Environmental and Microbial Technology
https://www.sciltp.com/journals/emt

Article

Anaerobic Co-Digestion of Thermal Hydrolysis Pretreated Sludge and Food Waste: Insights of Different Solids Content on Methane Production, Substrate Metabolism, and Microbial Functional Profiles

Chao Yao [1,2], Lexiang Huang [1,2], Zhicheng Wei [1,2], Xiang Zou [1,2], Chenxin Zhao [1,2], Qin Zhang [3,4,*], Jiashun Cao [1,2] and Jingyang Luo [1,2,*]

[1] Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Nanjing 210098, China
[2] College of Environment, Hohai University, Nanjing 210098, China
[3] School of Energy and Environment, Anhui University of Technology, Ma'anshan 243000, China
[4] Engineering Research Center of Biofilm Water Purification and Utilization Technology of Ministry of Education, Anhui University of Technology, Ma'anshan 243032, China
* Correspondence: Zq198601@126.com (Q.Z.); luojy2016@hhu.edu.cn (J.L.); Tel.: +86-25-83786701 (J.L.)

How To Cite: Yao, C.; Huang, L.; Wei, Z.; et al. Anaerobic Co-Digestion of Thermal Hydrolysis Pretreated Sludge and Food Waste: Insights of Different Solids Content on Methane Production, Substrate Metabolism, and Microbial Functional Profiles. *Environmental and Microbial Technology* **2026**, *1*(1), 10. https://doi.org/10.53941/emt.2026.100010

Received: 10 March 2026
Revised: 26 March 2026
Accepted: 30 March 2026
Published: 10 April 2026

Abstract: This study assessed the anaerobic co-digestion (AcoD) potential of thermal hydrolysis (TH) pretreated waste activated sludge (WAS) and food waste (FW) across various solid contents (SC). Results demonstrated that along with the SC increased from 2 to 6%, the cumulative CH_4 production was ascending from 85.0 to 96.3 mL/g VSS, and this value further improved from 99.2 to 122.0 mL/g VSS in the corresponding TH-treated reactors. TH improved soluble carbohydrate and protein concentrations from 34.2–80.7 and 51.1–380.1 mg/L in the untreated group to 59.3–172.1 and 129.3–731.9 mg/L in the corresponding TH-treated reactors. The surge in bioaccessible substrates contributed to the enrichment of hydrolysis acidogenic bacteria (e.g., *Bacillota*) and methanogens (e.g., *Methanospirillum*), and meanwhile stimulated their cooperative symbiosis (e.g., positive (93.14%)). Further explorations demonstrated that, accompanied with the more bioavailable organics induced by TH, the critical metabolic pathways (e.g., fatty acid biosynthesis and acetoclastic methanogenesis) and functional gene expression (e.g., *mtrA*, *mtrB*, and *mtrC*) were stimulated and upregulated, maintaining optimal metabolic activity for CH_4 formation. This study investigated the response and mechanisms of CH_4 production to SC during AcoD of TH pretreated-WAS and FW, which provided optimized strategy for energy recovery from organic wastes.

Keywords: anaerobic co-digestion; solids content; thermal hydrolysis; microbial community; syntrophic relationship

1. Introduction

With the growth of population and economy, the generation of municipal solid waste (MSW) has precipitated an upward trajectory [1]. Of these, food waste (FW) and waste-activated sludge (WAS) are ranked among the top two [2], posing serious environmental problems and secondary pollutions if disposed of and treated improperly [3]. Conversely, if appropriate strategies (e.g., anaerobic digestion) are implemented to recycle resource (e.g., volatile

fatty acids (VFAs)) and energy (e.g., methane (CH_4)) from these wastes [4,5], the crisis of resource starvation will be alleviated, while substantial economic benefits will also be acquired, contributing to the goal of "carbon-neutrality".

Compared with mono-digestion, anaerobic co-digestion (AcoD) of WAS and FW exhibited superior performance advantages such as increased methane yields, optimized nutrient availability, enhanced process stability, and improved resource recovery [6,7]. Notably, the majority of previous studies have employed AcoD of FW and WAS, primarily focusing on blends in specified proportions, operational conditions (e.g., pH), the addition of specific materials, etc. For instance, An et al. used black phosphorus as a nanomaterial to enhance methane production during anaerobic co-digestion [8]. Apart from this, the solids content (CS) was also regarded as one of the regulatory strategies for optimizing AcoD. It is generally believed that an increase in SC contributed to improving the loading rate of organic substrates and reducing equipment volume, but it also faced some challenges, such as system viscosity, mixing, and mass transfer [9]. How to effectively address these issues under high SC conditions is of great significance for optimizing system performance.

Thermal hydrolysis (TH), which was carried out at high temperatures and high pressure, has been documented to be an effective strategy to reduce the viscosity of substrates by using water molecule permeation and ion dissociation [10,11]. Inspired by this, if this strategy were applied to pretreat the digestive substrate, it might be expected to alleviate those challenges mentioned above posed by high SC. Nevertheless, the stimulation effects of TH pretreatment on the performance of AcoD under high SC remain unclear. Moreover, four stages, namely solubilization, hydrolysis, acidification, and methanogenesis, were involved in the AcoD process. However, the effects of TH on the distinct stages of the AcoD of WAS and FW across varied SC are not yet understood. Meanwhile, the anaerobic metabolism of organic substrates depended not only on the abundance and composition of bacteria and archaea, but also was closely related to the syntrophic interactions between them [12,13]. How changes in them in response to SC and TH during the process of AcoD of WAS and FW are also "black box".

To answer these questions, the performances of CH_4 production during the AcoD of TH-treated WAS and FW under different SC were first investigated. Then, the specific processes, including solubilization, hydrolysis, and acidification under different operational environments were distinguished. Finally, the variations in microbial community composition, bacterial-archaeal symbiotic relationships, and metabolic functional profiles were revealed to elucidate the mechanisms of TH and varied SC on CH_4 production at the microbial level. This study clarified the performance and mechanisms of CH_4 production during AcoD of TH-treated WAS and FW under different SC, contributing to the optimization of energy recovery from organic wastes.

2. Materials and Methods

2.1. Source and Characteristics of WAS, FW, and Inoculum

WAS was collected from a wastewater treatment plant in Nanjing, China. After removing inorganic impurities, the WAS was allowed to settle for 24 h at 4 °C. FW was collected from a university canteen in Nanjing, China. After removing bone residues, the FW was pulped using a food blender and stored at 4 °C to prevent acidification. Additionally, the inoculum was obtained from an anaerobic digester in Lu'an, Anhui Province, China, which had been operated stably for a long time during the anaerobic co-digestion of waste activated sludge and food waste. The inoculum possessed a total suspended solids concentration of 52.67 g/L, a volatile suspended solids concentration of 24.41 g/L, a soluble chemical oxygen demand of 3.95 g/L, a soluble protein content of 0.58 g/L, and a soluble carbohydrate content of 0.29 g/L. The main characteristics of the concentrated WAS and FW were presented in Table 1.

Table 1. Characteristics of WAS and FW applied in this study.

Parameters	WAS	FW
Total suspended solids (TSS, g/L)	22.55 ± 0.08	35.92 ± 0.10
Volatile suspended solids (VSS, g/L)	13.98 ± 0.14	32.8 ± 0.15
VSS/TSS	0.62	0.91
Total chemical oxygen demand (TCOD, g/L)	20.03 ± 0.24	31.25 ± 0.38
Proteins (g/L)	7.43 ± 0.29	5.89 ± 0.34
Carbohydrates (g/L)	1.18 ± 0.15	19.3 ± 0.45

2.2. Performance of CH_4 Production during AcoD of TH-Treated WAS and FW under Different SC

The influences of different SC on the AcoD of FW and TH-treated/untreated WAS were conducted in six identical serum bottles (Working volume: 600 mL). Three reactors with SC of 2, 4, and 6% were named as R1–R3 (WAS unpretreated by TH), while the other three reactors with the corresponding SC (WAS pretreated by TH)

were referred to as TH1–TH3, respectively. TH was conducted in a 500 mL stainless steel high-temperature and high-pressure reactor. The reactor was heated in an oven at 120 °C for 30 min, and the pressure inside was controlled at 6–8 bar using an explosion-proof pressure relief valve and a pressure gauge [14]. After the reactor was cooled to room temperature, the hydrolyzed product was taken out and stored at 4 °C for subsequent use. In each reactor, the ratios of FW/WAS and the substrate/inoculum were both set at 1:1 (based on TSS) [15]. The anaerobic atmosphere was achieved by flushing with N_2 for 5 min. To ensure data reliability, each group of experiments was repeated three times.

During the whole stage of AcoD, biogas samples and VFAs were collected and monitored regularly. Meanwhile, the soluble proteins, soluble carbohydrates, and NH_4^+-N concentrations in the reactors were measured on days 2 and 4. Additionally, the three-dimensional excitation-emission matrix (EEM) fluorescence spectra of fermentation liquids in each reactor were also analyzed to clarify the variations in the structure of released fermentation substrates. Finally, microbial samples were extracted from reactors R1, R3, TH1, and TH3 and immediately stored at −80 °C for subsequent microbial analysis and metagenomic sequencing.

2.3. Analytical Methods

The TSS, VSS, TCOD, and NH_4^+-N were determined according to standard methods (Chinese NEPA, 2002). The protein was measured using a BCA protein assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China), and the carbohydrate was determined by the anthrone colorimetric method [5]. EEM spectroscopy was conducted by using an F7000 fluorometer (Hitachi, Tokyo, Japan). The composition and concentration of VFAs were analyzed using an Agilent 7820A gas chromatography system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA, USA) [16]. Biogas composition was determined using an Agilent 8890 GC system (Agilent Technologies Inc., Santa Clara, CA, USA) [17]. The dynamics of CH_4 production during the entire digestive process were simulated by the modified Gompertz model [17,18].

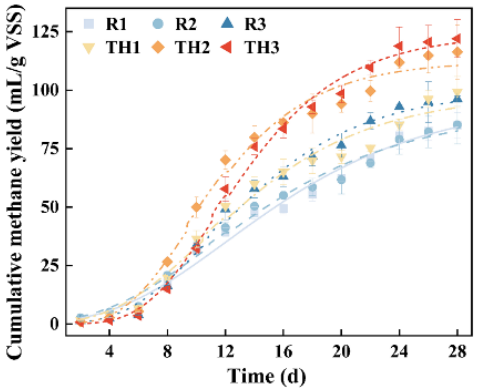
Furthermore, metagenomic analysis was performed using Illumina MiSeq sequencing by BioZerone Biotechnology (Shanghai, China) [19]. The specific procedures were documented in the previous literature [4]. Functional analyses were conducted by annotating genes against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Inter-domain ecological networks (IDENs) between bacterial and archaeal communities were constructed using the INAP platform (https://inap.denglab.org.cn/ (accessed on 20 January 2026)), and the networks were visualized using Gephi (version 0.10.1).

3. Results

3.1. CH_4 Production in Different Reactors

The profiles of CH_4 production in different reactors are shown in Figure 1a. Along with the increase in SC from 2 to 6%, the cumulative CH_4 production was ascending from 85.0 to 96.3 mL/g VSS, suggesting that a high SC contributed to the bioconversion of substrates and benefitted to energy recovery to a certain extent. Analogously, Li et al. also found that the increase in SC within a certain range (<8%) improved the CH_4 production [20]. The generation of CH_4 could be further improved after TH, especially in the reactor with 6% SC. The cumulative CH_4 production in the TH1 was improved by approximately 16.7% compared with R1, while that of the TH3 was increased by 43.5%. Clearly, the substantial enhancement in methane production was mainly driven by the synergistic effect of high SC and TH.

(a)



(b)

Reactor	Kinetic model parameters		
	Pm (mL/g VSS)	Rm (mL/(g VSS·d))	λ (d)
R1	95.95 ± 7.13	4.53 ± 0.37	4.27 ± 0.72
R2	91.51 ± 5.46	4.54 ± 0.36	3.60 ± 0.68
R3	100.00 ± 3.61	6.51 ± 0.47	5.30 ± 0.50
TH1	98.56 ± 6.15	5.78 ± 0.64	4.27 ± 0.84
TH2	112.35 ± 3.85	9.24 ± 0.96	5.03 ± 0.61
TH3	124.22 ± 3.34	9.42 ± 0.61	6.39 ± 0.40

Figure 1. (**a**) Cumulative methane production and (**b**) Gompertz model fitting results in different reactors.

Furthermore, the CH_4 production in each reactor fitted the modified Gompertz model (Figure 1b). The maximum CH_4 yield (Pm) and CH_4 production rate (Rm) increased from 98.56 mL/g VSS and 5.78 mL/(g VSS·d) to 100.00 mL/g VSS and 6.51 mL/(g VSS·d) when SC varied from 2 to 6%. In the TH-treated reactors, the values of Pm and Rm further increased, especially in the high SC reactor. Compared with those in R1, the increased ratios of Pm and Rm in the TH1 were merely 2.72 and 27.59%, but they were up to 24.22 and 44.70% by comparing R3 and TH3. All this evidence suggested that the increase in SC facilitated CH_4 production during AcoD of WAS and FW, with more pronounced increases in the reactors when TH was applied.

3.2. Distinguish the Particular Impact on Specific Processes Triggered by Different Treatments

As the important intermediate products that link substrate metabolism to the performance of digestion, the dynamics of VFAs production and consumption were closely associated with CH_4 generation [21]. In comparison with the reactors without pretreatment, the VFAs concentrations in the TH-treated reactors increased significantly at the initial stage and then decreased gradually (Figure 2a). As the precursor for CH_4 production, the dynamics of VFAs were aligned with the profiles of CH_4 production (Figure 1a).

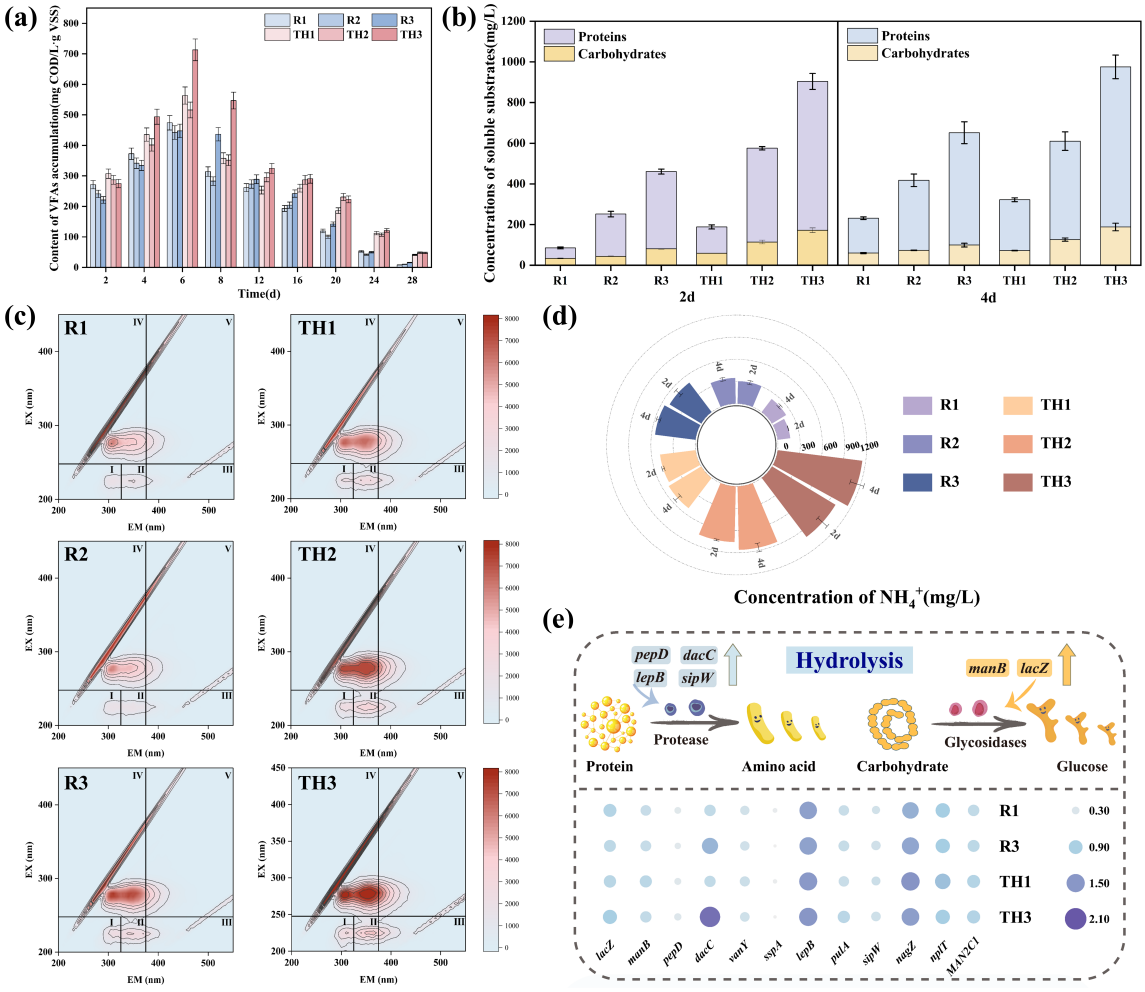


Figure 2. (**a**) Concentration of VFAs; (**b**) polysaccharide and protein concentration; (**c**) three-dimensional fluorescence of supernatants; (**d**) ammonia nitrogen and phosphate concentration; and (**e**) the expression of key genes responsible for encoding hydrolytic enzymes in different reactors.

The solubilization and hydrolysis processes are the limited steps during anaerobic digestion, and their performance has a significant impact on the production of VFAs. Fortunately, these thorny issues could be resolved effectively by TH. As shown in Figure 2b, the carbohydrate concentration in the R1–R3 reactors ranged from 34.2 to 80.7 mg/L, but it surged to 59.3–172.1 mg/L in the corresponding reactors with TH. Similarly, the soluble protein contents in the TH-treated reactors were up to 129.3–731.9 mg/L, which was increased 92.5–152.9% when compared with the reactors without pretreatment. The positive effects of TH on the solubilization of the substrate could also be reflected by the EEM of the supernatants in different reactors (Figure 2c). The fluorescence intensity,

which was directly correlated with the concentrations of fluorescent compounds [22], in the TH-treated reactors was higher than that in the untreated reactors. The remarkable increase in the concentration of soluble substrates could be attributed to the disruption of WAS structure by the TH, which contributed to the release of ex- and intracellular material.

Subsequently, the hydrolytic properties of soluble substrates were also stimulated due to the TH. As indicated in Figure 2d, the concentration of NH_4^+-N, which originated from the biodegradation of proteinaceous matter [4], was remarkably improved from 171.5–498.5 mg/L in the R1–R3 reactors to 483.8–1002.2 mg/L in the corresponding TH-treated reactors. The enhancement of the hydrolysis performance could be further substantiated by the expression of key genes (Figure 2e). For example, the relative abundance of *pepD* (encoding dipeptidyl peptidase) and *dacC* (encoding serine-type carboxypeptidase) in the TH3 was respectively 0.28 and 1.88‰, increased by 24.4 and 55.0% compared to R3. Analogously, *lepB* and *sipW*, which are involved in encoding serine endopeptidases, increased from 1.41 and 0.30‰ in the R1 to 1.48 and 0.35‰ in the TH1. Furthermore, the genes encoding glycosidases, including β-mannosidase (*manB*) and β-galactosidase (*lacZ*), exhibited relative abundances of 0.55 and 0.68‰ in R3, increasing to 0.61 and 0.94‰ in TH3, respectively. The high expression of these functional genes in the TH-treated reactors might be attributed to the great quantities of bioavailable fermentation substrates (e.g., soluble carbohydrates and proteins), which can stimulate bioactivity for substrate hydrolysis [5].

Collectively, the application of TH alleviated the constraints on the solubilization and hydrolysis of substrate, instead providing more bioaccessibility with higher biodegradability of the substrate for VFAs production and subsequent CH_4 production.

3.3. Reshaping of the Microbial Community and Alteration in Bacterial-Archaeal Symbiosis

Since the metabolism of organic substrates under anaerobic conditions was executed by anaerobic flora, the alteration of their composition would actually affect system performance [23]. As shown in Figure 3a, *Chloroflexi* (27.22–36.4%), *Bacillota* (5.28–29.25%), *Pseudomonadota* (9.31–16.23%), and *Actinomycetota* (9.48–15.66%) were the dominant phyla in each reactor, suggesting their critical roles in the bioconversion of fermentation substrates. Nevertheless, their specific abundances were changed due to the varied SC and TH. For instance, the relative abundance of *Chloroflexi*, which plays an important role in the decomposition and transformation of organic materials [24], increased by 10.3% in the TH1 reactor compared to that in the R1. The relative abundance of *Bacillota*, which work along with methanogenic bacteria to generate spaghetti-like structures that aid in organic matter degradation and methanogenesis [25], increased from 5.28% in the R1 to 10.20% in the TH1 and further improved to 29.25% in the TH3. As a result, the significant enrichment of these bacteria promoted the hydrolysis and acidification of the substrate, aligning with the profiles of VFAs production (Figure 2a). For the archaea community, *Halobacteriota*, which colonizes high-salt habitats (e.g., FW) and participates in the methanogenesis process [26], was the primary phylum. Its relative abundance in the R1, R3, TH1, and TH3 reactors was 77.52%, 88.47%, 83.30%, and 93.40%, respectively.

The distribution of functional microorganisms could be more in-depth reflected at the genus level. *Proteiniphilum*, which was primarily utilized to ferment proteins and amino acids, resulting in the generation of a wide range of organic acids [27], increased from 32.67% in the R1 to 38.12% in the TH1 and further improved to 41.17% in the TH3. Its enrichment could stem from the massive release of proteins in the TH-treated reactors, making them become the primary substrates for metabolism. Analogously, the methanogenic genera were also prevalent in the TH-treated reactors and further enriched with the increased SC. Compared to the R3, *Methanospirillum* in the TH3 increased by 94.5%. Similarly, *Methanosarcina*, which has multi-substrate metabolism and can synthesize CH_4 not only through the cleavage pathway of methyl compounds (methanol, methylamine) and acetic acid, but also through the hydrotrophic metabolic pathway to complete the process of methanogenesis [28], in the TH1 was 2.1-fold higher than that in the R1.

Apart from these, the overall performance of AcoD also relies on the cooperative symbiosis among bacterial-archaeal through IDEN analysis [29]. As illustrated in Figure 4, network topology indices indicated that the total number of nodes and connection density in R3 were higher than those in R1, consistent with those compared between TH1 and TH3. This suggests that bacterial-archaeal interactions in the high SC reactors were complex and strongly interconnected, especially after TH. Further exploration found that the increase in SC reduced the negatively correlated links between bacteria and archaea, but increased their positive correlations. The negative and positive correlations in the R1 were 14.18 and 85.82%, but varied to 10.66 and 89.34% in the R3, respectively. In the corresponding TH-treated reactors, this phenomenon was even more pronounced, with their values being respectively 8.11 and 6.86% (negative) and 91.89 and 93.14% (positive) in the TH1 and TH3. It has been demonstrated that the decrease in negative correlations and increase in positive correlations imply reduced

antagonism and enhanced material exchange between bacteria and archaea [30]. This co-operation enhancement might also be one of the key reasons for effective CH_4 production in the R3 and TH3 reactors.

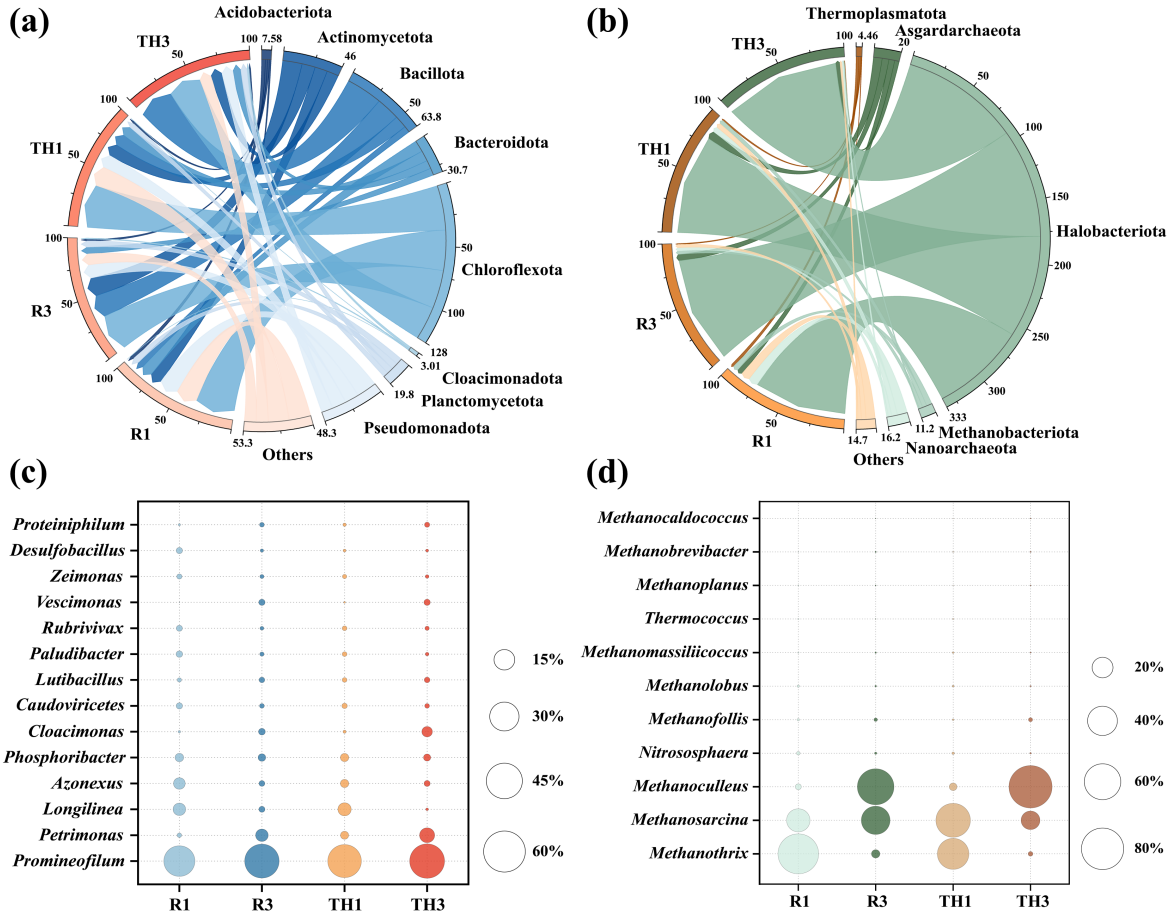


Figure 3. Relative abundance of (**a**) bacteria and (**b**) archaea (phylum level) and (**c**) bacteria and (**d**) archaea (genus level) during WAS and FW co-digestion in different reactors.

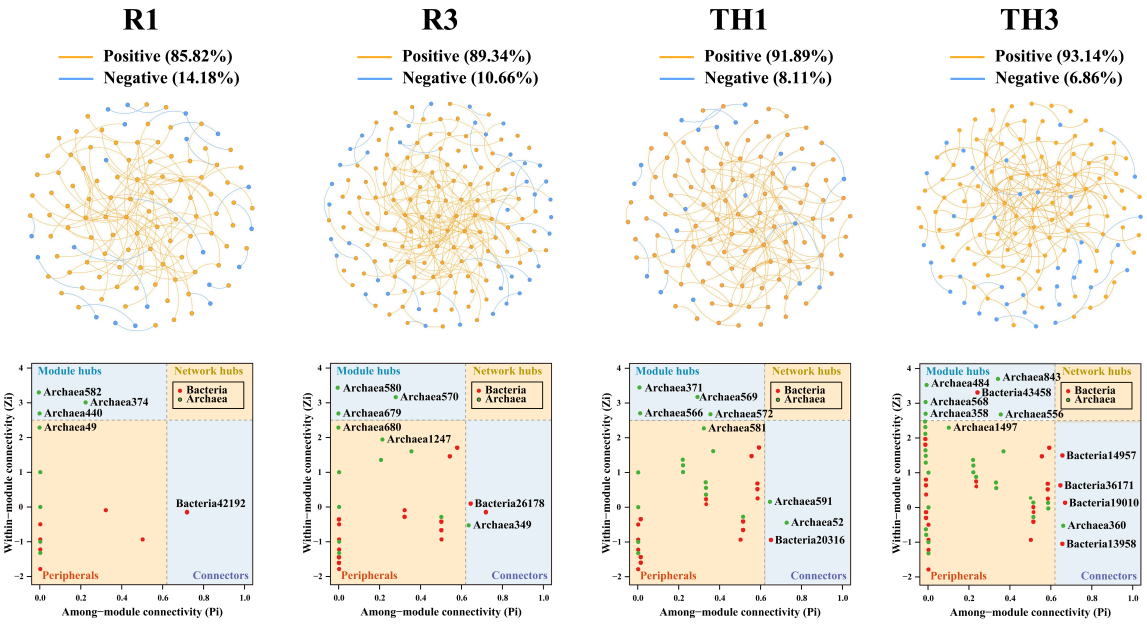


Figure 4. Cross-domain ecological network diagram of species interactions in the bacterial-archaeal community during WAS and FW co-digestion in different reactors.

In summary, the increase of SC contributed to the enrichment of functional bacteria and was favorable for their cooperation. These effects were more pronounced occurring in the TH-treated reactors, thereby resulting in the high-efficiency bioconversion of WAS and FW into CH_4.

3.4. Reconstruction of the Microbial Metabolic Activity for CH_4 Production Enhancement

Except for the enrichment of functional microbes, the effective CH_4 production during AcoD also relied on the microbial metabolic activity. For this purpose, the genetic expression levels related to microbial metabolic activities involved in the pathway of CH_4 biosynthesis were further analyzed via metagenomic analysis.

As shown in Figure 5, most of the critical genes involved in VFAs production were upregulated along with the increase in SC, and further improved by TH. The relative abundance of *pfkA*, which encodes the fructose 6-phosphate kinase 1 and was responsible for catalyzing the production of fructose-1,6-bisphosphate from fructose-6-phosphate [31], was improved from 0.80‰ in the R1 reactor to 0.82‰ in R3and further increased to 0.90‰ and 0.91‰ in the TH1 and TH3 reactors. The *E4.3.1.17* encoding L-serine dehydratase, which catalyzes the dehydration of L-serine to produce pyruvate [32], exhibited a similar distribution pattern. Its relative abundance in the R1 and R3 reactors was respectively 0.43 and 0.70‰, while up to 0.47 and 0.91‰ occurred in TH1 and TH3 reactors, respectively. The high expression of these critical genes contributed to the bioconversion of the primary fermentation substrates (e.g., glucose and amino acids) into pyruvate. Beyond this, the increase in the SC and TH also stimulated the expression of genes that participate in VFAs production. The *korB* gene, which catalyzes the conversion of pyruvate to acetyl coenzyme A, was improved from 2.07‰ (R1) to 2.17‰ (TH1) and further ascended to 2.25‰ (TH3). The distribution of *ackA*, which catalyzes the production of acetate from acetylphosphate [33], increased from 0.56‰ (R1) and 0.65‰ (R3) to 0.60‰ (TH1) and 0.80‰ (TH3). Undoubtedly, the upregulation of these genes contributed to VFAs production, especially acetic acid, aligning with Figure 2a.

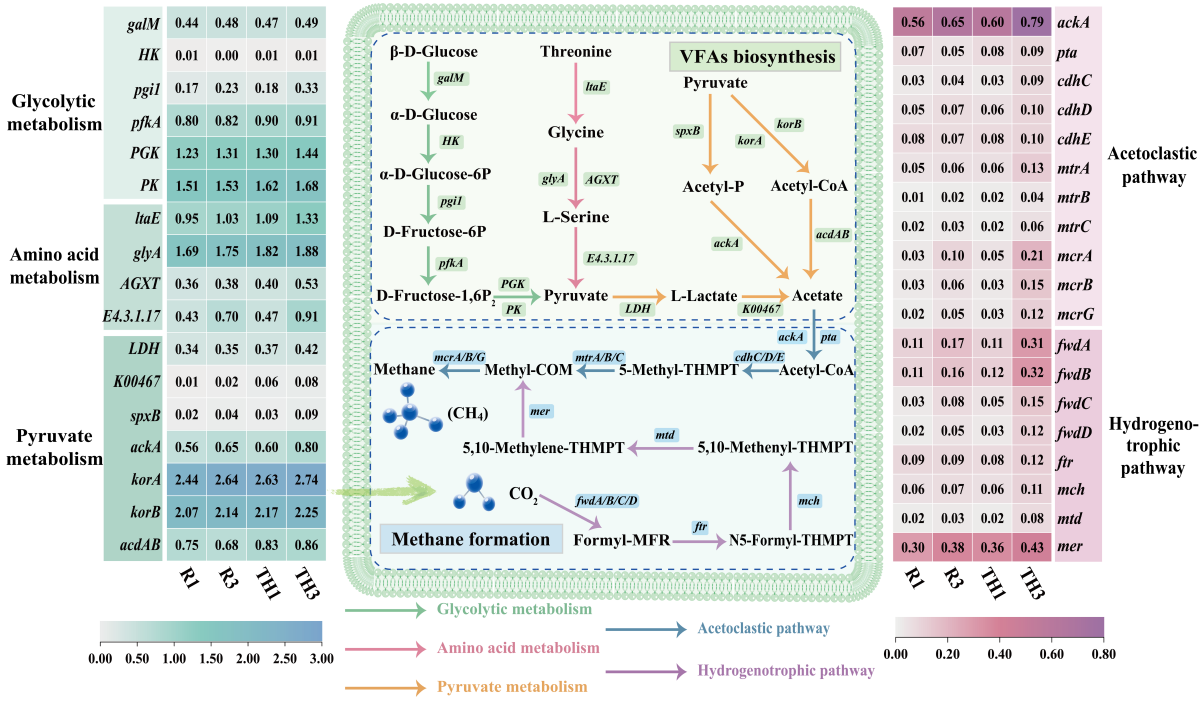


Figure 5. Changes in relative abundance of key coding genes involved in VFAs biosynthesis and methane formation pathways (‰).

Regarding methanogenesis, the generation of CH_4 occurs primarily through the pathways of acetoclastic and hydrogenotrophicmethanogenesis by utilizing acetate and CO_2 as substrates [34]. As expected, the variation in SC and TH also stimulated these pathways, especially acetoclastic methanogenesis. The *mtr* gene cluster plays a key role in converting 5-Methyl-THM(S)PT to Methyl-CoM [35]. In R1, the relative abundances of *mtrA*, *mtrB*, and *mtrC* were merely 0.048, 0.0098, and 0.018‰, while they were raised to 0.061, 0.021, and 0.031‰ in the R3 reactor. With TH, they were further increased to 0.127, 0.0387, and 0.0572‰ in the TH3 reactor. Analogously, the *mcr* gene cluster, which was responsible for converting Methyl-CoM to CH_4 [34], was also upregulated by SC and TH. Apart from this, the associated genes, such as *fwd*, *ftr*, *mch*, and *mtd*, that are involved in the CO_2/H_2-

reduced-type methanogenesis pathway, also showed a significant up-regulation in the reactor with a high SC and TH treatment.

Overall, the increase in SC had a promoting effect on the related genes involved in VFAs biosynthesis and CH_4 production, and such an effect could be further stimulated after TH treatment.

3.5. Overall Understanding of TH Pretreatment Impacts on AcoD of WAS and FW with Different Solids Content

This study demonstrated that with the increase of SC (2–6%), the cumulative CH_4 production rose from 85.0 to 96.3 mL/g VSS, and this value further increased to 99.2–122.0 mL/g VSS in the corresponding TH-treated reactors. The increase in SC and TH pretreatment induced large amounts of organic substrates release, which in turn led to the functional microorganisms occupying the dominant ecological niche. Redundancy analysis (RDA) showed that the VFAs production was positively correlated with the contents of proteins, carbohydrates, and functional bacteria (e.g., *Chloroflexi*, *Bacillota,* and *Proteiniphilum*), and they were clustered closely in TH1 and TH3, providing more substrates for subsequent CH_4 production (Figure 6a). As decisive factors for CH_4 production, methanogenic archaea (e.g., *Methanospirillum* and *Methanosarcina*) also dominated in reactors with higher SC, and TH pretreatment further promoted their enrichment. Meanwhile, archaea and bacteria in TH1 and TH3 exhibited a strong positive correlation, indicating strengthened their symbiotic co-operation for efficient CH_4 production. PLS-PM analysis further confirmed the key roles of TH pretreatment, and SC are the critical factors governing substrates metabolism and microbial community structure (path coefficients: 0.774 and 0.693 for TH & substrates metabolism and bacterial community, and 0.688 and 0.557 for SC & substrates metabolism and bacterial community, respectively) (Figure 6b). Collectively, the increase in SC and TH pretreatment improved CH_4 production by promoting substrate release, functional microbial enrichment, syntrophic interactions, and metabolic function. In addition, the wastes after digestion (digestate and biogas residue) can be recycled through various approaches, such as preparing biochar and recovering nitrogen and phosphorus resources [7,36]. Considering the experimental conditions and product characteristics of this study, the feasibility of resource recovery from digestate after thermal hydrolysis pretreatment should be further investigated in future work.

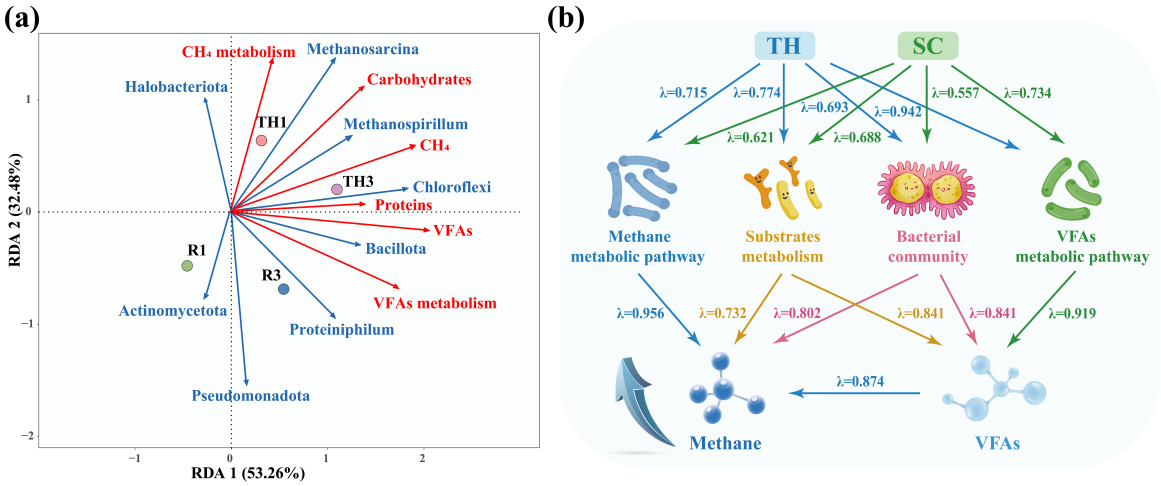


Figure 6. (**a**) RDA correlation analysis between microorganisms and methane yield, VFAs production, and other influencing factors; and (**b**) PLS-PM analysis of the contributions of multi-parameters to methane production during the AcoD of WAS and FW.

4. Conclusions

The increase in SC contributed to CH_4 production during AcoD of WAS and FW, and this performance could be further improved after TH. The enhancement of substrates concentration and bioavailability not only promoted the enrichment of hydrolytic-acidogenic bacteria and methanogenic archaea but also stimulated their cooperative symbiosis. Meanwhile, these effects also stimulated the expression of functional genes, maintaining optimal metabolic activity and ultimately facilitating CH_4 formation. This study provides insights into the effects and mechanisms of SC and TH on the production of CH_4 during anaerobic co-digestion of WAS and FW, suggesting operation at a SC of 6% combined with TH is recommended to achieve the optimal methane yield and energy recovery efficiency of the anaerobic co-digestion system.

Author Contributions

C.Y.: writing—original draft, methodology, investigation, data curation, conceptualization, visualization; L.H.: methodology, investigation, data curation, formal analysis, writing—original draft; Z.W. and X.Z.: conceptualization, investigation, data curation, and validation; C.Z.: methodology, investigation, and visualization; Q.Z.: conceptualization, methodology, investigation, funding acquisition, formal analysis, and visualization; J.C.: methodology, investigation, and visualization; J.L.: writing—review & editing, visualization, methodology, investigation, funding acquisition, formal analysis, conceptualization. All authors have read and agreed to the published version of the manuscript.

Funding

The work is financially supported by the "National Natural Science Foundation of China (No.52470146)", "Engineering Research Center of Biofilm Water Purification and Utilization Technology of Ministry of Education, Anhui university of technology (No. BWPU2023ZY03)" and "Basic Research Program of Jiangsu (BK20250189)".

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Uthirakrishnan, U.; Godvin Sharmila, V.; Merrylin, J.; et al. Current Advances and Future Outlook on Pretreatment Techniques to Enhance Biosolids Disintegration and Anaerobic Digestion: A Critical Review. *Chemosphere* **2022**, *288*, 132553.
2. Moretto, G.; Russo, I.; Bolzonella, D.; et al. An Urban Biorefinery for Food Waste and Biological Sludge Conversion into Polyhydroxyalkanoates and Biogas. *Water Res.* **2020**, *170*, 115371.
3. Li, Y.; Chen, Y.; Wu, J. Enhancement of Methane Production in Anaerobic Digestion Process: A Review. *Appl. Energy* **2019**, *240*, 120–137.
4. Zhang, Q.; Xu, Y.; Yang, H.; et al. Performance and Underlying Mechanisms of Zero-Valent Iron and Percarbonate Co-Regulation for Improved Volatile Fatty Acids Production from Food Waste Anaerobic Fermentation. *Bioresour. Technol.* **2025**, *431*, 132645.
5. Zhang, Q.; Cao, W.; Liu, Z.; et al. Performance and Mechanisms of Urea Exposure for Enhancement of Biotransformation of Sewage Sludge into Volatile Fatty Acids. *Bioresour. Technol.* **2023**, *388*, 129776.
6. Shao, M.; Zhang, C.; Wang, X.; et al. Co-Digestion of Food Waste and Hydrothermal Liquid Digestate: Promotion Effect of Self-Generated Hydrochars. *Environ. Sci. Ecotechnol.* **2023**, *15*, 100239.
7. Wu, Y.; Cao, J.; Zhang, Q.; et al. Continuous Waste Activated Sludge and Food Waste Co-Fermentation for Synchronously Recovering Vivianite and Volatile Fatty Acids at Different Sludge Retention Times: Performance and Microbial Response. *Bioresour. Technol.* **2020**, *313*, 123610.
8. An, J.; Yun, S.; Wang, W.; et al. Enhanced Methane Production in Anaerobic Co-Digestion Systems with Modified Black Phosphorus. *Bioresour. Technol.* **2023**, *368*, 128311.
9. Li, Z.; Chen, H. Elucidating the Role of Solids Content in Low-Temperature Thermal Hydrolysis and Anaerobic Digestion of Sewage Sludge. *Bioresour. Technol.* **2022**, *362*, 127859.
10. Ruiz, H.A.; Conrad, M.; Sun, S.-N.; et al. Engineering Aspects of Hydrothermal Pretreatment: From Batch to Continuous Operation, Scale-Up and Pilot Reactor under Biorefinery Concept. *Bioresour. Technol.* **2020**, *299*, 122685.

11. Martín, C.; Dixit, P.; Momayez, F.; et al. Hydrothermal Pretreatment of Lignocellulosic Feedstocks to Facilitate Biochemical Conversion. *Front. Bioeng. Biotechnol.* **2022**, *10*, 846592.
12. Xu, R.; Fang, S.; Zhang, L.; et al. Revealing the Intrinsic Drawbacks of Waste Activated Sludge for Efficient Anaerobic Digestion and the Potential Mitigation Strategies. *Bioresour. Technol.* **2022**, *345*, 126482.
13. Abbas, Y.; Yun, S.; Wang, Z.; et al. Recent Advances in Bio-Based Carbon Materials for Anaerobic Digestion: A Review. *Renew. Sustain. Energy Rev.* **2021**, *135*, 110378.
14. Han, W.; Liu, X.; Wang, Y.; et al. The Interaction between Sludge and Microplastics during Thermal Hydrolysis of Sludge. *J. Hazard. Mater.* **2024**, *480*, 135816.
15. Luo, L.; Pradhan, N. Anaerobic Mono-Digestion and Co-Digestion of Food Waste and Mixed Sewage Sludge: A Comparative Analysis of Metabolic Patterns and Taxonomic Profiles. *Chem. Eng. J.* **2024**, *489*, 151397.
16. Fang, S.; Cao, W.; Wu, Q.; et al. Multifaceted Roles of Methylisothiazolinone Intervention in Sludge Disintegration and Acidogenic and Methanogenic Pathways for Efficient Carboxylate Production during Anaerobic Fermentation. *Chem. Eng. J.* **2023**, *472*, 145022.
17. Luo, J.; Cao, W.; Guo, W.; et al. Antagonistic Effects of Surfactants and CeO_2 Nanoparticles Co-Occurrence on the Sludge Fermentation Process: Novel Insights of Interaction Mechanisms and Microbial Networks. *J. Hazard. Mater.* **2022**, *438*, 129556.
18. Abbas, Y.; Yun, S.; Wang, K.; et al. Static-Magnetic-Field Coupled with Fly-Ash Accelerant: A Powerful Strategy to Significantly Enhance the Mesophilic Anaerobic-Co-Digestion. *Bioresour. Technol.* **2021**, *327*, 124793.
19. Wu, Y.; Yu, Q.; Zhou, X.; et al. MXene-Coated Piezoelectric Poly-L-Lactic Acid Membrane Accelerates Wound Healing by Biomimicking Low-Voltage Electrical Pulses. *Int. J. Biol. Macromol.* **2024**, *278*, 134971.
20. Li, Z.; You, Z.; Zhang, L.; et al. Effect of Total Solids Content on Anaerobic Digestion of Waste Activated Sludge Enhanced by High-Temperature Thermal Hydrolysis. *J. Environ. Manage.* **2024**, *359*, 120980.
21. Chanthong, S.; Kongjan, P.; Jariyaboon, R.; et al. Synergistic Integration of Hydrothermal Pretreatment and Co-Digestion for Enhanced Biogas Production from Empty Fruit Bunches in High Solids Anaerobic Digestion. *Heliyon* **2024**, *10*, e34817.
22. Zhang, L.; Tian, S.; Yang, S.; et al. Low Intensity Ultrasound Enhances Volatile Fatty Acid Production of Waste Activated Sludge: Insights into Extracellular Polymeric Substances and Sludge Rheological Properties. *J. Environ. Manage.* **2025**, *391*, 126658.
23. Lee, E.; Min, K.J.; Park, K.Y. Changes in Anaerobic Digestion Performance and Microbial Community by Increasing SRT through Sludge Recycling in Food Waste Leachate Treatment. *Sci. Rep.* **2025**, *15*, 19845.
24. Wu, J.; Wang, L.; Du, J.; et al. Biogeographic Distribution, Ecotype Partitioning and Controlling Factors of *Chloroflexi* in the Sediments of Six Hadal Trenches of the Pacific Ocean. *Sci. Total Environ.* **2023**, *880*, 163323.
25. You, J.; Farghali, M.; Osman, A.I.; et al. Mechanisms of Biochar-Mediated Reduction of Antibiotic-Resistant Bacteria and Biogas Production Enhancement in Anaerobic Digesters. *Biochem. Eng. J.* **2024**, *211*, 109465.
26. Oren, A. Novel Insights into the Diversity of Halophilic Microorganisms and Their Functioning in Hypersaline Ecosystems. *NPJ Biodivers.* **2024**, *3*, 18.
27. Zeibich, L.; Staege, M.; Schmidt, O.; et al. Amino Acids and Ribose: Drivers of Protein and RNA Fermentation by Ingested Bacteria of a Primitive Gut Ecosystem. *Appl. Environ. Microbiol.* **2019**, *85*, e01297-19.
28. Wang, G.; Fu, P.; Zhang, B.; et al. Biochar Facilitates Methanogens Evolution by Enhancing Extracellular Electron Transfer to Boost Anaerobic Digestion of Swine Manure under Ammonia Stress. *Bioresour. Technol.* **2023**, *388*, 129773.
29. Cheng, X.; Xiang, X.; Yun, Y.; et al. Archaea and Their Interactions with Bacteria in a Karst Ecosystem. *Front. Microbiol.* **2023**, *14*, 1068595.
30. Huang, W.; Cheng, X.; Li, Y.; et al. Signaling Molecule Alleviates Inhibitory Impacts of Surfactant on Methane Production during Sludge and Food Waste Co-Digestion: Insights of Electron Bifurcation and Quorum Sensing. *J. Hazard. Mater.* **2025**, *484*, 136810.
31. Wu, L.; Zhu, R.; Han, X.; et al. Sulfite Altered Permanganate Effects on Acetate-Enriched Short-Chain Fatty Acids Production during Sludge Anaerobic Fermentation. *Bioresour. Technol.* **2023**, *371*, 128589.
32. Fang, S.; Li, Y.; Wang, F.; et al. Promoting Effects of Bioplastics and Sludge Anaerobic Co-Fermentation for Carboxylates Production with pH Regulation: Insights into the Plastic Structure, Microbial Metabolic Traits, and Adaptive Mechanism. *Bioresour. Technol.* **2024**, *413*, 131564.
33. Zhao, Y.; Yuan, F.; Su, Y.; et al. Metagenomic and Proteomic Insights into Graphene Oxide-Boosted Anaerobic Co-Fermentation of Food Waste and Sewage Sludge for Volatile Fatty Acids Production. *Chem. Eng. J.* **2025**, *504*, 158706.
34. Wang, J.; Cao, W.; Fang, S.; et al. Maximizing Methane Production in Sludge Anaerobic Digestion: Multifaceted Roles of Choline Chloride in Substrate Release, Microbial Adaptation and Syntrophic Interactions. *Chem. Eng. J.* **2025**, *520*, 166286.

35. Murakami, E.; Ragsdale, S.W. Evidence for Intersubunit Communication during Acetyl-CoA Cleavage by the Multienzyme CO Dehydrogenase/Acetyl-CoA Synthase Complex from *Methanosarcina thermophila*: Evidence That the Beta Subunit Catalyzes C–C and C–S Bond Cleavage. *J. Biol. Chem.* **2000**, *275*, 4699–4707.
36. Yun, S.; Fang, W.; Du, T.; et al. Use of Bio-Based Carbon Materials for Improving Biogas Yield and Digestate Stability. *Energy* **2018**, *164*, 898–909.