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Effects of high solid content and straw proportion on volatile fatty acids production from straw, sludge and food wastes: performance and microbial community characteristics

Yu-Wei Chen[1,2,3], Gao Feng[2,3], Xia Hong[4], Meng Wang[5], Quan Zhang[5], Zhao-Yong Sun[2,3,6], Ya-Ting Chen[1,2,3*] and Yue-Qin Tang[2,3,6]

Abstract

Anaerobic digestion (AD) is an efficient technology for treating organic solid wastes, and the volatile fatty acids (VFAs) produced during AD have significant value due to their wide range of applications and higher added value compared to methane. This study investigated the long-term effects of high solid content and straw proportion in mixed substrates (straw, sludge, and food wastes) on VFAs production through semi-continuous reactors under thermophilic and mesophilic conditions. Results showed that both reactors achieved a maximum VFAs concentration of ~ 22 g/L as the straw proportion increased to 50%. Acetate (48.3 – 64.5%) was the main component of produced VFAs in both reactors, while butyrate and propionate production in thermophilic temperature were superior compared to mesophilic conditions. Microbial community analysis revealed that *Defluviitoga* plays a pivotal role in acidogenesis within both reactors; besides, unclassified Hungateiclostridiaceae and *Caproiciproducen* were found to be dominant in thermophilic reactor, while Lachnospiraceae_NK3A20_group and Rikenellaceae_RC9_gut_group were essential for VFAs production under mesophilic conditions. These findings provide valuable insights for the biotechnological exploration of acidogenic fermentation for large-scale mechanized production of VFAs from agricultural wastes.

Keywords Anaerobic co-digestion, Lignocellulosic biomass, Target VFAs production, Semi-continuous reactor, Microbial community

*Correspondence:
Ya-Ting Chen
cytscu1101@scu.edu.cn
[1] Institute for Disaster Management and Reconstruction, Sichuan University, Chengdu 610207, Sichuan, China
[2] College of Architecture and Environment, Sichuan University, Chengdu 610065, Sichuan, China
[3] Sichuan Environmental Protection Key Laboratory of Organic Wastes Valorisation, Chengdu, Sichuan, China
[4] Sinopec Shanghai Engineering Co. Ltd, Shanghai, China
[5] Sinopec (Dalian) Research Institute of Petroleum and Petrochemicals Co. Ltd, Dalian, Liaoning, China
[6] Engineering Research Centre of Alternative Energy Materials and Devices, Ministry of Education, Chengdu, Sichuan, China

Introduction

Anaerobic digestion (AD) has been considered an efficient technology to treat organic solid wastes, and the product methane produced in the process can be used as fuel for energy recovery [1, 2]. Based on the relatively limited application for methane, the main intermediate products in AD process, volatile fatty acids (VFAs), hold greater appeals [3, 4]. VFAs are regarded as more valuable products than methane due to their wider application and higher add-value, such as they can be further converted into biofuel and bioplastic polyhydroxyalkanoates (PHA), used as carbon sources for denitrification during

sewage treatment [5, 6]. Approximately 90% of VFAs in the market are supplied by petrochemical-derived processes [7].

Current studies of acidogenic fermentation most focus on readily degradable feedstocks such as fruit and food waste [6, 8]. Compared to which, lignocellulosic biomass is considered as one of the most potential and abundant renewable resources [4, 9]. Among various kinds of lignocellulosic biomass, agriculture straw currently represents the largest reserves in China, which production could reach about 800 million tons per year, with one fifth of the world's total straw resources [4, 10].

The main drawback of using agriculture straw for VFAs production is its recalcitrance to biological degradation [4]. Previous studies have shown that co-fermentation of straw with sludge or manure can promote the anaerobic fermentation, which showed that mixed substrates are supposed to improve the hydrolysis phase of AD process, as such wastes are rich in nitrogen, organic matter, and trace elements necessary for microbial growth [11, 12]. In addition, the mixing ratio and solid content of substrates have been considered as important factors governing the efficiency of anerobic co-digestion (AcoD) process and VFAs concentration [13, 14]. Due to the presence of straw in the mixed substrates, most studies have controlled the total solids (TS) content of the substrates to less than 5%, high solid content of mixed substrates has poorly been studied for producing VFAs [7, 15, 16].

Currently, most studies have focused on the short-term feasibility of VFAs production from mixed straw substrates fermentation through batch experiments, while which is relatively unrealistic for applications on a large scale due to its ambiguous influence on engineering applications [7, 9, 11]. Based on this, there are significant prospects to investigate the long-term operation of acidogenic fermentation with continuous reactors to achieve large-scale mechanized production. Anaerobic co-digestion of various agricultural wastes in CSTR has been successfully implemented at practical scales [17, 18]. However, there are rarely relevant continuous reactors while many treated other readily degradable substrates [8, 19]. VFAs composition is the most important in acidogenic fermentation, while microbial community governing the metabolic pathway of substrates, then further bio-convert to target VFAs [20, 21]. Therefore, clarifying the influence of the long-term acidogenic fermentation process on microorganisms is the basis for regulating the generation of target acids, while batch experiments are limited.

This study aimed to investigate the long-term effects of high solid content and the proportion of straw in mixed substrates (straw, sludge, and food wastes) on VFAs production by conducting semi-continuous reactors in thermophilic and mesophilic conditions, respectively. The knowledge obtained will enable biotechnological exploration of manipulating the microbial community to improve the yield of target VFAs.

Materials and methods

Bioreactor fermentation system

Two completely stirred-tank AD reactors (3.0 L with a working volume of 2.4 L, MDL, B. E. Marubishi Co., Ltd.) utilized in this study were identical and equipped with an improved universal mixer bracket (Fig. S1). It has been demonstrated that thermophilic conditions offer greater advantages for bioreactor efficiency compared to mesophilic conditions, as they can facilitate the dissolution and production of hydrated organic matters, resulting in higher digestion rates [22]. However, mesophilic conditions are more conducive to microbial survival to produce higher concentrations of VFAs than thermophilic conditions [23]. Therefore, two reactors were respectively operated under thermophilic (53 ± 1 ℃, H) and mesophilic (35 ± 1 ℃, L) conditions to investigate the optimal conditions for VFAs production from straw, sludge and food wastes. Each reactor was equipped with a thermostat regulator and a regulated stirrer. The agitation rate of the two reactors was kept at 100 rpm and pH was controlled at 5.5–6.0 by pumping in 2 M NaOH.

Seed sludge gathered from different anaerobic digesters treating various wastes were used as inoculum. The two reactors were fed with the same substrates, including straw, food waste, and sludge, as shown in Table 1. The reactors were operated by feeding the mixed substrates every 2-day using the draw-and-fill method. For every feeding cycle, the fermentation liquid was taken from the reactor before feeding. Following the initial addition of the seed sludge (total solids, TS: ~ 5%) into the bioreactor, no feed or withdrawal was allowed for the first 2 days. When finishing the first cycle of reaction, we discharged 480 mL of fermentation liquid from the system, remained mixture was retained and then we fed substrates while started the next cycle. The amount of gas was measured using a pneumatic trough. The effluent was sampled and used for the analysis of TS, volatile total solids (VTS), soluble total organic carbon (STOC), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), and VFAs. Samples for microbial community analysis were periodically taken (as shown in Figs. 1 and 2) and stored at − 20 ℃.

The long-term operational process consisted of three phases (F1, F2, and F3; Table 2). The F1 phase (Days 0–105) was the startup phase of reactors with a hydraulic retention time (HRT) of 100 days; in the F2 phase (Days 105–189), the TS of substrate was adjusted to 10% with organic loading rate (OLR) of 1 g VTS/L/d and HRT of

Table 1 TS and VTS of inoculum and substrates

	Materials	TS (%FM)	VTS (%FM)
Inoculum (seed sludge)	Anaerobic digesters treating grain wastes	2.52	1.85
	Anaerobic digesters treating food wastes	2.13	1.45
	Anaerobic mesophilic digesters treating cellulose wastes	1.09	0.57
	Anaerobic thermophilic digesters treating cellulose wastes	0.92	0.36
	Anaerobic digesters treating fruit wastes	7.94	4.54
Substrate	Straw	23.8	22.9
	Food wastes	12.8	12.5
	Sludge	16.5	10.0

100 days; during the F3 phase (Days 189–368), the reactors were operated at the OLR of 2 g VTS/L/d and HRT of 50 days, and the VTS ratio of straw, food and sludge in the feeding changed from 1:1:1 to 3:2:1.

Analytical methods

TS and VTS were analyzed on the basis of methods. After centrifugation (12,000 r/min for 10 min) of the fermented liquid, the supernatant filtered through 0.22 μm membrane was used for analysis. STOC was measured by a TOC auto-analyzer (TOC-VE, Shimadzu, Japan) [24]. TCOD and SCOD in the supernatant were measured via a DR/2400 spectrophotometer system (HACH, USA).The VFAs concentrations were determined by a high-performance liquid chromatography (SCL-10A VP, Shimadzu, Japan). Organic elements (C, N) were measured by an elemental analyzer (Vario Elcube, Elementar) after the samples were dried with oven and ground. Methane, H_2, and CO_2 contents were measured by gas chromatography (GC-2014C, Shimadzu, Japan).

Microbial community analysis

Total DNA and RNA of microbial community were extracted using cetyl-trimethyl ammonium bromide (CTAB) method on day 0 (H1, L1), 9 (H2, L2), 33 (H3, L3), 69 (H4, L4), and 103 (H5, L5) of the F1 phase, days 129 (H6, L6), 157 (H7, L7), and 185 (H8, L8) of the F2 phase, and days 213 (H9, L9), 229 (H10, L10), 249 (H11, L11), 269 (H12, L12), and 289 (H13, L13) of the F3 phase [25]. The quality and concentration of DNA and RNA were measured by agarose gel electrophoresis (1% w/v) and spectrophotometer (NanoDrop 2000, Thermo, Japan), respectively. Total RNA extracts were reverse transcribed with random hexamer primers using the PrimeScriptRT Reagent Kit with gDNA Eraser (Takara, Kusatsu, Japan) according to the manufacturer's protocol. The V4-V5 hypervariable regions of the 16S rRNA genes were amplified using primer set 515F (5′-GTG CCAGCMGCCGCGGTAA-3′) and 909R (5′-CCCCGY CAATTCMTTTRAGT-3′). PCR of each sample was performed in triplicate and PCR products were pooled to eliminate PCR bias. The equimolar ratio of PCR products for each sample was combined, purified with a QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA, USA), and sequenced on Illumina MiSeq™ platform by Majorbio (Shanghai, China). The raw data were processed based on the i-sanger cloud platform (www.i-sanger.com). Raw fastq files were demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH. Operational taxonomic units (OTU) were clustered at a 97% similarity cutoff by UPARSE. A taxonomic analysis was conducted using a Bayesian classifier based on the Ribosomal Database Project (RDP), according to the Silva 132 reference database. The sequencing data are available at the NCBI database (PRJNA1117803 and PRJNA1117795).

Statistic analysis

Spearman rank correlation coefficients and corresponding *p*-values were calculated by R (https://www.r-project.org/). Figures in this study were constructed using Origin 2021 (Origin Lab, Massachusetts, USA), and Adobe Illustrator 2021 (Adobe Illustrator, Ireland).

Results and discussion

Physicochemical and acidogenic performance in H and L reactors

The physicochemical parameters of H and L reactors are shown in Figs. 1 and 2. The performance of thermophilic and mesophilic reactors remained relatively stable and comparable through the F1 and F2 phases. In the F3 phase, both H and L reactors achieved maximum VFAs concentrations of 22.3 g/L and 22.7 g/L, respectively. In reactor H, the composition analysis of VFAs revealed that acetate was the predominant component (50.1 ± 4.3%), followed by butyrate (25.6 ± 7.6%) and propionate (19.1 ± 8.3%) (Fig. 1C). In reactor L, the main components of VFAs were primarily acetate (53.8 ± 2.1%), butyrate (20.7 ± 2.3%), and propionate (15.2 ± 3%) (Fig. 2C).

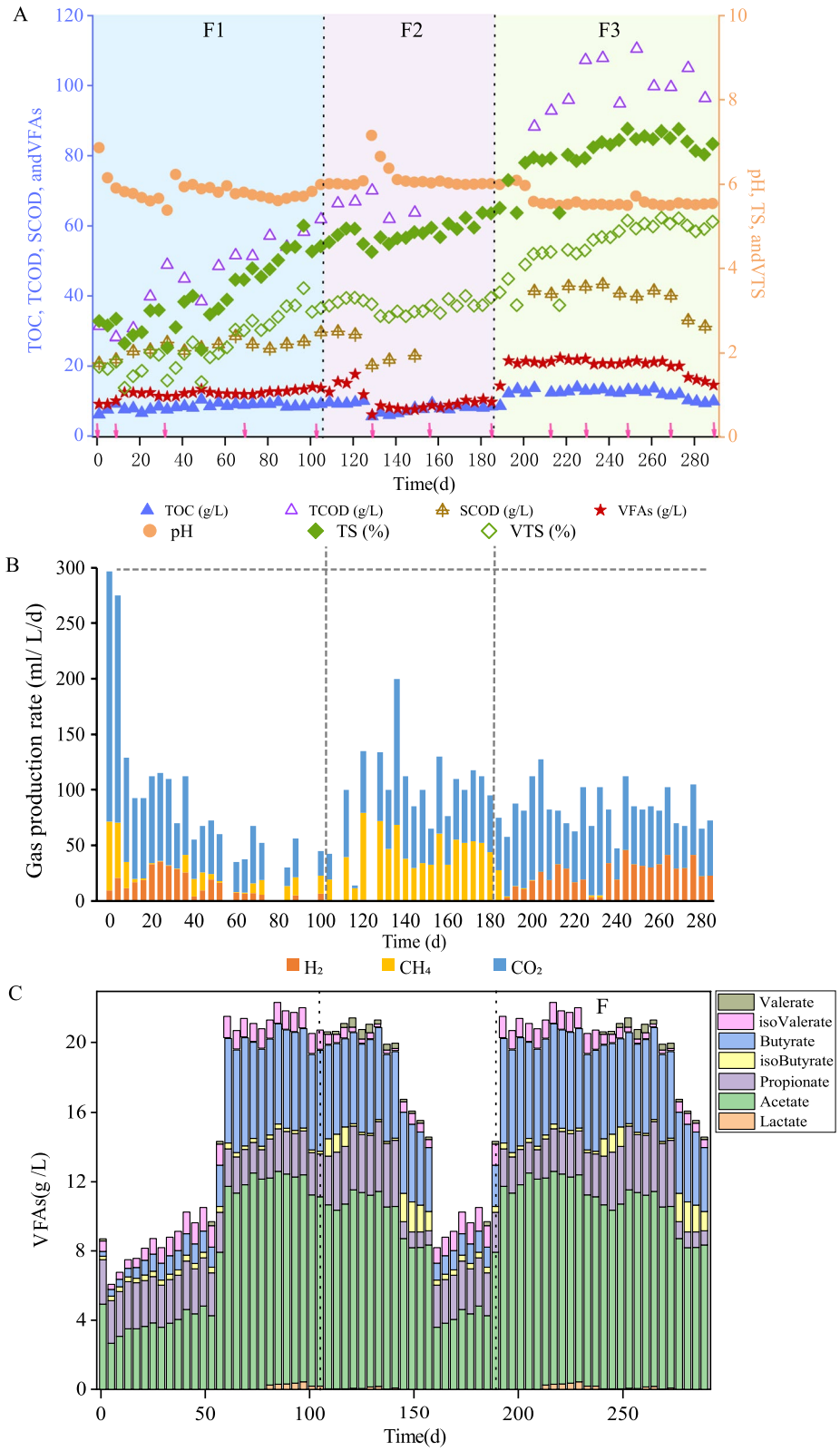

Fig. 1 The operating conditions and parameters of thermophilic reactor H during straw, food wastes and sludge co-fermentation

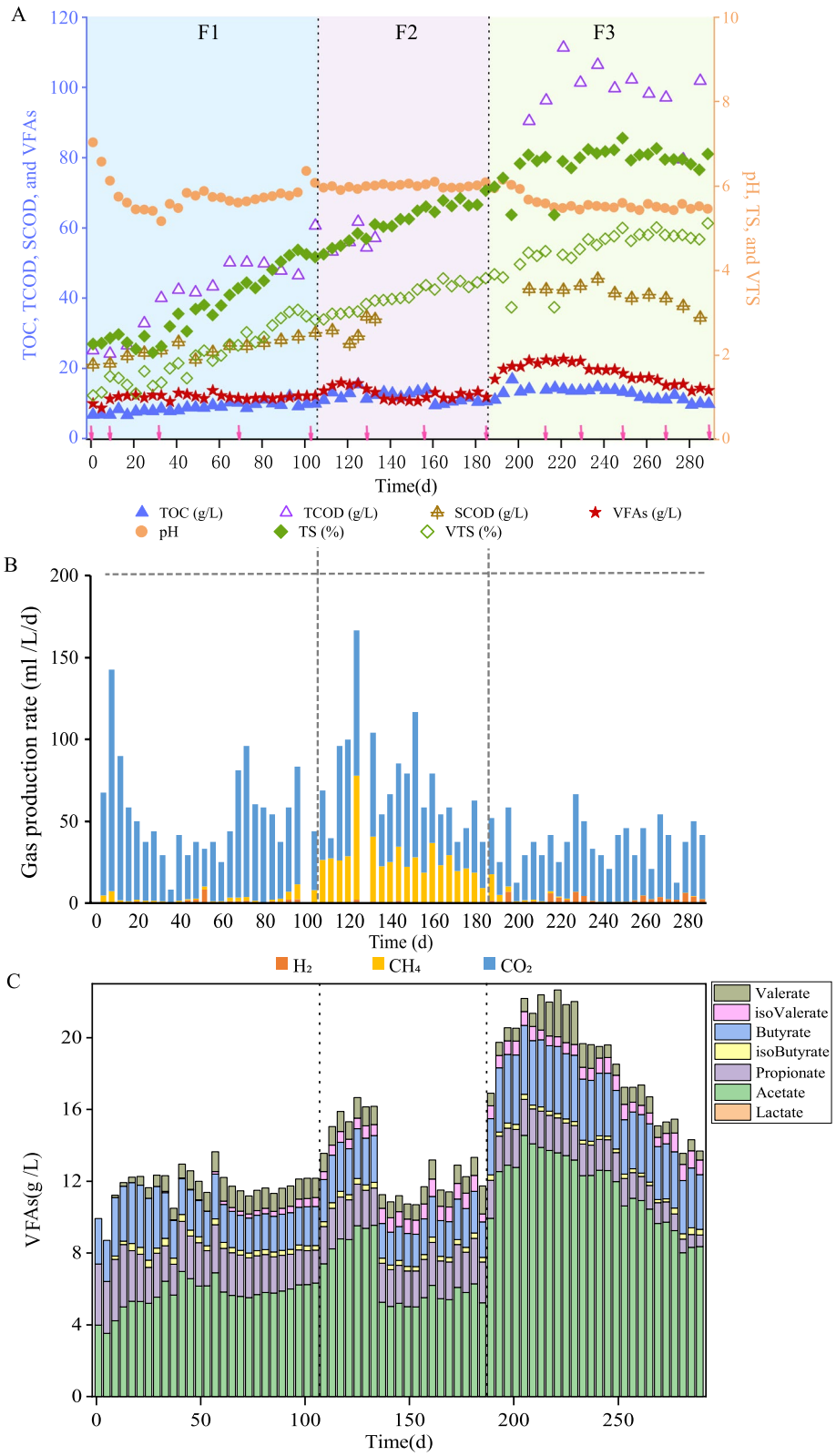

Fig. 2 The operating conditions and parameters of mesophilic reactor L during straw, food wastes and sludge co-fermentation

Table 2 Operating parameters of thermophilic and mesophilic reactors at different phases

	F1	F2	F3
Proportion of straw, food wastes, and sludge VTS in substrates	1:1:1	1:1:1	3:2:1
Substrates TS (%)	5.2	10.3	9.7
Substrates VTS (%)	4.1	8.2	8.3
Substrates C/N ratio	20	20	40
Organic loading rate (g VTS/L/day)	0.5	1	2
Hydraulic retention time (day)	100	100	50

Furthermore, throughout phase F3, progressive stabilization was achieved in both H and L reactors, with VTS degradation efficiency maintained at approximately 44.6 – 45.5%. Simultaneously, the STOC reached about 12.3 – 12.6 g/L. In terms of gas production, minimal methane generation was observed during phase F3, with CO_2 respectively accounting for 70.2% and 90.9% as the primary gas components in H and L reactors. Additionally, the solubilization of mixed substrates during the AD process was represented as the ratio of released SCOD to TCOD [26]. In this study, the substrate release degrees in H reactor during the three phases ranged from 0.38 to 0.58 g SCOD/g TCOD, while in L were 0.40 – 0.65 g SCOD/g TCOD.

In this study, both thermophilic and mesophilic reactors achieved a maximum concentration of VFAs when the VTS ratio of straw, food, and sludge in the feeding was adjusted from 1:1:1 to 3:2:1. This suggests that increasing the proportion of straw in mixed substrates fermentation may be advantageous for obtaining higher VFAs production and yields. To support this, Zhou et al. [11] demonstrated that increasing the proportion of straw in the mixed substrates (activated sludge and corn straw) from 0 to 50% resulted in a significant increase of up to 69% in VFAs production [11]. Lian et al. [7] observed a notable rise in VFAs concentration from ~ 10 to 15 g/L by changing the ratio of cattle manure and corn straw silage from 3:1 to 1:3 [7]. Sivagurunathan et al. [27] reported that at a low enzyme dosage of 1.5 FPU g^{-1}, a straw solid load of 15% could yield approximately 48.3 g/L of lactic acid, which increased to 59.3 g/L with an elevated straw load of 20% [27]. However, in comparison to the maximum VFAs concentration through semi-continuous fermentation of straw silage and cow manure, Lian et al. [7] achieved an obviously higher maximum VFAs concentration of 28.3 g/L in a batch experiment [7]. This suggests that further enhancement of the bioconversion efficiency for long-term VFAs production in reactors is necessary by optimizing the process parameters and operational methods, such as improving feeding strategies [14].

Regarding the distribution of VFAs, despite variations in the solid content of the substrate and the proportion of straw, acetate (50.0 – 53.8%) was the main VFA in both thermophilic and mesophilic reactors, consistent with previous studies on VFAs production during straw co-fermentation [28, 29]. According to previous studies, for carbohydrate-rich substrates, low pH and OLR (< 8 g VS/L/day) conditions are more conducive to acetate production [30, 31]. Additionally, mesophilic conditions resulted in higher acetate yield, while butyrate was more abundant under thermophilic conditions, particularly during the F3 phase with an increased proportion of straw (Table 3). Notably, isovalerate contributed primarily to valerate concentration in H reactor (83.2%), whereas n-valerate predominated in L reactor (65.7%) (Figs. 2 and 3). Garcia et al. [22] found that in the batch experiments using food waste as substrate, butyrate yield (~ 3.5 g COD/L) was markedly higher under thermophilic conditions (55 ℃) compared to mesophilic conditions (35 ℃), and the distribution of valerate is consistent with the results found in our study [22].Valerate can undergo isomerization to form isovalerate, a step that releases 2.02 kJ of free energy. The shift towards isovalerate forming may confer a

Table 3 The distributions of VFAs in reactors H and L (Mean ± standard deviation)

	H			L		
	F1	F2	F3	F1	F2	F3
VFAs(g/L)	12.1 ± 11.1	10.1 ± 3.2	20.1 ± 2.0	11.7 ± 0.9	13.3 ± 2.1	18.7 ± 2.9
Lactate (%)	0	0	0.50 ± 0.60	0	0	0
Acetate (%)	48.5 ± 3.3	47.4 ± 3.7	54.2 ± 2.3	48.3 ± 4.6	50.6 ± 5.0	62.5 ± 2.0
Propionate (%)	19.7 ± 5.5	26.5 ± 8.4	12.1 ± 4.2	20.5 ± 5.2	16.9 ± 2.4	8.22 ± 1.43
Iso-Butyrate (%)	1.10 ± 0.50	2.80 ± 1.14	2.77 ± 3.29	2.36 ± 0.81	2.14 ± 0.21	1.33 ± 0.44
Butyrate (%)	27.6 ± 4.3	14.6 ± 8.5	26.5 ± 1.3	20.6 ± 4.7	17.1 ± 0.5	18.5 ± 1.8
Iso-Valerate (%)	2.04 ± 1.97	8.31 ± 3.22	3.71 ± 1.80	1.31 ± 1.37	5.71 ± 1.72	3.96 ± 1.29
Valerate (%)	1.14 ± 0.87	0.53 ± 0.71	0.73 ± 0.86	6.94 ± 3.28	7.52 ± 1.58	5.53 ± 2.45

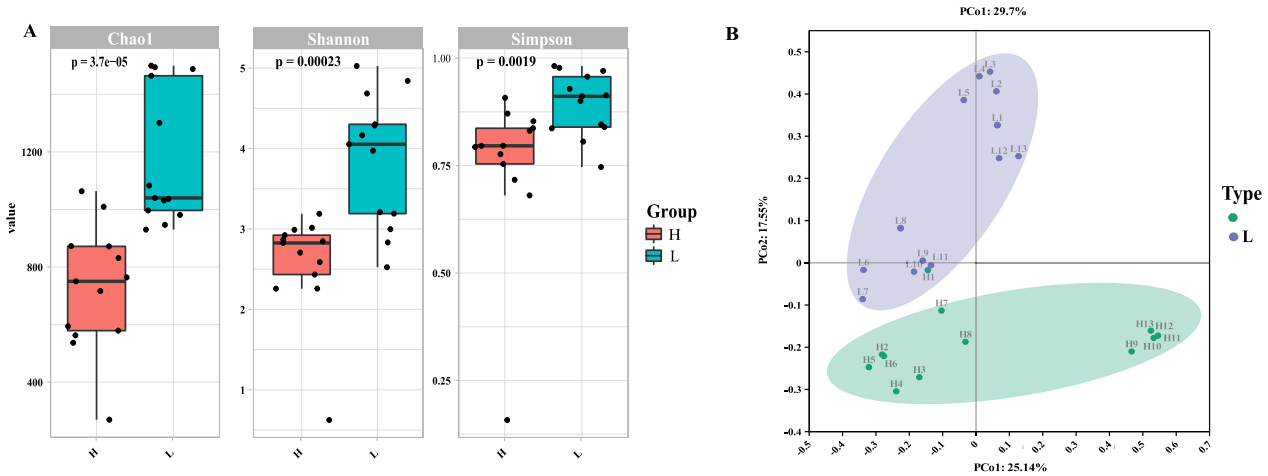

Fig. 3 Chao1, Shannon, and Simpson index analysis (**A**) and PCoA analysis (**B**) based on based on 16S rRNA gene amplicon sequencing at RNA level in reactors H and L

competitive advantage for microorganisms growing at thermophilic temperature to overcome the inhibitory effects of accumulated valerate, as branched-chain fatty acids are less toxic compared to their straight-chain isomers [32]. Additionally, increasing mixed substrate solid content and OLR led to steady increases in acetate concentration for both reactors. Dogan and Demirer. [33] demonstrated that an elevated OLR (20 g VS/L/day) enhanced acetate production (75%) in a continuous reactor [33]. Process parameters significantly influence the distribution of microbial community and their metabolic pathways, and it has been shown that the combined effect of process variables greatly influences waste streams abundant in carbohydrates (e.g., straw) [22, 34]. Therefore, increasing the solid contents of substrate and the proportion of straw in this study proves advantageous for acetate accumulation.

Microbial community structure analysis

The alpha diversity (Chao1, Shannon, and Simpson indices), as well as PCoA analysis of the microbial communities in thermophilic and mesophilic reactors based on the 16S rRNA gene analysis are shown in Fig. 3. The microbial alpha diversity in L reactor was significantly higher than that in H reactor ($P < 0.05$), which is consistent with previous study on anaerobic co-digestion using straw and sludge as substrates [35]. The PCoA analysis revealed distinct differences in the composition of microbial community between thermophilic and mesophilic temperatures, suggesting that temperature may exert a pronounced influence on the VFAs-producing microbial communities [36, 37].

As shown in Fig. 4, the dominant phyla in F1, F2, and F3 phases of the H reactor were Firmicutes (58.4% at RNA level) and Thermotogota (23.2%). In contrast, for the L reactor, the dominant phyla in three phases

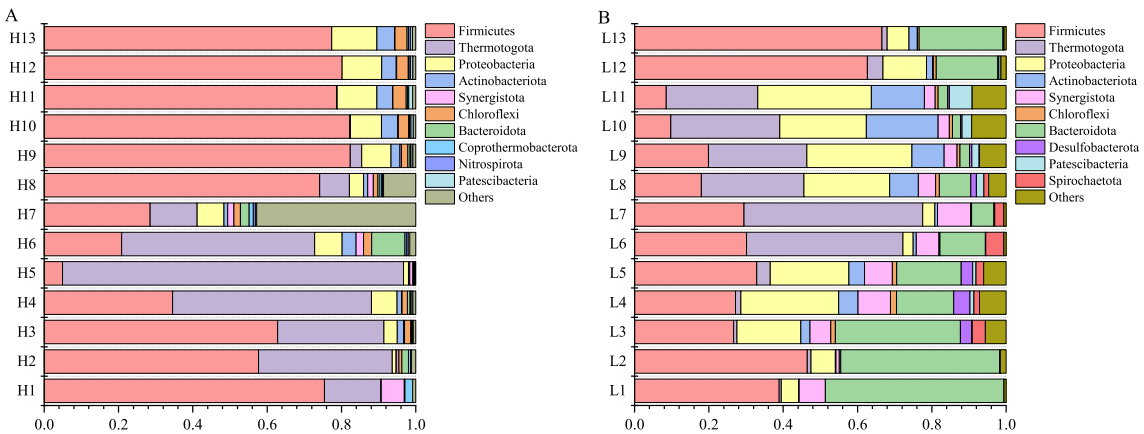

Fig. 4 Relative abundance of bacterial phylum based on 16S rRNA gene amplicon sequencing at RNA level in reactors H (**A**) and L (**B**)

included Firmicutes (31.2%), Bacteroidota (15.9%), Thermotogota ((33%), and Proteobacteria (17%).

The distribution of dominant genera in H reactor is shown in Fig. 5A. The microbial community compositions based on DNA and RNA were found to be highly consistent (Fig. S4), with *Defluviitoga*, *Caproiciproducens*, and unclassified Hungateiclostridiaceae as the dominant genera. In phases F1 and F2, *Defluviitoga* was predominant, with relative abundances ranging from 24.2 to 45.0% (RNA-based), while it was undetectable in phase F3. *Caproiciproducens* (RNA: 16.3%) and unclassified Hungateiclostridiaceae (RNA: 17.1%) belonging to Firmicutes became dominant in the F3 phase, with their relative abundances markedly increased at H9.

The dominant genera in L reactor included *Defluviitoga*, Lachnospiraceae_NK3A20_group, Rikenellaceae_RC9_gut_group, and *Petrimonas* (Fig. 5B). In the F1 phase, *Petrimonas* (RNA: 10.8%), *Dysgonomonas* (RNA: 6.5%), and *Prevotella* (RNA: 5.6%) belonging to Bacteroidota were dominant. During the F2 and F3 phases, *Defluviitoga* (RNA: 33.0%) displayed high abundance during L6-L11, while Lachnospiraceae_NK3A20_group (RNA: 32.3%) was obviously enriched during L12-L13. Notably, Rikenellaceae_RC9_gut_group was dominant across all three phases with average relative abundances of 6.9% (RNA-based).

In this study, Firmicutes were consistently dominant in both H and L reactors, indicating the pivotal role of this phylum in acidogenesis process under both thermophilic and mesophilic conditions. Previous studies showed that Firmicutes are essential for the degradation of lignocellulosic materials and proteins during the co-fermentation of straw and cow manure for anaerobic VFAs production, as well as in the mechanism studies on anaerobic degradation of rice straw [7, 38, 39]. At the genus level, *Defluviitoga* exhibited remarkable adaptability to both thermophilic and mesophilic temperatures. Members of this genus can produce carbohydrate-hydrolyzing enzymes and generate acetate, H_2, and CO_2 from polysaccharides such as cellulose and chitin [40]. During the anaerobic co-digestion of pig manure and straw, *Defluviitoga* is found to be capable of cellulose degradation and acetate production [16, 20, 41]. Thus, in this study, the high-titer production of acetate during mixed substrates fermentation may be supported by the high abundance of *Defluviitoga* in both reactors. In addition, the predominant genera in H reactor were *Caproiciproducens* and unclassified Hungateiclostridiaceae, which are uniquely dominant. *Caproiciproducens* is capable of metabolizing glucose through the Embden-Meyerhof-Parnas pathway, resulting in the production of lactate, butyrate, acetate, and H_2 [42, 43]. Previous studies have shown that under thermophilic conditions (50 ℃) with a 1:1 or 1:2 mixture ratio of cow manure and straw silage, *Caproiciproducens* predominantly produces lactate as a facultative anaerobe [7]. Members of Hungateiclostridiaceae can ferment complex carbohydrates and protein substrates to produce VFAs [44, 45]. By contrast, under mesophilic conditions, populations such as Rikenellaceae_RC9_gut_group, Lachnospiraceae_NK3A20_group, and *Prevotella*, commonly found in the rumen environment [21], are polysaccharide-degrading bacteria that possess the ability to produce various enzymes for cellulose and hemicellulose degradation. These populations also play a significant role in the production of acetate and propionate. For instance, *Prevotella* is able to degrade xylan, xyloglucan, and pectin, converting sugars into acetate, succinate, and propionate [9, 46–48]; while the dominant genera in the F1 phase, *Petrimonas* and *Dysgonomonas*, have saccharolytic activity and the ability to utilize monosaccharides and disaccharides, with the

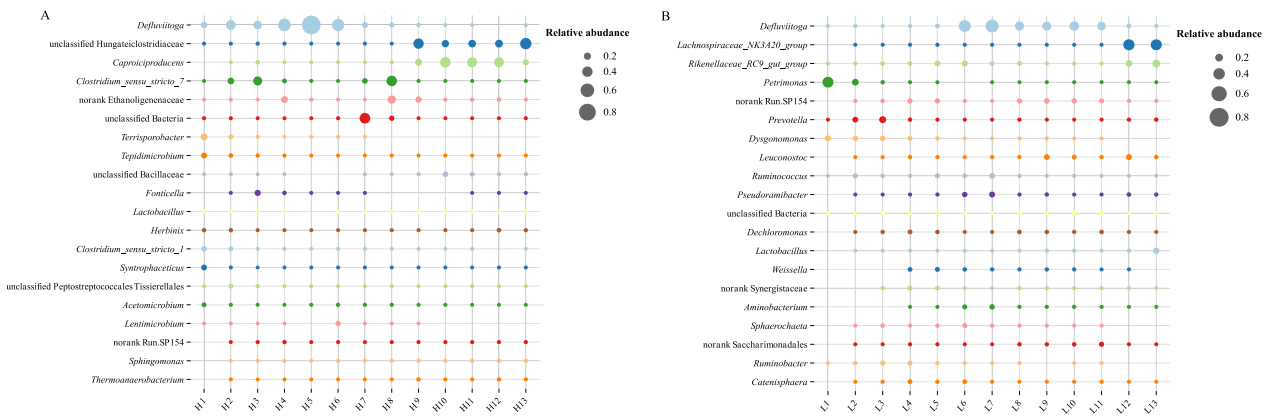

Fig. 5 Relative abundance of bacterial genera based on 16S rRNA gene amplicon sequencing at RNA level in reactors H (**A**) and L (**B**)

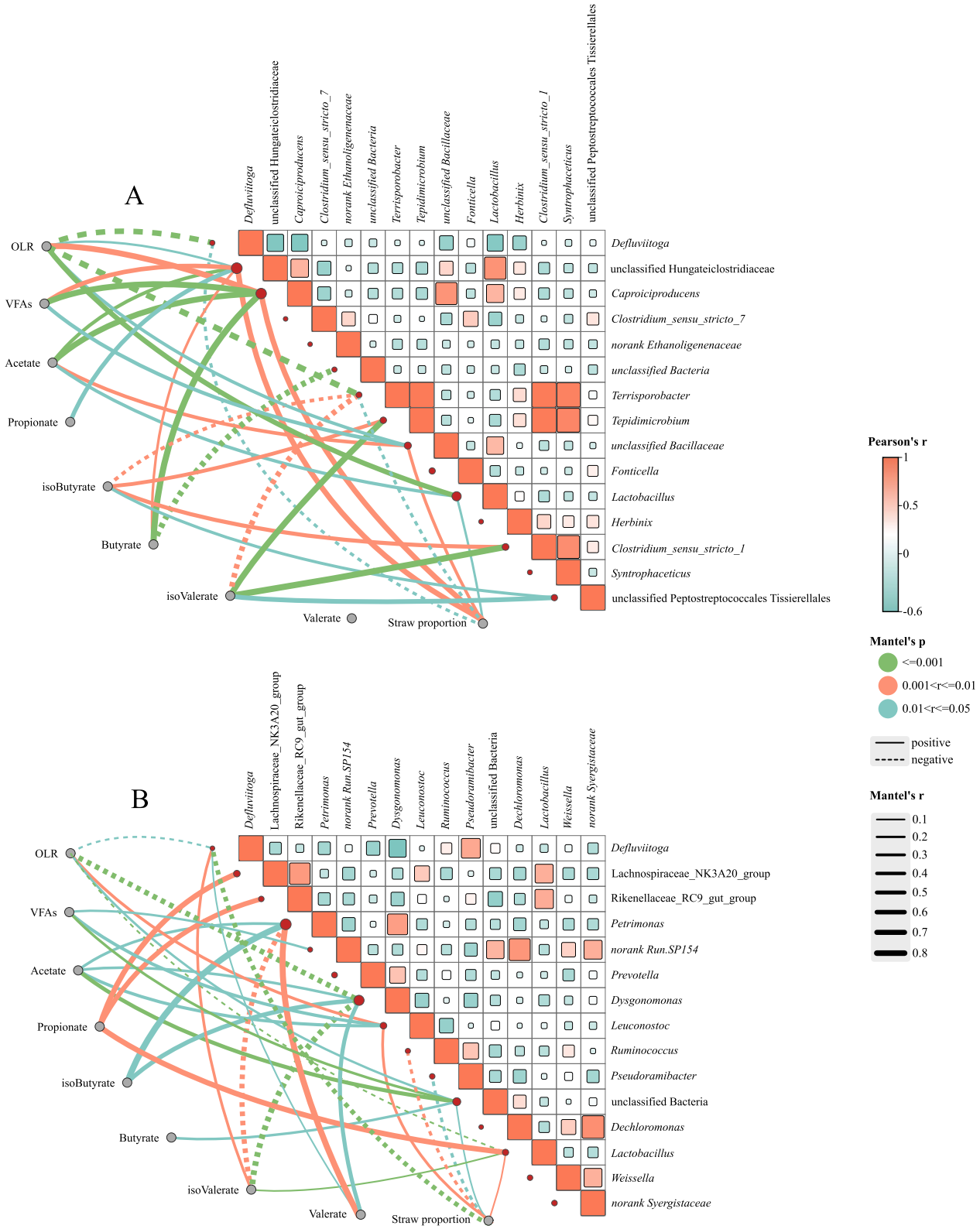

Fig. 6 Heatmap showing the Spearman's correlation analysis of RNA-based on top 15 dominant bacterial genera. Distance correlation plots of top 15 dominant bacterial genera and OLR, straw proportion, and VFAs parameters in reactors H (**A**) and L (**B**)

main metabolic products being acetate and propionate, glucose can promote its growth [49–51].

Interaction between operational parameters and microbial community

To investigate the correlation between VFAs-producing populations and key operational parameters, a Mantel test analysis was conducted based on the 16 s RNA gene analysis (RNA-based) (Fig. 6). In H reactor, there was a significant positive correlation ($P \leq 0.01$) between OLR and unclassified Hungateiclostridiaceae as well as *Caproiciproducens*. Furthermore, both of these microbial groups showed a strong positive association with acetate production, while propionate production was significantly related to unclassified Hungateiclostridiaceae ($P \leq 0.05$), and butyrate production displayed an obviously positive correlation with *Caproiciproducens* ($P \leq 0.001$; Fig. 6A). Genera such as *Terrisporobacter*, *Tepidimicrobium*, *Clostridium_sensu_stricto_1*, and *Syntrophaceticus* were highly positively correlated with each other ($r > 0.5$), suggesting a potential collaborative effort among these microorganisms in the production of VFAs during straw, food wastes, and sludge co-fermentation. Furthermore, the proportion of butyrate in H reactor obviously increased in the F3 phase, and based on the Mantel test analysis, *Caproiciproducens* exhibited a significant correlation with butyrate production ($P \leq 0.001$). While the RNA abundance of *Caproiciproducens* and unclassified Hungateiclostridiaceae increased with the straw proportion. Therefore, *Caproiciproducens* might be the targeted butyricogenic bacteria under thermophilic conditions in this study.

In the L reactor (Fig. 6B), unclassified Bacteria was significantly positively correlated with acetate production ($P \leq 0.01$), while propionate production exhibited a significant positive correlation ($P \leq 0.01$) with Lachnospiraceae_NK3A20_group and Rikenellaceae_RC9_gut_group. Consequently, the gradual decline in the proportion of propionate could be attributed to the decrease in abundance of Rikenellaceae_RC9_gut_group. Notably, in both thermophilic and mesophilic reactors, *Defluviitoga* showed a negative correlation with OLR and the proportion of straw; as OLR and straw proportion increased, the relative abundance of *Defluviitoga* decreased in both reactors. This finding is consistent with previous study that increasing the reactor OLR resulted in a decrease in the relative abundance of *Defluviitoga* from 65.76% to 50.56% [20].

The present study investigated the long-term stability of VFAs production through the co-fermentation of straw, food waste, and sludge using semi-continuous reactors at different temperatures and OLRs. The results showed that elevating the straw load led to increase concentrations of VFAs (22 ± 2 g/L) in both thermophilic and mesophilic reactors, with no significant effect of temperature on VFAs yield. Acetate was found to be the main component of produced VFAs in both reactors, while butyrate and propionate production was higher under the thermophilic conditions compared to the mesophilic conditions. Moreover, high solid content and increased straw proportion significantly enhanced acetate production. Microbial community analysis indicated that *Defluviitoga* played a crucial role in acidogenesis process in both reactors, besides, unclassified Hungateiclostridiaceae and *Caproiciproducen* were dominant in thermophilic reactor, while Lachnospiraceae_NK3A20_group and Rikenellaceae_RC9_gut_group were essential for VFAs production under mesophilic conditions. Building upon these findings, it is possible to improve the yield of target VFAs by manipulating process parameters and selectively enriching the microbial community.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00935-1.

Additional file 1.

Author contributions

Yu-Wei Chen: Data curation, Methodology, Visualization, Writing—original draft & editing. Feng Gao: Data curation, Conceptualization, Investigation. Xia Hong: Data curation, Methodology. Meng Wang: Formal analysis, Software. Quan Zhang: Resources, Software. Zhao-Yong Sun: Data curation. Ya-Ting Chen: Data curation, Methodology, Visualization, Writing—original draft & editing. Yue-Qin Tang: Conceptualization, Writing—review and editing, Supervision, Funding acquisition.

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Data availability

Data will be made available on request.

Declarations

Competing interests

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

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References

1. Kim J, Kim J, Lee C (2019) Anaerobic co-digestion of food waste, human feces, and toilet paper: methane potential and synergistic effect. Fuel 248:189–195. https://doi.org/10.1016/j.fuel.2019.03.081
2. Nag R, Auer A, Markey BK, Whyte P, Nolan S, O'Flaherty V, Russell L, Bolton D, Fenton O, Richards K, Cummins E (2019) Anaerobic digestion of agricultural manure and biomass—Critical indicators of risk and knowledge

gaps. Sci Total Environ 690:460–479. https://doi.org/10.1016/j.scitotenv.2019.06.512
3. Xin Y, Liu W, Chen C, Wang D (2022) Effect of biochar on methane production and structural characteristics in the anaerobic digestion (AD) of rape straw. BioResources 17:5632–5644. https://doi.org/10.15376/biores.17.4.5632-5644
4. Yu Q, Liu R, Li K, Ma R (2019) A review of crop straw pretreatment methods for biogas production by anaerobic digestion in China. Renew Sustain Energy Rev 107:51–58. https://doi.org/10.1016/j.rser.2019.02.020
5. Fang W, Zhang P, Zhang X, Zhu X, van Lier JB, Spanjers H (2018) White rot fungi pretreatment to advance volatile fatty acid production from solid-state fermentation of solid digestate: efficiency and mechanisms. Energy 162:534–541. https://doi.org/10.1016/j.energy.2018.08.082
6. Sun J, Zhang L, Loh K-C (2021) Review and perspectives of enhanced volatile fatty acids production from acidogenic fermentation of lignocellulosic biomass wastes. Bioresour Bioprocess 8:68. https://doi.org/10.1186/s40643-021-00420-3
7. Lian T, Zhang W, Cao Q, Wang S, Dong H, Yin F (2022) Improving production of lactic acid and volatile fatty acids from dairy cattle manure and corn straw silage: effects of mixing ratios and temperature. Bioresour Technol 359:127449–127449. https://doi.org/10.1016/j.biortech.2022.127449
8. Li B-Y, Xia Z-Y, Gou M, Sun Z-Y, Huang Y-L, Jiao S-B, Dai W-Y, Tang Y-Q (2022) Production of volatile fatty acid from fruit waste by anaerobic digestion at high organic loading rates: performance and microbial community characteristics. Bioresour Technol 346:126648. https://doi.org/10.1016/j.biortech.2021.126648
9. Liang J, Zhang H, Zhang P, Zhang G, Cai Y, Wang Q, Zhou Z, Ding Y, Zubair M (2021) Effect of substrate load on anaerobic fermentation of rice straw with rumen liquid as inoculum: Hydrolysis and acidogenesis efficiency, enzymatic activities and rumen bacterial community structure. Waste Manage 124:235–243. https://doi.org/10.1016/j.wasman.2021.02.017
10. Cai J, Liu J, Wang N, Zhang Y, Wang J (2021) Research progress on comprehensive utilization of agricultural straw. J Anhui Agric Sci 49:11–14. https://doi.org/10.3969/j.issn.0517-6611.2021.09.003
11. Zhou A, Guo Z, Yang C, Kong F, Liu W, Wang A (2013) Volatile fatty acids productivity by anaerobic co-digesting waste activated sludge and corn straw: effect of feedstock proportion. J Biotechnol 168:234–239. https://doi.org/10.1016/j.jbiotec.2013.05.015
12. Ji J-L, Chen F, Liu S, Yang Y, Hou C, Wang Y-Z (2022) Co-production of biogas and humic acid using rice straw and pig manure as substrates through solid-state anaerobic fermentation and subsequent aerobic composting. J Environ Manag 320:115860. https://doi.org/10.1016/j.jenvman.2022.115860
13. Ma H, Liu H, Zhang L, Yang M, Fu B, Liu H (2017) Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol Biofuels 10:137. https://doi.org/10.1186/s13068-017-0821-1
14. Strazzera G, Battista F, Garcia NH, Frison N, Bolzonella D (2018) Volatile fatty acids production from food wastes for biorefinery platforms: a review. J Environ Manag 226:278–288. https://doi.org/10.1016/j.jenvman.2018.08.039
15. Li P, Wang Y, He X, Liang D, Liu X, He C, Shen D, Jiao Y (2024) Investigation into the effects of different recycled magnetic additives on anaerobic co-digestion of sludge and straw. Fuel 358:130245. https://doi.org/10.1016/j.fuel.2023.130245
16. Chen H, Zhang W, Wu J, Chen X, Liu R, Han Y, Xiao B, Yu Z, Peng Y (2021) Improving two-stage thermophilic-mesophilic anaerobic co-digestion of swine manure and rice straw by digestate recirculation. Chemosphere 274:129787. https://doi.org/10.1016/j.chemosphere.2021.129787
17. Yu Q, Sun C, Liu R, Yellezuome D, Zhu X, Bai R, Liu M, Sun M (2021) Anaerobic co-digestion of corn stover and chicken manure using continuous stirred tank reactor: the effect of biochar addition and urea pretreatment. Bioresour Technol 319:124197. https://doi.org/10.1016/j.biortech.2020.124197
18. Wei Y, Gao Y, Yuan H, Chang Y, Li X (2022) Effects of organic loading rate and pretreatments on digestion performance of corn stover and chicken manure in completely stirred tank reactor (CSTR). Sci Total Environ 815:152499. https://doi.org/10.1016/j.scitotenv.2021.152499
19. Wang Z, Fang Q, Luo J, Wei T, Chen Y, Ping Q, Xiao Y, Ding W (2022) Optimized process for the odd-carbon volatile fatty acids (OCFA) directional production: anaerobic co-digestion of disused grease with sludge by anaerobic sequencing batch reactor. J Water Process Eng 46:102592. https://doi.org/10.1016/j.jwpe.2022.102592
20. Guo X, Wang C, Sun F, Zhu W, Wu W (2014) A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. Bioresour Technol 152:420–428. https://doi.org/10.1016/j.biortech.2013.11.012
21. Liang J, Fang W, Wang Q, Zubair M, Zhang G, Ma W, Cai Y, Zhang P (2021) Metagenomic analysis of community, enzymes and metabolic pathways during corn straw fermentation with rumen microorganisms for volatile fatty acid production. Bioresour Technol 342:126004. https://doi.org/10.1016/j.biortech.2021.126004
22. Garcia-Aguirre J, Aymerich E, de Goñi JGM, Esteban-Gutiérrez M (2017) Selective VFA production potential from organic waste streams: assessing temperature and pH influence. Bioresour Technol 244:1081–1088. https://doi.org/10.1016/j.biortech.2017.07.187
23. He M, Sun Y, Zou D, Yuan H, Zhu B, Li X, Pang Y (2012) Influence of temperature on hydrolysis acidification of food waste. Proced Environ Sci 16:85–94. https://doi.org/10.1016/j.proenv.2012.10.012
24. Wu Z-L, Lin Z, Sun Z-Y, Gou M, Xia Z-Y, Tang Y-Q (2020) A comparative study of mesophilic and thermophilic anaerobic digestion of municipal sludge with high-solids content: reactor performance and microbial community. Bioresour Technol 302:122851. https://doi.org/10.1016/j.biortech.2020.122851
25. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000
26. Wang Q, Sun J, Liu S, Gao L, Zhou X, Wang D, Song K, Nghiem LD (2019) Free ammonia pretreatment improves anaerobic methane generation from algae. Water Res 162:269–275. https://doi.org/10.1016/j.watres.2019.06.065
27. Sivagurunathan P, Raj T, Chauhan PS, Kumari P, Satlewal A, Gupta RP, Kumar R (2022) High-titer lactic acid production from pilot-scale pretreated non-detoxified rice straw hydrolysate at high-solid loading. Biochem Eng J 187:108668. https://doi.org/10.1016/j.bej.2022.108668
28. Ngwa AT, Nsahlai IV, Iji PA (2003) Effect of feeding legume pods or alfalfa in combination with poor quality grass straw on microbial enzyme activity and production of VFA in the rumen of South African Merino sheep. Small Rumin Res 48:83–94. https://doi.org/10.1016/S0921-4488(03)00009-9
29. Cavinato C, Da Ros C, Pavan P, Bolzonella D (2017) Influence of temperature and hydraulic retention on the production of volatile fatty acids during anaerobic fermentation of cow manure and maize silage. Bioresour Technol 223:59–64. https://doi.org/10.1016/j.biortech.2016.10.041
30. Rossi E, Pecorini I, Paoli P, Iannelli R (2022) Plug-flow reactor for volatile fatty acid production from the organic fraction of municipal solid waste: influence of organic loading rate. J Environ Chem Eng 10:106963. https://doi.org/10.1016/j.jece.2021.106963
31. Wainaina S, Awasthi MK, Horváth IS, Taherzadeh MJ (2020) Anaerobic digestion of food waste to volatile fatty acids and hydrogen at high organic loading rates in immersed membrane bioreactors. Renew Energy 152:1140–1148. https://doi.org/10.1016/j.renene.2020.01.138
32. Yu H-Q, Mu Y, Fang HHP (2004) Thermodynamic analysis of product formation in mesophilic acidogenesis of lactose. Biotechnol Bioeng 87:813–822. https://doi.org/10.1002/bit.20190
33. Doğan E, Demirer GN (2009) Volatile fatty acid production from organic fraction of municipal solid waste through anaerobic acidogenic digestion. Environ Eng Sci 26:1443–1450. https://doi.org/10.1089/ees.2009.0062
34. Zheng M, Zheng M, Wu Y, Ma H, Wang K (2015) Effect of pH on types of acidogenic fermentation of fruit and vegetable wastes. Biotechnol Bioprocess Eng 20:298–303. https://doi.org/10.1007/s12257-014-0651-y
35. Sun L, Pope PB, Eijsink VGH, Schnürer A (2015) Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure. Microb Biotechnol 8:815–827. https://doi.org/10.1111/1751-7915.12298
36. Greses S, Gaby JC, Aguado D, Ferrer J, Seco A, Horn SJ (2017) Microbial community characterization during anaerobic digestion of Scenedesmus

spp. under mesophilic and thermophilic conditions. Algal Res 27:121–130. https://doi.org/10.1016/j.algal.2017.09.002
37. Vanwonterghem I, Jensen PD, Rabaey K, Tyson GW (2015) Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. Sci Rep 5:8496. https://doi.org/10.1038/srep08496
38. Lu J, Jia Z, Wang P, Yang X, Lin P, Ren L, Farghali M (2022) Restoration of acidified dry anaerobic digestion of food waste: bioaugmentation of butyric acid-resistant microbes. J Environ Chem Eng 10:106935. https://doi.org/10.1016/j.jece.2021.106935
39. Huang J, Gao K, Yang L, Lu Y (2023) Successional action of Bacteroidota and Firmicutes in decomposing straw polymers in a paddy soil. Environ Microb 18:76. https://doi.org/10.1186/s40793-023-00533-6
40. Conners SB, Mongodin EF, Johnson MR, Montero CI, Nelson KE, Kelly RM (2006) Microbial biochemistry, physiology, and biotechnology of hyperthermophilic Thermotoga species. FEMS Microbiol Rev 30:872–905. https://doi.org/10.1111/j.1574-6976.2006.00039.x
41. Wu X, Tian Z, Lv Z, Chen Z, Liu Y, Yong X, Zhou J, Xie X, Jia H, Wei P (2020) Effects of copper salts on performance, antibiotic resistance genes, and microbial community during thermophilic anaerobic digestion of swine manure. Bioresour Technol 300:122728. https://doi.org/10.1016/j.biortech.2019.122728
42. Feng K, Li H, Zheng C (2018) Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresour Technol 270:180–188. https://doi.org/10.1016/j.biortech.2018.09.035
43. Flaiz M, Baur T, Brahner S, Poehlein A, Daniel R, Bengelsdorf FR (2020) Caproicibacter fermentans gen. nov., sp. nov., a new caproate-producing bacterium and emended description of the genus Caproiciproducens. Int J Syst Evol Microbiol 70:4269–4279. https://doi.org/10.1099/ijsem.0.004283
44. Degueurce A, Dabert P, Argence V, Blondel L, Le Bihan A, Lebreton M, Peu P, Sarrazin M, Picard S, Trémier A (2022) An innovative solid-state micro-anaerobic digestion process to valorize food waste: technical development constraints and consequences on biological performances. Waste Biomass Valoriz 13:617–630. https://doi.org/10.1007/s12649-021-01555-2
45. Prem EM, Duschl SF, Wagner AO (2023) Effects of increasing phenyl acid concentrations on the AD process of a multiple-biogas-reactor system. Biomass Bioenergy 168:106686. https://doi.org/10.1016/j.biombioe.2022.106686
46. Xing B-S, Han Y, Wang XC, Wen J, Cao S, Zhang K, Li Q, Yuan H (2020) Persistent action of cow rumen microorganisms in enhancing biodegradation of wheat straw by rumen fermentation. Sci Total Environ 715:136529. https://doi.org/10.1016/j.scitotenv.2020.136529
47. Ahmad AA, Yang C, Zhang J, Kalwar Q, Liang Z, Li C, Du M, Yan P, Long R, Han J (2020) Effects of dietary energy levels on rumen fermentation, microbial diversity, and feed efficiency of yaks (Bos grunniens). Front Microbiol 11:625
48. Accetto T, Avguštin G (2021) Non-oral Prevotella stepping into the spotlight. Anaerobe 68:102321. https://doi.org/10.1016/j.anaerobe.2021.102321
49. Hahnke S, Langer T, Koeck DE, Klocke M (2016) 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 Evolut Microbiol 66:1466–1475. https://doi.org/10.1099/ijsem.0.000902
50. Tao Y, Li J, Rui J, Xu Z, Zhou Y, Hu X, Wang X, Liu M, Li D, Li X (2014) Prokaryotic communities in pit mud from different-aged cellars used for the production of Chinese strong-flavored liquor. Appl Environ Microbiol 80:2254–2260. https://doi.org/10.1128/aem.04070-13
51. Soares LA, Silva Rabelo CAB, Sakamoto IK, Silva EL, Varesche MBA (2020) Screening and bioprospecting of anaerobic consortia for biofuel production enhancement from sugarcane bagasse. Appl Biochem Biotechnol 190:232–251. https://doi.org/10.1007/s12010-019-03074-2

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