂ .	[22]
<i>Bacteroides</i>	Secrete different hydrolyzing enzymes, such as cellulase, to hydrolyze a complex insoluble substrate, such as polysaccharides.	[27]
<i>Sphaerochaeta</i>	Ferment carbohydrates to produce acetate, formate, and ethanol.	[28]
<i>Unclassified_f__Anaerolineaceae</i>	Anaerobic fermentative and acetogenic bacteria.	[29]
<i>Propionimicrobium</i>	Growth under anaerobic conditions and produce propionic acid.	[30]
<i>Norank_f__Anaerolineaceae</i>	Convert glycerol into VFAs.	[31]
<i>Proteiniphilum</i>	Ferment organic substrates to produce acetic and propionic acids.	[32]
<i>Petrimonas</i>	Anaerobic fermentative and acetogenic bacteria.	[29]
<i>Norank_f__V2072-189E03</i>	Ferment polysaccharides to produce acetate, formate, and ethanol.	[33]
<i>Microbacter</i>	Fermentative bacteria in the anaerobic digestion system.	[34]
<i>Aeromonas</i>	Ferment glucose to produce acetate in the anaerobic digestion system.	[35]
<i>Norank_f__Porphyromonadaceae</i>	Ferment glucose to produce lactate, acetate, butyrate, and isobutyrate.	[23]
<i>Defluviitoga</i>	Degrade biomass to produce acetate.	[36]
<i>Thermoanaerobacterium</i>	Degrade lignocellulose to produce acetic acid, butyric acid, and lactic acid.	[37]
<i>Anaerobaculum</i>	Ferment a range of amino acids to acetate, propionate, and hydrogen.	[38]
<i>Clostridium_sensu_stricto_8</i>	Acetogens; conduct extracellular electron transfer and reduce Fe ³⁺ .	[39]

Genus	Functional description	Reference
<i>Clostridium_sensu_stricto_1</i>	Acetogens; conduct extracellular electron transfer and reduce Fe ³⁺ .	[39]
<i>Ruminiclostridium</i>	Hydrolyze cellulosic substrates and ferment the resulting sugars to ethanol.	[40]
<i>Ruminiclostridium_1</i>	Hydrolyze cellulosic substrates and ferment the resulting sugars to ethanol.	[40]
<i>Mobilitalea</i>	Hydrolyze celluloses, peptides and fermenting amino acids, and carbohydrates to produce acetate, butyrate, lactate, CO ₂ , and H ₂ .	[41]
<i>Bacillus</i>	Fermentative bacteria; functioned as lignin degraders as they can decompose lignin with little consumption of cellulose.	[42]
<i>Anaerolinea</i>	Use carbohydrates to produce hydrogen and acetic acid.	[43]
<i>Candidatus_Caldatribacterium</i>	Thermophilic bacteria; produce acetate from sugar fermentation.	[44]
<i>Norank_o_D8A-2</i>	Syntrophic acetate-oxidizing bacteria (SAOB).	[45]
<i>Norank_o_MBA03</i>	SAOB.	[46]
<i>Gelria</i>	SAOB.	[12]
<i>Syntrophaceticus</i>	SAOB	[47]
<i>Defluviitalea</i>	Acid-producing bacteria.	[28]
<i>Norank_f_Syntrophomonadaceae</i>	Syntrophic butyrate oxidizers in anoxic environments.	[48]
<i>Norank_f_Synergistaceae</i>	Can degrade amino acids into volatile fatty acids and contribute to acidogenesis and acetogenesis via syntrophic relationships with methanogens.	[49]
<i>Norank_f_Lentimicrobiaceae</i>	Denitrification under anaerobic condition.	[50]
<i>Coprothermobacter</i>	Protein-degrading bacteria that established a syntrophy with hydrogenotrophic methanogens.	[51]
<i>Norank_p_Atribacteria</i>	Specialize in either primary fermentation of carbohydrates or secondary fermentation of organic acids, such as propionate.	[52]
<i>Mesotoga</i>	Anaerobic mesophilic acetogens.	[53]
<i>Hydrogenispora</i>	Ferment glucose to produce acetate, ethanol, and hydrogen.	[54]

Genus	Functional description	Reference
<i>Norank_f__Ruminococcaceae</i>	Fermentative bacteria with the ability of fiber degradation.	[55]
<i>Methanosaeta</i>	Acetoclastic methanogens that use acetate to produce methane.	[23]
<i>Methanosarcina</i>	Mixotrophic methanogens that can perform acetoclastic, hydrogenotrophic, and methylotrophic methanogenesis.	[23]
<i>Methanothermobacter</i>	Hydrogenotrophic methanogens that can use H ₂ as a precursor to produce methane.	[56]

The most abundant genera from T_{Stable} were *Methanosarcina* (30.58%) and *Anaerobaculum* (12.59%), followed by *Bacillus* (9.65%), *Syntrophaceticus* (4.84%), *Defluviitalea* (4.66%), *Mobilitalea* (3.97%), *Ruminiclostridium* (3.65%), *norank_o__D8A-2* (3.29%), *Methanothermobacter* (3.17%), and *norank_f__Syntrophomonadaceae* (3.08%). For $T_{\text{Collapsed}}$, the most abundant genera were *Defluviitoga* (48.93%) and *Thermoanaerobacterium* (29.08%), followed by *Clostridium_sensu_stricto_8* (4.92%), *Anaerolinea* (3.46%), *Methanosarcina* (2.77%), *Methanothermobacter* (2.70%), *Candidatus_Caldatibacterium* (1.33%), *norank_f__Anaerolineaceae* (1.29%), *Coprothermobacter* (1.27%), and *norank_f__Lentimicrobiaceae* (1.12%). Based on the summary of functional microorganisms in Table 3, the genera of *Anaerobaculum*, *Bacillus*, *Defluviitalea*, *Mobilitalea*, and *Ruminiclostridium* functioned as fermentative bacteria, and *Syntrophaceticus*, *norank_o__D8A-2*, and *norank_f__Syntrophomonadaceae* were classified as syntrophic bacteria. *Methanothermobacter* acted as hydrogenotrophic methanogen that can use H₂ as a precursor to produce methane. *Syntrophaceticus* and *norank_o__D8A-2* have been reported to be syntrophic acetate-oxidizing bacteria (SAOB), which possess the syntrophic acetate-oxidizing ability in cocultivation with a hydrogen-utilizing methanogen. The coexistence of *Methanothermobacter*, *Syntrophaceticus*, and *norank_o__D8A-2* showed that a syntrophic acetate-oxidizing association was developed in the thermophilic stable stage. When the process failure of the thermophilic AD of VW occurred at the $T_{\text{Collapsed}}$, the genus *Defluviitoga* were dominant, and the abundance increased from 4.66% at T_{Stable} to 48.93% at $T_{\text{Collapsed}}$. *Defluviitoga* was revealed to act as acetogens that degraded various biomass to produce acetate under the anaerobic condition. Another dominant genus was *Thermoanaerobacterium*, which uniquely existed in $T_{\text{Collapsed}}$. Meanwhile, *Thermoanaerobacterium* exhibited the ability to degrade lignocellulose to produce acetic, butyric, and lactic acids, and was classified as fermentative and acidogenic bacteria. These results indicate that both *Defluviitoga* and *Thermoanaerobacterium* were more tolerant to a highly acidic environment. Furthermore, the dominant functional microbial consortium changed from methanogens and fermentative bacteria at the stable stage to fermentative bacteria at the collapsed stage. All the methanogens decreased at the collapsed stage, particularly for the abundance of *Methanosarcina* that decreased from 30.58% at the stable stage to 2.77% at the collapsed stage. The sharp decrease of methanogens and the sharp increases of fermentative and acidogenic bacteria both explain the occurrence of the severe

accumulation of organic acids in the thermophilic collapsed stage. These results also verify that methanogens are vulnerable to the accumulation of VFAs.

3.5 Dynamics of methanogen communities

The dynamics of methanogens among the archaea at different temperatures are presented in Fig. 4. The acetoclastic methanogen of *Methanosaeta* (59.16%) and the mixotrophic methanogen of *Methanosarcina* (34.73%) were the dominant methanogens at the mesophilic stable condition, reflecting that the most dominant methanogen in the mesophilic reactor was acetoclastic methanogen, which uses acetate to produce methane. Meanwhile, the most dominant methanogens that existed in the thermophilic reactor were *Methanosarcina* and *Methanothermobacter*. *Methanothermobacter* was reported to function as a hydrogenotrophic methanogen, which can use H₂ as a precursor to produce methane (Table 3). The relative abundance of *Methanosarcina* accounted for 90.71% at the thermophilic stable condition, but sharply decreased to 63.61% when the severe acidification occurred at $T_{\text{Collapsed}}$. Nonetheless, the relative abundance of *Methanothermobacter* dramatically increased from 9.16% at T_{Stable} to 36.01% at $T_{\text{Collapsed}}$, indicating that *Methanothermobacter* is more favorable to survive under a severe acidic environment. These results demonstrate that temperature can change the methanogenesis pathway in a large extent. Furthermore, methanogenesis might shift from acetoclastic in the mesophilic stable stage to a hydrogenotrophic pathway in the thermophilic stage. However, this result should be further confirmed via genomic and metabonomics analyses.

3.6 Differences in microorganisms at mesophilic and thermophilic stable stages

The differences in the microorganisms at the mesophilic and thermophilic stable stages were compared to determine the effect of temperature on the distribution of dominant genera at the stable operational condition of the AD of VW (Fig. 5). The abundances of *Candidatus_Cloacamonas* ($P < 0.01$), *Propionimicrobium* ($P < 0.01$), *Proteiniphilum* ($P < 0.05$), *unclassified_f__Anaerolineaceae* ($P < 0.05$), and *Petrimonas* ($P < 0.05$) were significantly higher in the mesophilic stable stage. In contrast, the abundances of *Anaerobaculum* ($P < 0.01$) and *Mobilitalea* ($P < 0.05$) were significantly higher in the thermophilic stable stage. All of these genera were classified as fermentative and acidogenic bacteria (Table 3). *Candidatus_Cloacamonas* functioned as both syntrophic and fermentative bacteria. *Propionimicrobium*, *Proteiniphilum*, *unclassified_f__Anaerolineaceae*, and *Petrimonas* were all reported to ferment biomass to produce acetate. *Anaerobaculum* and *Mobilitalea* were both found to hydrolyze celluloses and other polysaccharides, peptides and fermenting amino acids, and carbohydrate to produce acetate, butyrate, lactate, CO₂, and H₂. The abundances of syntrophic bacteria of *Syntrophaceticus* ($P < 0.01$) and *norank_f__Syntrophomonadaceae* ($P < 0.05$) were both significantly higher in the thermophilic stable condition. *Norank_f__Syntrophomonadaceae* functioned as syntrophic butyrate oxidizers, and *Syntrophaceticus* belonged to SAOB, which are highly associated with hydrogen-utilizing methanogens via syntrophic acetate-oxidizing to produce methane [24]. The abundance of *Methanosaeta* ($P < 0.05$)

was significantly higher in the mesophilic stable condition, while the abundance of *Methanosarcina* ($P < 0.05$) was significantly higher in the thermophilic stable condition.

An overall comparison of the functional microbial consortium is exhibited in Fig. 6. The microorganisms were classified into five functional types, including fermentative and acidogenic bacteria, syntrophic organic acid oxidation bacteria, SAOB, homoacetogenic bacteria, and methanogens. The syntrophic acetate-oxidizing relationship between the syntrophic bacteria of *Syntrophaceticus* and the hydrogenotrophic methanogens of *Methanothermobacter* and *Methanosarcina* developed under the thermophilic stable condition. For the mesophilic reactor, acetoclastic methanogenesis was the dominant process in the overall stable operation. Pap et al. indicated that a high temperature will accelerate the degradation of most types of organic substrate; thus, speeding up the accumulation of VFAs [25]. Generally, *Methanosaeta* was more vulnerable to the stress of VFAs compared to *Methanosarcina* [13]. Therefore, the dynamic shifts of the functional microbial community from acetoclastic methanogenesis to syntrophic acetate-oxidizing coupled with hydrogenotrophic methanogenesis resulted in the accumulation of VFAs under the thermophilic condition.

Conclusion

With the severe accumulation of VFAs, system failure occurred due to the imbalance between fermentative bacteria and methanogens. The diversity and richness of the microbial community under the mesophilic condition were higher than those under the thermophilic condition. Temperature changes significantly affected microbial shifting. Furthermore, syntrophic acetate-oxidizing coupled with hydrogenotrophic methanogenesis was the dominant anaerobic reaction in the thermophilic reactor, while it was mainly acetoclastic methanogenesis in the mesophilic reactor. The dominant acidogens, syntrophic bacteria, and methanogens were *Candidatus_Cloacamonas*, *norank_f__Synergistaceae*, *Methanosaeta*, and *Methanosarcina* in the mesophilic stable stage, and *Anaerobaculum*, *Syntrophaceticus*, *Methanosarcina*, and *Methanothermobacter* in the thermophilic stable stage.

Abbreviations

VW: Vegetable waste; AD: Anaerobic digestion; VS: Volatile solid; TS: Total solid; CSTR: Continuous stirred-tank reactor; OLR: Organic loading rate; HRT: Hydrolytic retention time; TAN: Total ammonium nitrogen; VFA: Volatile fatty acids; VMPPR: Volumetric methane production rate; TVFA: Total volatile fatty acids.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published Article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Notes

^aDiversity of the microbial community, ^bRichness of microbial species, ^cDistribution evenness of the microbial community.

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Authors' contributions

Tianjie Ao, Liping Wan, and Dong Li designed this study. Tianjie Ao, Zhijie Xie, and Pan Zhou performed the experiments. Tianjie Ao and Dong Li analyzed the data. Tianjie Ao wrote the manuscript. Dong Li and Liping Wan supervised the project and revised the manuscript. All authors read and approved the final manuscript.

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References

1. Li D, et al. Instability diagnosis and syntrophic acetate oxidation during thermophilic digestion of vegetable waste. *Water Res.* 2018;139:263–71.

2. Arhoun B, et al. Anaerobic co-digestion of mixed sewage sludge and fruits and vegetable wholesale market waste: Composition and seasonality effect. *J Water Process Eng.* 2019;31:100848.
3. Perazzolo F, et al. Effect of mechanical separation on emissions during storage of two anaerobically codigested animal slurries. *Agr Ecosys Environ.* 2015;207:1–9.
4. Zuo Z, et al. Performance enhancement of leaf vegetable waste in two-stage anaerobic systems under high organic loading rate: Role of recirculation and hydraulic retention time. *Appl Energy.* 2015;147(0):279–86.
5. Li D, et al. Instability mechanisms and early warning indicators for mesophilic anaerobic digestion of vegetable waste. *Bioresour Technol.* 2017;245(Pt A):90–7.
6. Vats N, Khan AA, Ahmad K. Effect of substrate ratio on biogas yield for anaerobic co-digestion of fruit vegetable waste & sugarcane bagasse. *Environ Technol Innovation.* 2019;13:331–9.
7. Zhang L, et al. Mesophilic and thermophilic anaerobic digestion of soybean curd residue for methane production: Characterizing bacterial and methanogen communities and their correlations with organic loading rate and operating temperature. *Bioresour Technol.* 2019;288:121597.
8. Ryue J, et al. Comparative effects of GAC addition on methane productivity and microbial community in mesophilic and thermophilic anaerobic digestion of food waste. *Biochem Eng J.* 2019;146:79–87.
9. APHA. *Standard Methods for the Examination of Water and Wastewater.* 21st Edition[R], APHA, 1998.
10. Li D, et al. Instability diagnosis and syntrophic acetate oxidation during thermophilic digestion of vegetable waste. *Water Res.* 2018;139:263–71.
11. Cao Q, et al. Methane oxidation coupled to denitrification under microaerobic and hypoxic conditions in leach bed bioreactors. *Sci Total Environ.* 2019;649:1–11.
12. Zhu X, et al., *Differences of methanogenesis between mesophilic and thermophilic in situ biogas-upgrading systems by hydrogen addition.* *J Ind Microbiol Biotechnol*, 2019.
13. Lee J, Hwang S. Single and combined inhibition of *Methanosaeta concillii* by ammonia, sodium ion and hydrogen sulfide. *Bioresour Technol.* 2019;281:401–11.
14. Jiang Y, Heaven S, Banks CJ. Strategies for stable anaerobic digestion of vegetable waste. *Renew Energ.* 2012;44:206–14.
15. Chen H, et al. Nitrogen removal performance and microbial community of an enhanced multistage A/O biofilm reactor treating low-strength domestic wastewater. *Biodegradation.* 2018;29(3):285–99.
16. Greses S, et al. Microbial community characterization during anaerobic digestion of *Scenedesmus* spp. under mesophilic and thermophilic conditions. *Algal Res.* 2017;27:121–30.
17. Vanwonterghem I, et al. Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. *Sci Rep.* 2015;5:8496.
18. Wang M, et al. Methane production characteristics and microbial community dynamics of mono-digestion and co-digestion using corn stalk and pig manure. *Int J Hydrogen Energ.* 2017;42(8):4893–901.

19. Militon C, et al. Ecological significance of Synergistetes in the biological treatment of tuna cooking wastewater by an anaerobic sequencing batch reactor. *Environ Sci Pollut Res.* 2015;22(22):18230–8.
20. Nobu MK, et al., *Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor.* *Isme J*, 2015. 9.
21. Zamorano-López N, et al. Thermophilic anaerobic conversion of raw microalgae: Microbial community diversity in high solids retention systems. *Algal Res.* 2019;41:101533.
22. Gulhane M, et al. Study of microbial community plasticity for anaerobic digestion of vegetable waste in Anaerobic Baffled Reactor. *Renew Energ.* 2017;101:59–66.
23. Wang M, et al. The dynamic changes and interactional networks of prokaryotic community between co-digestion and mono-digestions of corn stalk and pig manure. *Bioresour Technol.* 2017;225:23–33.
24. Werner JJ, et al., *Microbial community dynamics and stability during an ammonia-induced shift to syntrophic acetate oxidation.* *Appl Environ Microbiol*, 2014. 80.
25. Pap B, et al. Temperature-dependent transformation of biogas-producing microbial communities points to the increased importance of hydrogenotrophic methanogenesis under thermophilic operation. *Bioresour Technol.* 2015;177:375–80.
26. Li L, et al. Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: Relationship between community structure and process stability. *Bioresour Technol.* 2015;189:113–20.
27. MerlinChristy P, Gopinath LR, Divya D. *A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms.* *Renew Sust Energ Rev.*, 2014. 34.
28. He Q, et al. Investigation of foaming causes in three mesophilic food waste digesters: reactor performance and microbial analysis. *Sci Rep.* 2017;7(1):13701.
29. Lu Q, et al. Organic matter rather than salinity as a predominant feature changes performance and microbiome in methanogenic sludge digesters. *J Hazard Mater.* 2019;377:349–56.
30. Ikeda M, et al. *Propionimicrobium lymphophilum* and *Actinotignum schaalii* bacteraemia: a case report. *New Microbes New Infect.* 2017;18:18–21.
31. Nakasaki K, et al., *Characterizing the microbial community involved in anaerobic digestion of lipid-rich wastewater to produce methane gas.* *Anaerobe*, 2019: p. 102082.
32. Hahnke S, et al. Description of *Proteiniphilum saccharofermentans* sp. nov., *Petrimonas mucosa* sp. nov. and *Fermentimonas caenicola* gen. nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus *Proteiniphilum*. *Int J Syst Evol Microbiol.* 2016;66(3):1466–75.
33. Yang H, et al. Comparison of three biomass-retaining reactors of the ASBR, the UBF and the USR treating swine wastewater for biogas production. *Renew Energ.* 2019;138:521–30.
34. Harb M, et al. Differences in microbial communities and performance between suspended and attached growth anaerobic membrane bioreactors treating synthetic municipal wastewater. *Environmental Science: Water Res Technol.* 2015;1(6):800–13.

35. Goncalves Pessoa RB, et al. The genus *Aeromonas*: A general approach. *Microb Pathog.* 2019;130:81–94.
36. Stolze Y, et al. Identification and genome reconstruction of abundant distinct taxa in microbiomes from one thermophilic and three mesophilic production-scale biogas plants. *Biotechnol Biofuels.* 2016;9(1):156.
37. Maus I, et al. Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. *Biotechnol Biofuels.* 2016;9:171.
38. Stolze Y, et al. Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. *Biotechnol Biofuels.* 2015;8(1):14.
39. Xiao Y, et al. Improved biogas production of dry anaerobic digestion of swine manure. *Bioresour Technol.* 2019;294:122188.
40. Singh N, et al. Influence of substrate loadings on the consolidated bioprocessing of rice straw and sugarcane bagasse biomass using *Ruminiclostridium thermocellum*. *Bioresour Technol Rep.* 2019;7:100138.
41. FitzGerald JA, et al. Trace element supplementation is associated with increases in fermenting bacteria in biogas mono-digestion of grass silage. *Renew Energ.* 2019;138:980–6.
42. Caruso M, et al. Recent Updates on the Use of Agro-Food Waste for Biogas Production. *Appl Sci.* 2019;9(6):1217.
43. Feng S, et al. Insights into the microbial community structure of anaerobic digestion of municipal solid waste landfill leachate for methane production by adaptive thermophilic granular sludge. *Electron J Biotechnol.* 2019;39:98–106.
44. Lee J, et al. 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.* 2017;245(Pt A):689–97.
45. Lee J, et al. Magnetite as an enhancer in methanogenic degradation of volatile fatty acids under ammonia-stressed condition. *J Environ Manage.* 2019;241:418–26.
46. Zheng D, et al. Identification of novel potential acetate-oxidizing bacteria in thermophilic methanogenic chemostats by DNA stable isotope probing. *Appl Microbiol Biotechnol.* 2019;103(20):8631–45.
47. Westerholm M, Roos S, Schnurer A. *Syntrophaceticus schinkii* gen. nov., sp nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. *Fems Microbiol Letters.* 2010;309(1):100–4.
48. Liu PF, Qiu QF, Lu YH. Syntrophomonadaceae-Affiliated Species as Active Butyrate-Utilizing Syntrophs in Paddy Field Soil. *Appl Environ Microbiol.* 2011;77(11):3884–7.
49. Ferguson RMW, Coulon F, Villa R. Organic loading rate: A promising microbial management tool in anaerobic digestion. *Water Res.* 2016;100:348–56.

50. Tian Q, et al. Microbial uniqueness of architecture modified loofah sponge as biological filler for efficient nitrogen removal. *Bioresour Technol Rep.* 2018;3:95–101.
51. Gagliano MC, et al. Thermophilic anaerobic digestion of thermal pretreated sludge: role of microbial community structure and correlation with process performances. *Water Res.* 2015;68:498–509.
52. Nobu MK, et al. Phylogeny and physiology of candidate phylum /`Atribacteria/' (OP9/JS1) inferred from cultivation-independent genomics. *Isme J.* 2016;10(2):273–86.
53. Lee J, et al. Microbial communities underpinning mesophilic anaerobic digesters treating food wastewater or sewage sludge: A full-scale study. *Bioresour Technol.* 2018;259:388–97.
54. Liu Y, et al. *Hydrogenispora ethanolica* gen. nov., sp. nov., an anaerobic carbohydrate-fermenting bacterium from anaerobic sludge. *Int J Syst Evol Microbiol.* 2014;64(5):1756–62.
55. Liu C, et al. Dynamic Alterations in Yak Rumen Bacteria Community and Metabolome Characteristics in Response to Feed Type. *Front Microbiol.* 2019;10:1116.
56. Chen Y, et al. Digestive performance of sludge with different crop straws in mesophilic anaerobic digestion. *Bioresour Technol.* 2019;289:121595.

Figures

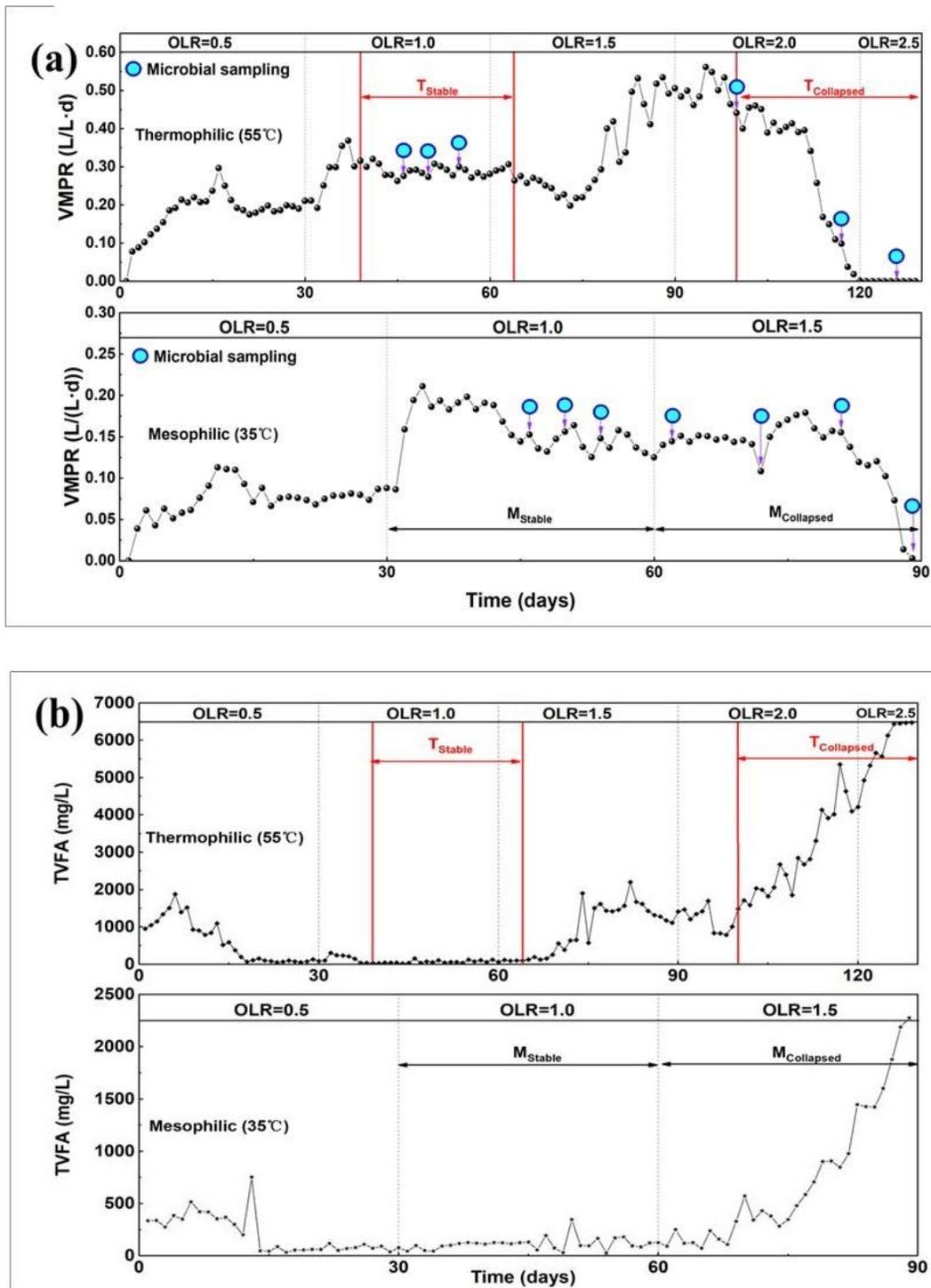


Figure 1

Daily changes of the (a) volumetric methane production rate (VMPR) and the (b) total volatile fatty acids (TVFA) under the mesophilic and thermophilic conditions (OLR: organic loading rate, g VS/(L·d)).

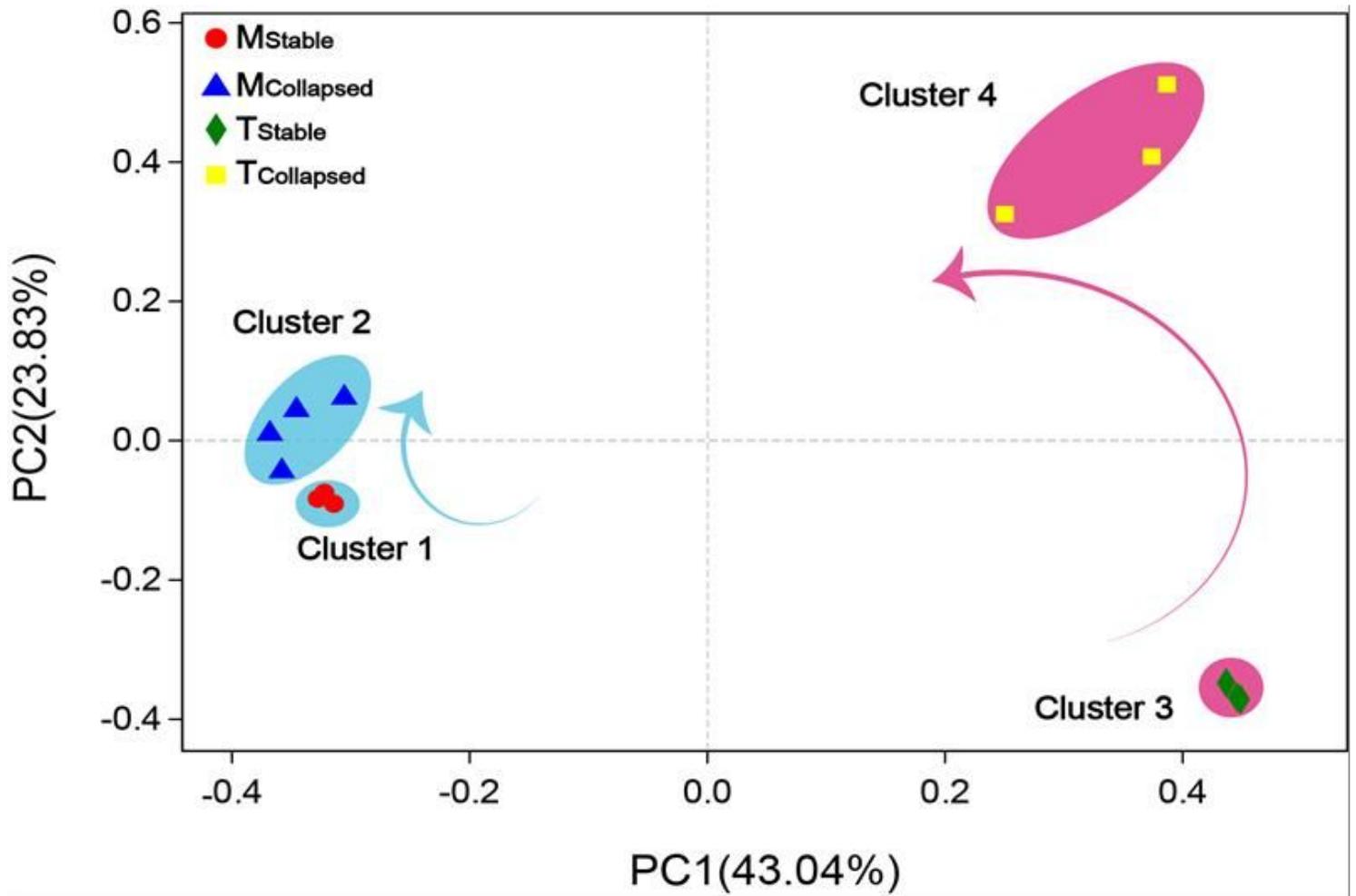


Figure 2

Difference of microbial communities at genus level under the mesophilic and thermophilic conditions revealed by the principal coordinate analysis (The blue arrow indicated that the mesophilic microbial consortium shifted over time and the pink arrow indicated that the thermophilic microbial consortium shifted over time).

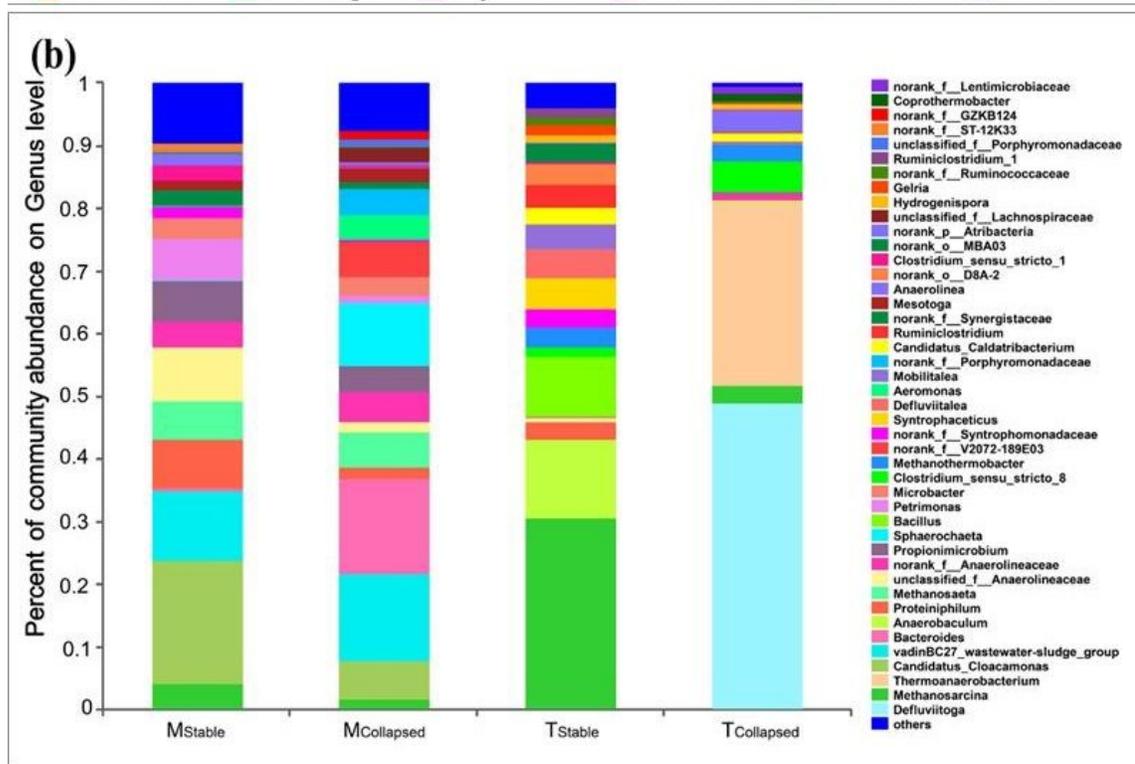
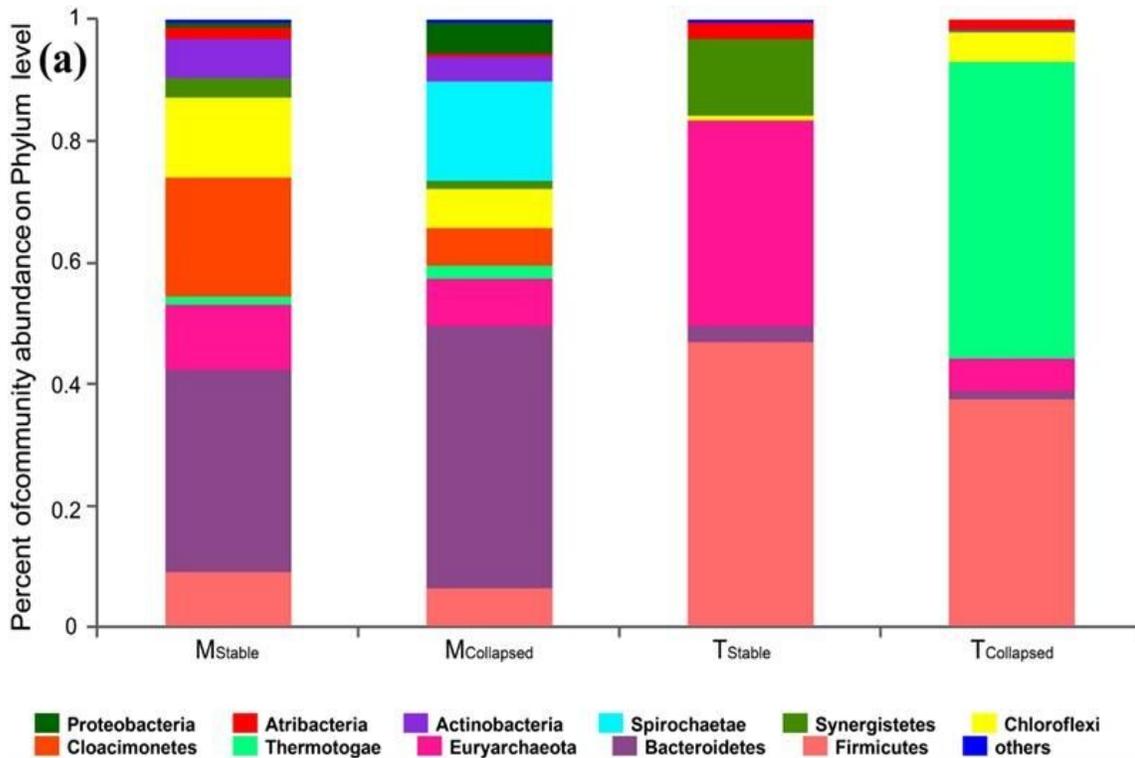


Figure 3

Community abundance and dynamics of microorganisms at (a) phylum and (b) genus levels under the mesophilic and thermophilic conditions (The data of the microbial abundance was exhibited via the average value of sampling point at each stage).

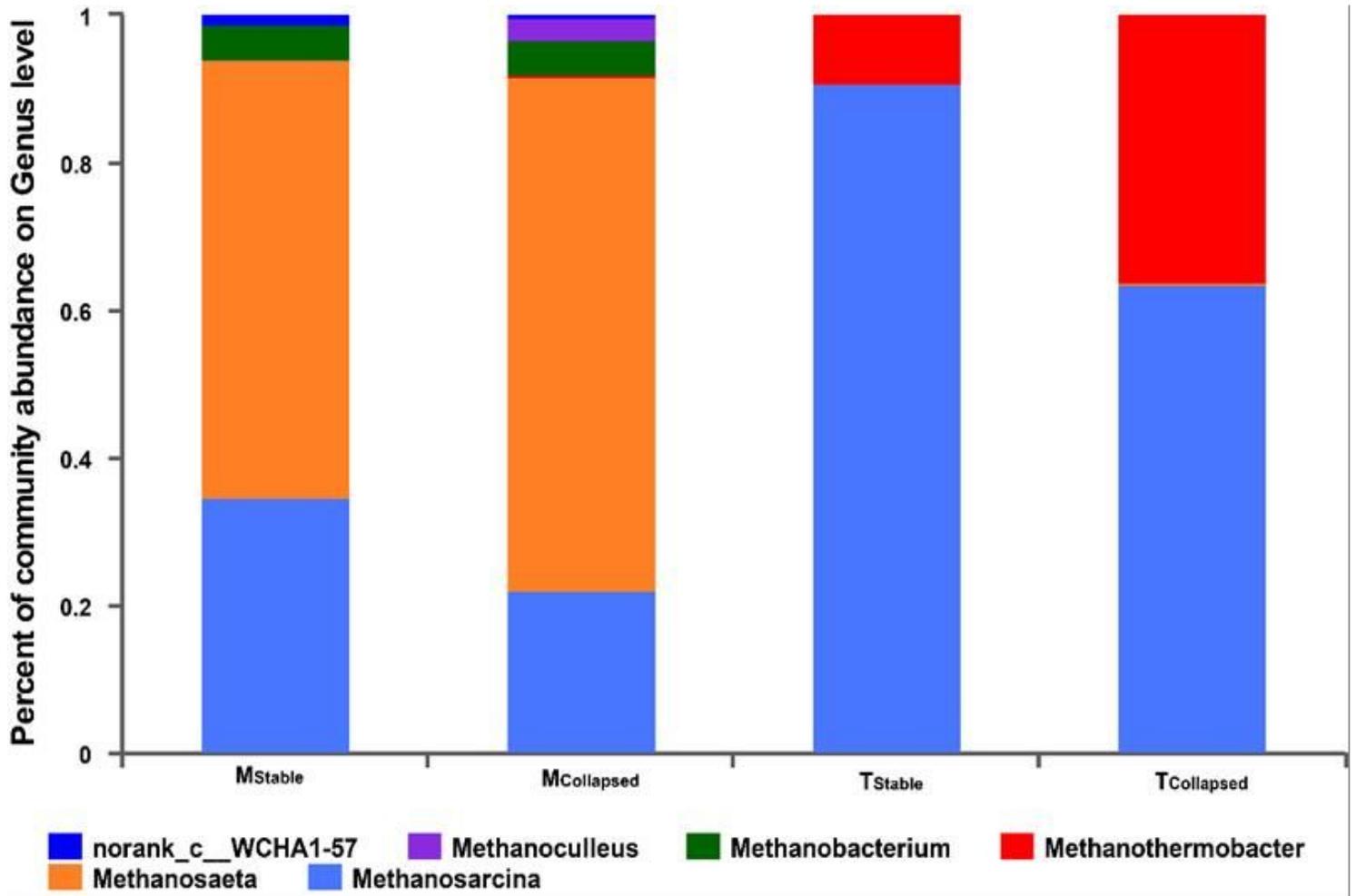


Figure 4

Community abundance and dynamics of methanogens at genus level under the mesophilic and thermophilic conditions (The data of the microbial abundance was exhibited via the average value of sampling point at each stage).

Welch's t-test bar plot on Genus level

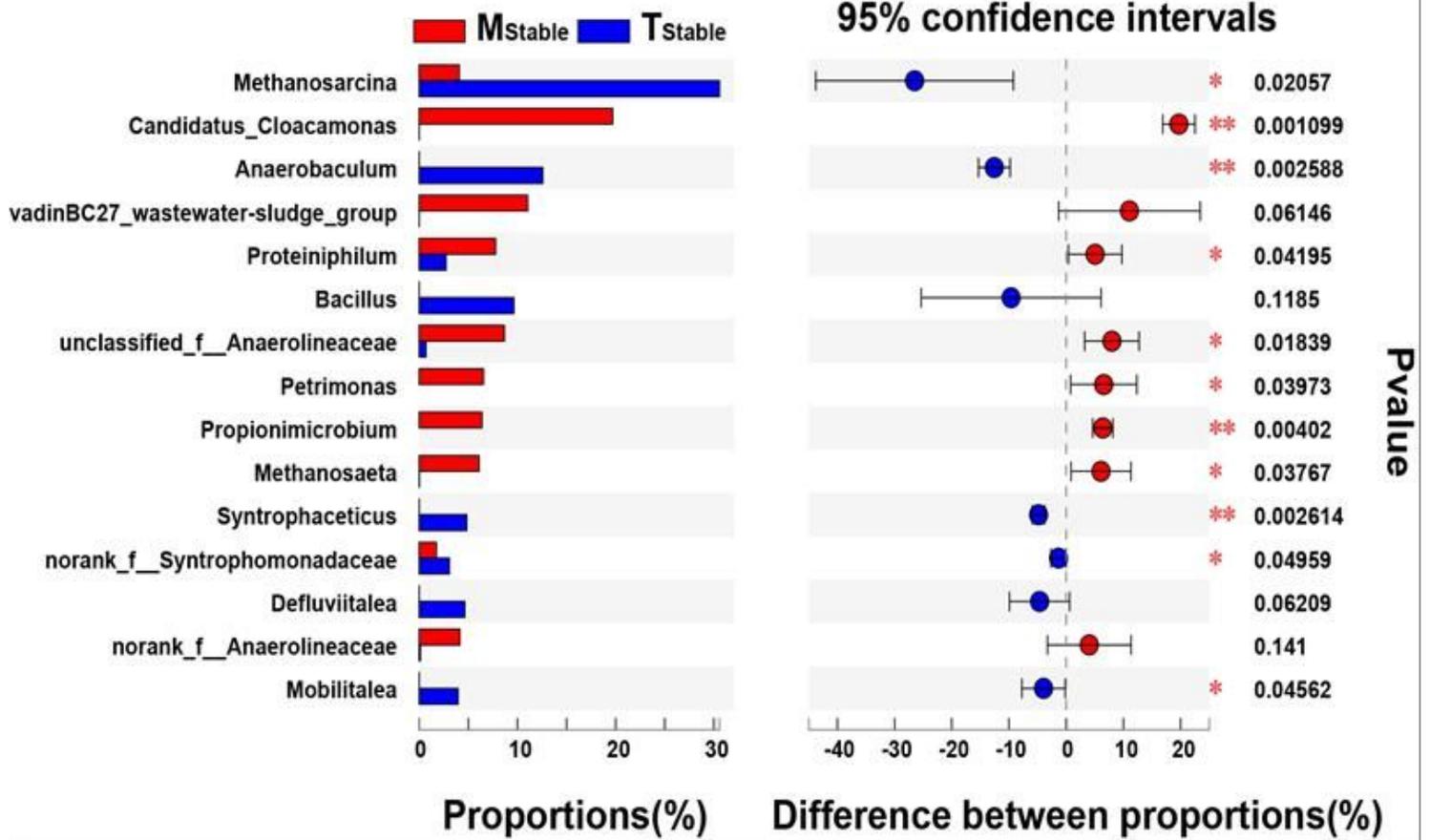


Figure 5

Microbial differences between the mesophilic stable (MStable) and thermophilic stable (TStable) stages. (* indicates $P \geq 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$).

