Effects of Different Acidification-resisting Strategies on Anaerobic Digestion of Kitchen Waste: Methanogenic Properties and Microbial Community Shift

Dongliang Hua,[a,b] Shuai Yuan,[b] Yuxiao Zhao,[a,b] Haipeng Xu,[a,b] Lei Chen,[a,b] Fuqiang Jin,[a,b] and Yan Li [a,b,]*

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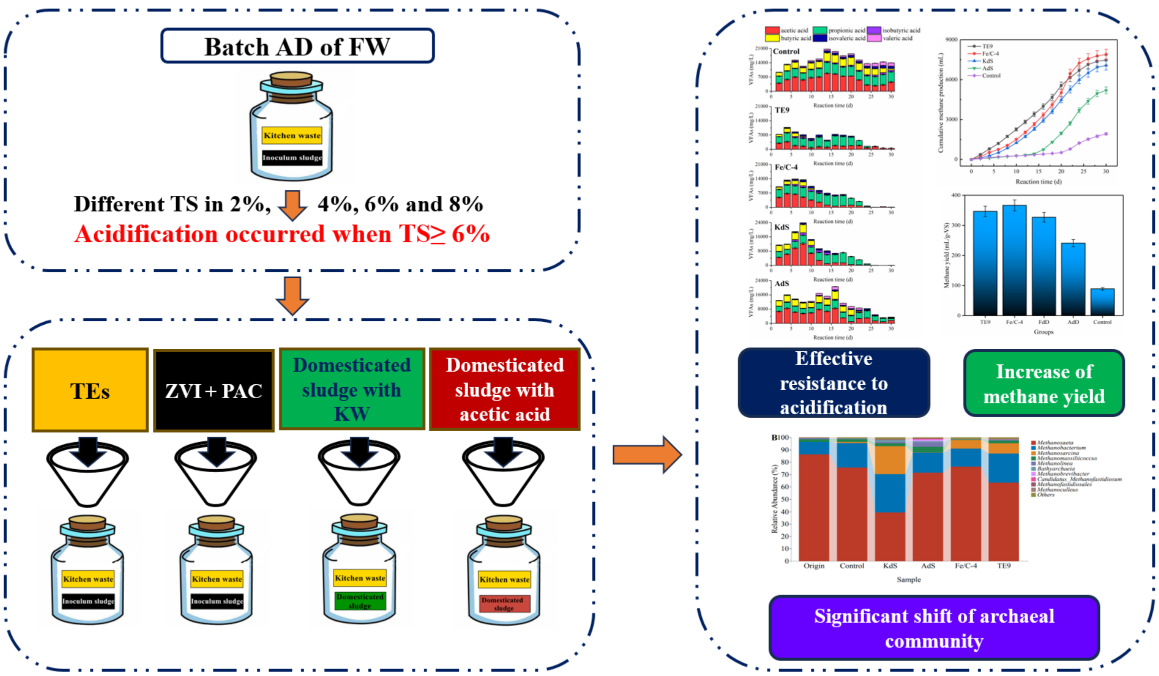
GRAPHICAL ABSTRACT



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Anaerobic digestion (AD) has been widely used as a promising technology for the treatment of kitchen waste (KW). The effects of several acidification-resisting methods were compared, which included the supplementation of trace elements (TEs) and zero-valent iron (ZVI) / powdered activated carbon (PAC), and the application of the sludge domesticated by acetic acid (HAc) and KW as inoculum. The results showed that the supplementation of TEs and ZVI/PAC at total solid (TS) content of 6% and optimal addition doses resulted in an increase in methane yield to 346 and 366 mL/g VS, respectively. In addition, the methane yields of 327 and 241 mL/g VS were obtained by applying the sludge domesticated with KW and HAc as inoculum, while the methane yield of the control was only 89.2 mL/g VS, representing a relative increase of 288%, 311%, 267%, and 170%. The acidification could be alleviated by applying these methods, and also the methanogenic profile was improved. Furthermore, microbial community analysis revealed that the enrichment of *Methanosarcina*, which enhanced the substrate utilization capacity and subsequently increased methane production, was achieved through the addition of TEs and ZVI/PAC, along with the application of sludge domesticated by KW.

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Keywords: Kitchen waste; Anaerobic digestion; Trace elements; Zero-valent iron / powder activated carbon; Microbial domestication

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INTRODUCTION

In China, kitchen waste (KW) has gained great concern due to its huge production. KW, an organic waste consisting of carbohydrates, proteins, and lipids, has the potential to generate renewable energy in the form of methane (Meng *et al.* 2013). If KW is not properly used or even left untreated, the environmental problems, such as water pollution, waste accumulation, and odor pollution would arise (Meng *et al.* 2022a). Anaerobic digestion (AD) is considered to be one of the most environmentally friendly alternatives for disposing of KW due to its minimal environmental impact, including low energy consumption and limited secondary pollution and high potential for bio-energy recovery (Li *et al.* 2018). However, due to the rich organic content of KW, rapid degradation of organic matter generates a significant amount of volatile fatty acids (VFAs), resulting in a rapid pH decrease in the reaction system. Methanogens, which are highly sensitive to pH fluctuations, experience inhibited activity, particularly in low pH environments. Consequently, during AD, this inhibition prolongs the lag phase of methane production and results in lower methane production. At present, the previous research on the methods of acidification-resisting in AD of KW mainly focused on the addition of functional additives and bioaugmentation technology. The functional additives commonly include trace elements (TEs) and conductive materials such as activated carbon (AC), biochar, and zero-valent iron (ZVI). The supplementation of TEs is regarded as an effective method for enhancement of AD of KW. Optimal amounts of TEs not only can stimulate the production of VFAs, but also they can optimize their conversion efficiency (Ortner *et al.* 2015). Shamurad *et al.* (2020) found that an anaerobic system of KW with the supplementation of TEs, including selenium (Se), iron (Fe), nickel (Ni), cobalt (Co), molybdenum (Mo), *etc.*, obtained high methane yield of 450 to 550 mL/g VS. Facchin *et al.* (2013) suggested that the methane yield of batch AD of KW increased by 45 to 65% with the supplementation of TEs (Co, Mo, Ni, Se, and tungsten (W)). Zhang *et al.* (2014) confirmed that the instable long-term AD of KW caused by propionate inhibition could be recovered by the supplementation of Fe, Co, Mo, and Ni. In addition, adding Se and Co improved the process performance and system stability at a high outgoing longwave radiation (5.0 g VS/L/d) and generated a high specific biogas production of 0.75 L/g VS (Banks *et al.* 2012). Some studies have indicated TEs supplementation was an effective approach for enhancement of AD of KW (Zhu *et al.* 2022). However, further research on the effect of different types and dosages of TEs is needed.

Many studies have shown that the addition of conductive materials to anaerobic digesters can accelerate and stabilize the conversion of organic matter to methane (Lim *et al.* 2020). The addition of AC favored VFAs consumption and shortened the lag phase of methanogenesis by improving microbial growth and promoting direct interspecies electron transfer (DIET) (Wu *et al.* 2022). Zhang *et al.* (2020a) reported that the methane yield was improved by 16.6% with supplementation of granular activated carbon (GAC). Additionally, other studies have ascribed the promotion effects of AC to its high specific surface area and abundant porous structure (Dai *et al.* 2022). ZVI has the potential to act as an electron donor for methanogens, resulting in an increase in methane production capabilities (Su *et al.* 2013). Wang *et al.* (2021) found that incorporating ZVI markedly increased the production of formic acid, acetic acid (HAc), and hydrogen (H_2) during the acidogenic phase in the AD of KW, leading to a notable increase in methane yield. The addition of ZVI and AC acting as electron transporter has a positive effect on AD process. The micro-electrolysis between iron and carbon could also work in the sewage treatment, which is known as hydrogen evolution corrosion (Tang *et al.* 2022).

There has been a lack of reports on the synergistic effects of ZVI/powder activated carbon (PAC) for the AD of KW. Moreover, research related to bioaugmentation technology has mainly focused on the domestication of microorganisms. Domestication can also improve the ability of microorganisms to adapt to complex environments, and bioaugmentation has been utilized in the metabolic regulation of AD. Previous research has shown that applying the sludge post-domesticated as inoculum increased the AD performance (Xing *et al.* 2020a). Cho *et al.* (2013) conducted the domestication of sludge and investigated the process of domestication. The results showed a decrease in the diversity of methanogens post-domesticated. Xing *et al.* (2020b) revealed the change in the microbiological characteristics of the anaerobic sludge during a 19-month domestication of KW digestion with cow manure addition. The domestication of microorganisms could create a specific environment and promote the rate of growth in microbial communities. However, the selection of methods and substrates of domestication still need to be investigated and further confirmed.

Three acidification-resisting methods including the supplementation of TEs (Fe^{3+}, Co^{2+}, Ni^{2+}), ZVI/ PAC, and the application of domesticated sludge as inoculum were selected to compare. One aim was to explore the effects of different methods on the performance of methane production during AD. Another objective was to dissect the transformation of the microbial communities in varying acidification-resisting methods. The results will provide valuable insights for relieving the inhibition of VFAs in AD of KW.

EXPERIMENTAL

Materials

The KW was collected from a centralized treatment plant in Shandong Province of China. The material had been screened and separated to remove plastic, chopsticks, bones, and aqueous phase. The total solid (TS) content was 34.25%, while the volatile solid (VS) content was 89.9% (% of TS). Granular anaerobic sludge was obtained from a sewage treatment plant in Laoling Shandong, China, which is characterized by TS of 9.71% and VS of 85.23%. The ZVI of 3 to 5μm was purchased from Kegong Gold Material Co., Ltd. The PAC of 200 mesh was obtained from Maclin Co., Ltd.

Research on the AD of KW with the addition of functional additives

A series of batch trials were carried out to investigate the effects of different types and concentrations of functional additives on methane production from KW. The experiment was performed using a 500 mL serum bottle with a working volume of 400 mL. The sludge and KW were mixed into the reactors with a ratio of 1:1 (based on VS). Afterwards, the mixture was diluted with deionized water to the TS concentrations of 2%, 4%, 6%, and 8%, and were named as X2, X4, X6, and X8, respectively. The pH was initially adjusted to 7.0 using 5 mol/L sodium hydroxide (NaOH) or 5 mol/L hydrogen chloride (HCl). All groups were placed in a water bath at 37 ℃ and shaken every 2 hours. The biogas was collected using an airbag and the volume of biogas was measured using a 200 ml syringe.

Table 1. The Addition of TEs and ZVI/PAC during AD of KW

TEs				ZVI/PAC		
	Fe^{3+} (mg/L)	Co^{2+} (mg/L)	Ni^{2+} (mg/L)		Fe (g/L)	C (g/L)
Control	0	0	0	Control	0	0
TE1	75	0.5	2.5	Fe/C-1	0.5	2.5
TE2	75	1.0	5.0	Fe/C-2	1.0	10.0
TE3	75	1.5	7.5	Fe/C-3	2.5	1.0
TE4	100	0.5	5.0	Fe/C-4	5.0	7.5
TE5	100	1.0	7.5	Fe/C-5	7.5	0.5
TE6	100	1.5	2.5	Fe/C-6	10.0	5.0
TE7	125	0.5	7.5	Fe (5)	5.0	-
TE8	125	1.0	2.5	C (5)	-	5.0
TE9	125	1.5	5.0			

The experiments with different TEs and ZVI/PAC addition schemes are listed in Table 1. The TS in each group were kept at 6%, and the operating conditions for the reactor were the same as those previously mentioned. All these experiments were conducted in triplicate.

Research on the AD of KW with the application of domesticated sludge as inoculum

In all experiments, a 2000 mL glass serum bottle was used, and 1500 g of sludge was added to the reactor. The KW and HAc were selected as the substrate to domesticate the sludge. The addition rate of KW and HAc was increased by 1% of TS and 1 g COD/L every 10 days, respectively.

The domestication process was finally completed on the 50th day. The application of sludge domesticated by KW and HAc was named as KdS and AdS, respectively and the operating conditions were consistent with those described in section 2.2.1. All these experiments were conducted in triplicate.

Methods

TS and VS contents of the KW and sludge were measured according to standard methods for the examination of water and wastewater. The contents of VFAs including HAc, propionic acid (HPr), isobutyric acid (IBu), butyric acid (HBu), isovaleric acid (IVa), and valeric acid (HVa) were quantitatively analyzed by the gas chromatography (Agilent, 7890A, USA) equipped with a HP-FFAP column (50 m*0.2 mm*0.3 mm). The detailed information refers to the previous work (Li *et al.* 2019). The volume and compositions of biogas was measured using a Micro-flow gas analyzer (U30, Shenzhen Angwei Electronics Co., LTD).

The multi-functional analyzer (5B-3B, Lianhua, China) was used to measure the ammonium (NH_4^+-N) during AD. The microbial community (archaea and bacteria) analysis was conducted by high throughput sequencing. The procedures were as follows: DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the recommended protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA quality was checked by1% agarose gel electrophoresis.

The V3-V4 regions of the bacteria 16S rRNA gene were amplified with primers (5'-ACTCCTACGGGAGGCAGCAG-3')/806R (5'-GGACTACHVGGGTWTCTAAT-3') for the V3 and V4 region of 16S rRNA and archaeal primer pairs Arch349F Arch806R. The PCR program consisted of an initial5 min denaturation step at 94 °C, and a total of 25 cycles (each including 30 s at 95 °C, 30 s at 50 °C, and 40 s at 72 °C) was followed by a final extension step of 7 min at 72 °C. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd.(Shanghai, China). Raw fastq files were demultiplexed. Moreover, the pH value was measured using a pH meter (JENCO MODEL 6010M). The experimental data were presented as the mean ± standard deviation. Statistical analyses were performed by Origin software.

RESULTS AND DISCUSSION

Effect of the Supplementation of Functional Additives in AD of KW

Methanogenic profile of KW at different TS content

As shown in Fig. 1(a), the daily methane volume of X2 to X8 reached the first peak on the 2nd to 4th days. Then, the methane volume in X2 and X4 remained constant. However, the lag phases during AD of KW in X6 and X8 were observed, and the daily methane volume declined dramatically. The ultimate methane yields of X2 to X8 were 277, 262, 89.2, and 42.0 mL/g VS, respectively. Combined with the changes in pH and VFAs concentration, the pH in all groups dropped to the range 5.8 to 6.4 in the early stage. The rapid decline of pH in the start-up process could be ascribed to the VFAs accumulation caused by the fast acidification. Subsequently, the pH in X2 and X4 was recovered from day 4 while the pH in X6 and X8 did not rise until day 20, which was related to the organic loading in AD process.

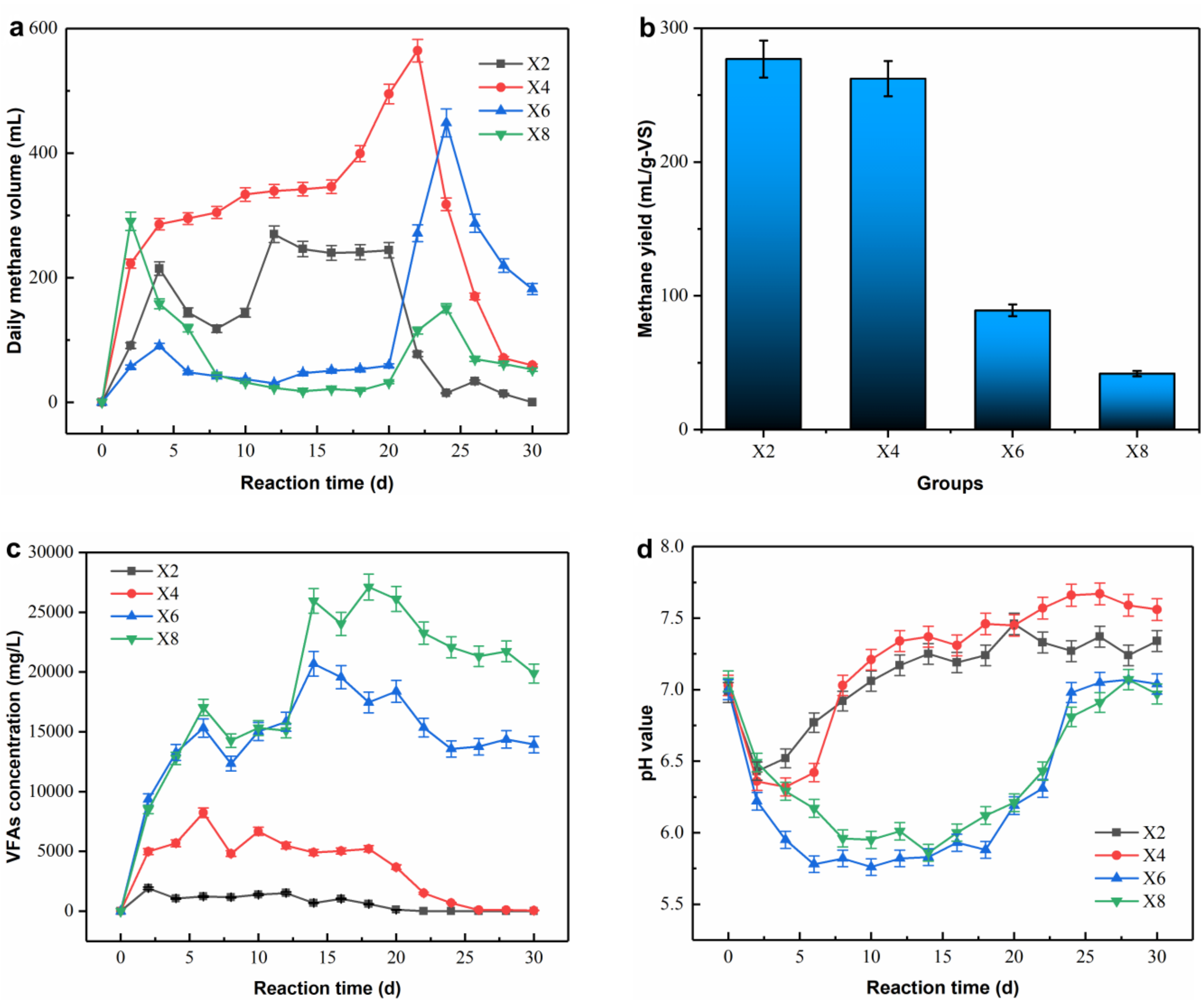

Fig. 1. Performances of batch AD of KW at different organic loadings conditions. Daily methane volume (a), methane yield (b), VFAs concentration (c), and pH (d)

The activity of methanogens was lightly inhibited at low TS content, and the VFAs were gradually converted into methane and CO_2. These findings indicated that the accumulation of abundant VFAs resulted in severe inhibition of methane production when the TS content was higher than 6% during the AD of KW. Therefore, as for such material with easily biodegradable property, the methods about the alleviation of acidification should be investigated.

Effects of TEs concentrations on AD of KW

As shown in Fig. 2(c), the methane yields of TE9 and TE7 were 346 and 298 mL/g VS, respectively, which were markedly higher than those of the other experimental groups. Furthermore, there was no lag phase of methane production in TE9 and TE7. The methane yields of TE1-TE6 ranging from 139 to 195 mL/g VS and the shortening of lag phase were achieved through the supplementation of TEs. It is noteworthy that there was no significant difference in methane yield observed between TE1-3 and TE4-6. This indicated that the simultaneous addition of Fe^{3+}, Co^{2+}, and Ni^{2+} within this concentration range did not significantly impact methane yield due to insignificant interactions among these elements. In addition, the significant difference in methane yield between TE8 and TE7/TE9, despite the addition of high concentrations of Fe^{3+} in TE8, was primarily attributed to the interaction that occurs when these elements were simultaneously added. Specifically, alterations in the dosages of any individual element added can significantly impact methane yield. The results clearly showed the positive effects of TEs supplementation on methane production from KW. The element of Fe promotes the synthesis and activation of various enzymes, as well as facilitates the precipitation of sulfide and reduces its toxicity (Romero-Güiza *et al.* 2016). The element of Co is an important component of carbon monoxide dehydrogenase, while the element of Ni plays an essential role in the synthesis of methyl-coenzyme reductase, a major enzyme for carbon monoxide dehydrogenase (Kida *et al.* 2001). It was found that the synergistic effect among the TEs played a crucial role in enhancing methane production. Notably, when Fe^{3+}, Co^{2+}, and Ni^{2+} were simultaneously added at concentrations of 125, 1.5, and 5.0 mg/L, respectively, methane production increased significantly. A comparable study conducted by Zhang *et al.* (2015) investigated the simultaneous addition of Fe, Co, Mo, and Ni during AD of KW. This study reported a substantial increase in methane production by 35.5% compared to the control. This finding further confirmed the synergistic influence of multiple metals on methane production.

During the early stages of AD, the pH value of all experimental groups decreased sharply, which was between 5.7 and 6.3. This decrease in pH was caused by the accumulation of VFAs, which were generated by the conversion of easily biodegradable substrates in KW. As digestion proceeded, the pH value showed a rising trend, with TE9 recovering at a faster rate. Combining with the analysis of microbial community, the abundance of *Syntrophomonas* and acetoclastic *Methanosaeta* increased, promoting the consumption of HAc and HBu. *Syntrophomonas* belongs to the *Proteobacteria* phylum, which plays a crucial role in the HBu fermentation (Meng *et al.* 2022b). The conversion of HBu to HAc and H_2 was promoted. On the other hand, the acetoclastic methanogens dominated in the archaea, thereby the transformation and utilization of HAc was facilitated and the decreasing trend of pH was mitigated. In addition, the pH recovery rate of the other groups was slower than that of TE9. By comparing the daily methane volume in Fig. 2(a), it was evident that the variation of pH and methane volume was essentially the same, and the lag period of methane production was shortened in different degrees.

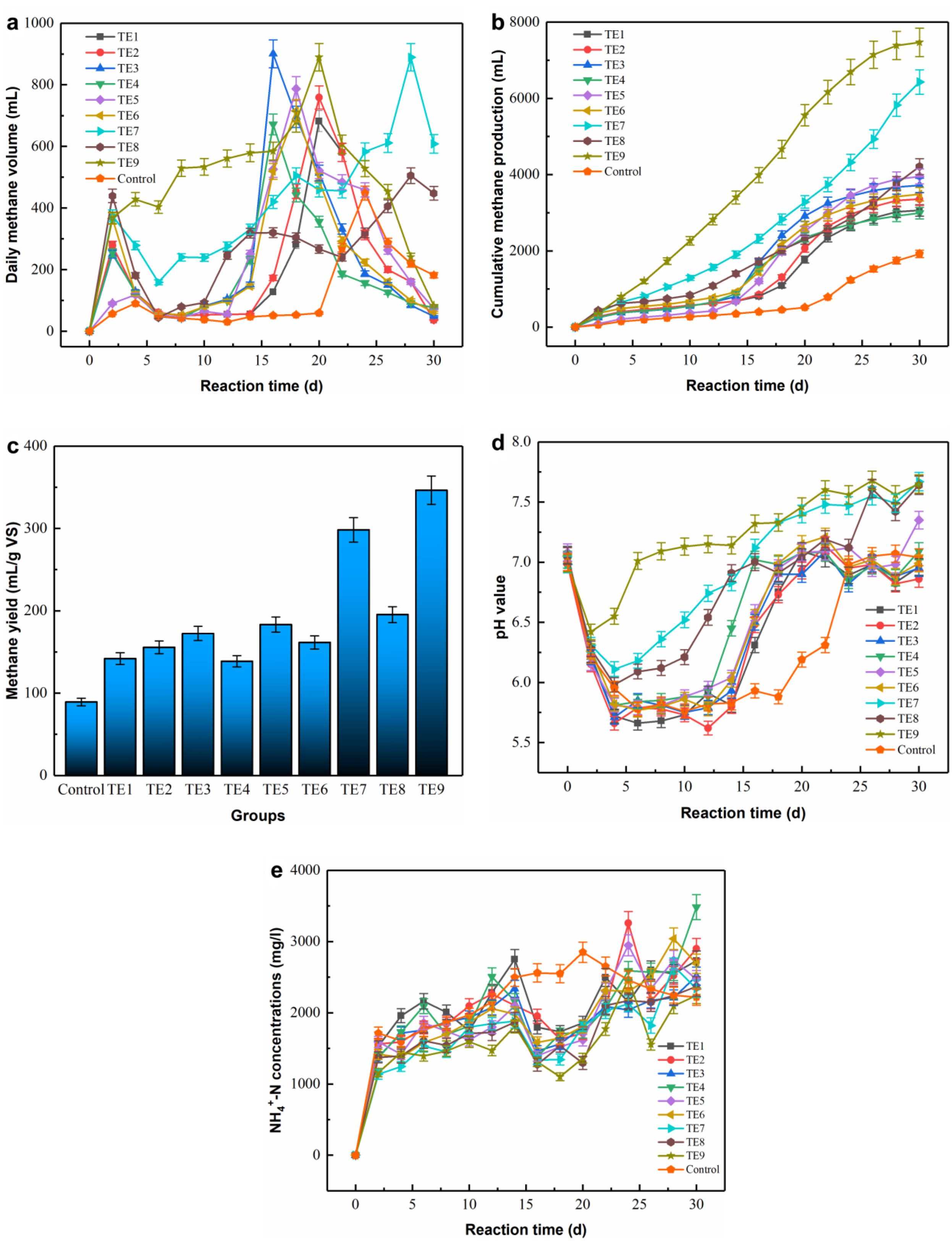

Fig. 2. Performances of batch AD of KW with varying TEs supplementation strategies. Daily methane volume (a), cumulative methane production (b), methane yield (c), pH (d) and NH_4^+-N (e)

The results showed that the supplementation of TEs could markedly enhance the buffering capacity of AD. Furthermore, TEs have been shown to play a crucial role in the acetotrophic pathway of methanogenesis (Fermoso *et al.* 2009). The results indicated that KW was deficient in essential TEs for sustaining basic stability under high load and TEs supplementation greatly enhanced the utilization of VFAs. This agreed with a previous study, in which the reactor adding TEs could maintain a high methane yield of 518 mL/g VS and low VFAs concentration of 438 mg/L (Zhang *et al.* 2020b). The supplementation of TEs with Fe^{3+} (125 mg/L), Co^{2+} (1.5 mg/L), and Ni^{2+} (5.0 mg/L) was the optimum condition.

Effect of ZVI/PAC addition on AD of KW

As indicated in Fig. 3(d), the methane yields of Fe (5) and C (5) were 269 and 152 mL/g VS, and the lag phase on the methane production was shortened by 12 and 6 days compared to the control. Due to the porous structure, large specific surface area and superior adsorption performance, the adhesion sites were provided by PAC for microorganisms, which could reduce the organic loading shock on the methanogenic process (Ma *et al.* 2020). Furthermore, the addition of GAC could promote methanogenesis by stimulating DIET (Ma *et al.* 2020). The addition of ZVI could create an environment conducive to the growth of methanogens, and Fe^{2+} released by the corrosion of ZVI under anaerobic conditions was an important element of various redox enzymes (Yuan *et al.* 2021). This result was similar to the previous research that the addition of AC led to a notable increase in methane yield, from 66.0 to 232 mL/g VS (Hu *et al.* 2023). Besides, Wang *et al.* (2021) observed a 23.9% increase in methane yield when ZVI was added to the AD of KW. The methane yields of Fe/C-1 to Fe/C-6 were 156, 323, 257, 366, 273, and 238 mL/g VS, respectively, and the lag phase on the methane production was shortened by 12 to 16 days compared to the control. This was due to the synergistic effect of ZVI/PAC. According to the principle of a galvanic cell, Fe^{2+} is released through the hydrogen evolution corrosion, which then participates in enzyme synthesis and enhances the metabolic activity of microorganisms. Simultaneously, H_2 reacts with CO_2 to generate methane (Yuan *et al.* 2021). The methane yield of Fe/C-4 showed a notable increase compared to the other groups and there was no lag period on methane production. The analysis of microbial community revealed an abundance of *Methanosarcina*, enabling the utilization of HAc and H_2. Zhang *et al.* (2021) discovered that the addition of ZVI and AC resulted in a 35% increase in methane yield and a 18.2% reduction in the lag phase of methane production. Furthermore, the methane yields of Fe/C-2, Fe/C-4, Fe/C-5, and Fe/C-6 were even higher than those of the experimental groups supplemented with ZVI and PAC alone. The addition of PAC could promote the dissolution, hydrolysis, and acidification of KW, which provided the substrates for methanogenesis (Ma *et al.* 2020). Besides, the addition of ZVI contributed to increasing methane levels and system stability. The synergistic effects were shown by the simultaneous addition of ZVI and PAC in the AD of KW. The methane yields of Fe/C-1 and Fe/C-3 were higher than that of C (5), but lower than that of Fe (5). It was determined that the lower doses of ZVI and PAC in Fe/C-1 and Fe/C-3 were responsible for the results in Table 1. According to Yuan *et al.* (2021), the appropriate dosage of ZVI in the AD of KW is 5g/L. Moreover, there was a positive correlation between the amount of GAC added (ranging from 0 to 5 g/L) and methane yield (Yang *et al.* 2017). Therefore, the addition of ZVI and PAC at appropriate doses could resist the acidification of system, enhance the stability of system, promote DIET, and improve methane production.

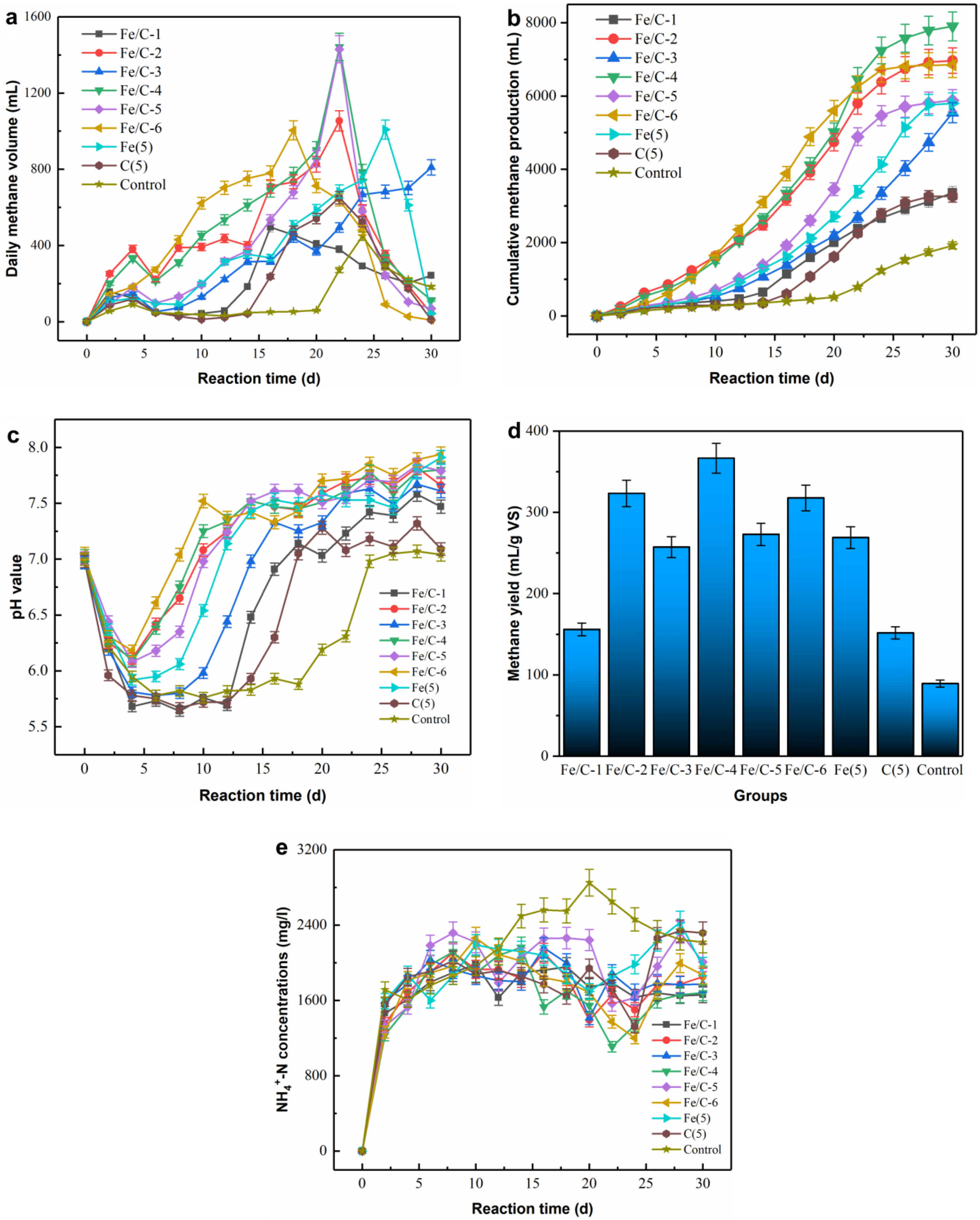

Fig. 3. Performances of batch AD of KW with varying ZVI/PAC supplementation strategies. Daily methane volume (a), cumulative methane production (b), pH (c), methane yield (d), and NH_4^+-N (e)

The pH was approximately recovered to 7.0 on the 8th day. Additionally, the buffering capacity of each group was markedly higher than that of the control group. The peak concentration of VFAs was reached between days 8 to 16. HAc, HPr and HBu were the main components of VFAs, accounting for 83.9% to 91.4% of the composition. Subsequently, the methanogens gradually adapted to the environment and activity recovered. Meanwhile, HAc, HPr, and HBu were rapidly utilized within 10 to 12 days. The conversion rates of VFAs were as follows: HAc > HBu > HPr. After HAc and HBu were completely consumed, HPr became the main component of VFAs in the system. The remaining HPr in Fe/C-2, Fe/C-4, Fe/C-5, Fe/C-6, and Fe (5) was almost completely degraded during the late period of AD. This was due to the production of HPr in the anaerobic system being affected by oxidation-reduction potential (ORP) and the addition of ZVI to reduce ORP possibly promoting the conversion of HPr (Wang *et al.* 2006). Meng *et al.* (2013) reported that the addition of ZVI can effectively increase the activity of dehydrogenase, so the conversion rate of HPr increased from 43 to 77% to 67 to 89%. Furthermore, a notable increase was observed in the abundance of *Methanosaeta* for Fe/C-4, which promoted the conversion of HAc. However, at the end of the experiment, a small amount of HAc and HPr in Fe/C-1, Fe/C-3 and C (5) was still not completely consumed. The reason was that the insufficient addition of ZVI and PAC in Fe/C-1 and Fe/C-3 contributed to the accumulation of VFAs, resulting in a drop in pH and the activity of methanogens was inhibited. The supplementation of ZVI/PAC markedly enhanced AD performance. The optimum condition was 5.0g/L of ZVI and 7.5g/L of PAC.

Effect of the Application of Domesticated Sludge as Inoculum in the AD of KW

Based on the characteristics of KW, the structure of microbial community plays a crucial role in the conversion of organic matter to methane. The domestication of critical microorganisms for specific functions will enhance the adaptability and efficiency of substrate conversion. As shown in Fig. 4(c), the methane yield of KdS was 327 mL/g VS, and there was no lag phase on the methane production. Despite the peak concentration of VFAs on day 8, there was no notable change in pH value. This indicated that microorganisms domesticated by KW had better substrate adaptability and better buffering ability at high concentrations of VFAs. De Oliveira *et al.* (2021) indicated that when the domesticated inoculum was used in AD of KW, the digestion system was stable with methane yield of 292.2 ± 9.8 mL/g VS at the organic loading of 20 g VS/L. This provided additional evidence that the domesticated microorganisms had superior adaptation to high organic loading. The methane yield of AdS was 241 mL/g VS, and the lag phase on methane production was shortened by 6 days compared to the control. Methane yield was lower, and the lag phase of methane production was longer for AdS than those for KdS. The reason might be that the complex components of KW made great contribution to the diversity of microbial community during the domestication.

In the phase of hydrolysis and acidification, the concentration of VFAs peaked at day 8 for KdS and day 16 for AdS. The main VFAs were HAc, HPr and HBu. Of these, HAc accounted for 51.1% and 40.9%, respectively. The concentrations of HPr were 19.7% and 22.3%, respectively. The consumption rate of HAc and HBu was markedly higher than that of HPr. When combined with the analysis of the microbial community, the abundance of *Syntrophomonas* in KdS was markedly increased, and HBu could be converted into H_2 and HAc by *Syntrophomonas*.

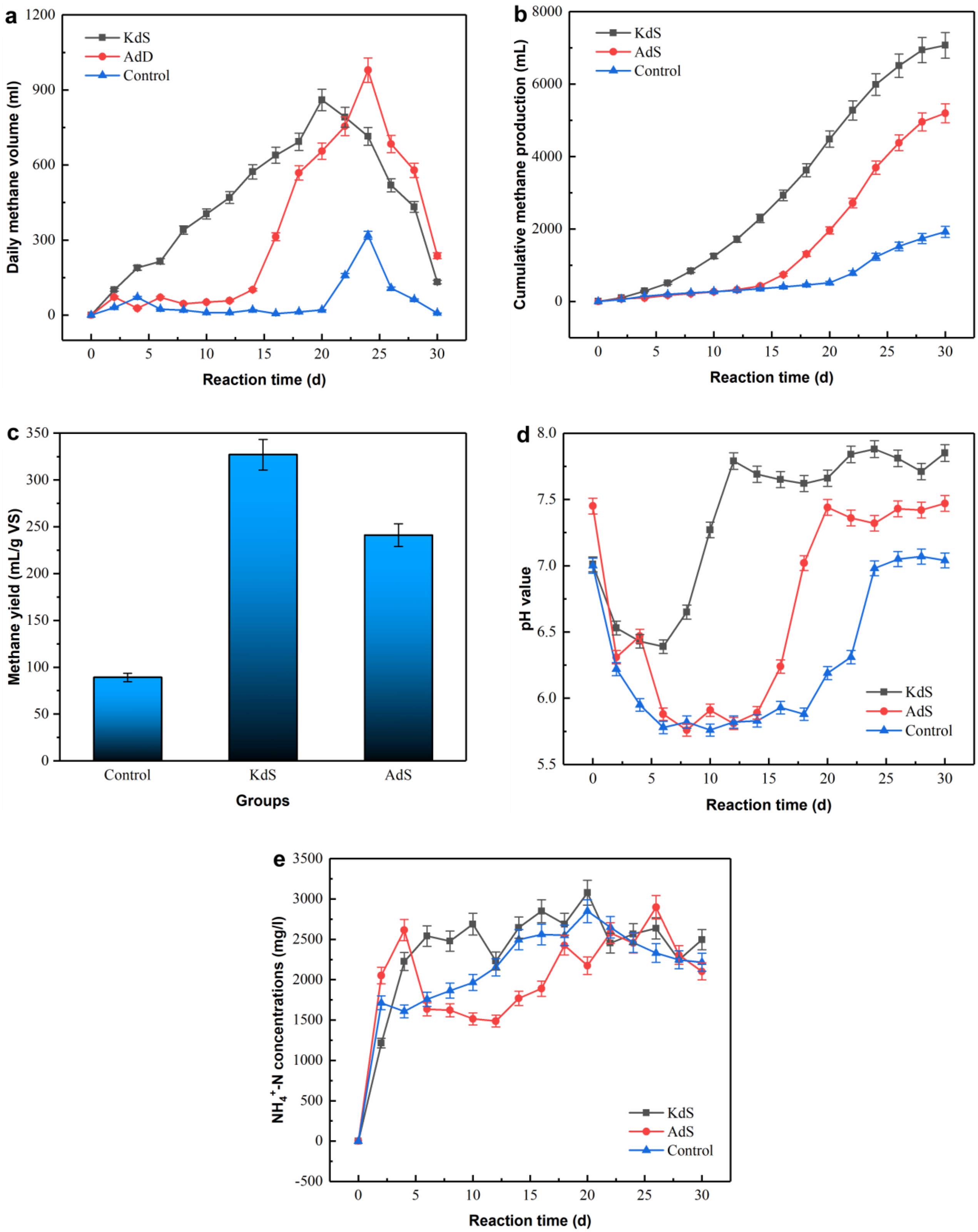

Fig. 4. Performances of batch AD of KW with the application of domesticated sludge by KW and HAc as inoculum. Daily methane volume (a), cumulative methane production (b), methane yield (c), pH (d) and NH4+-N (e)

In addition, the archaeal community in KdS was dominated by both acetotrophic *Methanosaeta* and hydrogenotrophic *Methanobacterium*, which greatly increased the consumption capacity of VFAs. Furthermore, *Methanosaeta* were dominant in AdS. Compared to the control, the sludge domesticated with HAc showed a notable improvement in HAc utilization capacity. By day 30, there was still a small amount of undecomposed VFAs in AdS, while the VFAs were completely degraded in KdS. The methanogenic performance, buffer capacity of the system and the acclimatization of VFAs for KdS were notably better than those for AdS.

Comparison of Acidification-Resisting Methods

Exhibiting the highest methane yields after the addition of TEs and ZVI/TAC, were TE9 and Fe/C-4, which were selected for comparison with KdS and AdS to assess the effects of varying anti-acidification methods on methane production from KW. The methane yields of TE9, Fe/C-4, KdS, and AdS were 346, 366, 327, and 241 mL/g VS, respectively, which was increased by 288%, 311%, 267%, and 170% compared to the control. There were no lag periods on methane production among other groups, except for AdS. These results indicated that the addition of TEs, ZVI/PAC, and the application of domesticated sludge by KW notably enhanced the performance of AD for KW. The methane yield of Fe/C-4 was increased by 311%. This could have resulted for the following reasons. Firstly, PAC could serve as an adsorbent for ammonium nitrogen and other harmful substances in AD systems due to the high specific surface area and excellent conductivity, as well as a carrier for microorganisms. In addition, PAC could promote DIET between microbial species, thus improving the efficiency of AD. Secondly, Fe^{2+} released by the corrosion of ZVI under anaerobic conditions was an important element of various redox enzymes. Hence, adding ZVI could improve the metabolic activity of microorganisms. Finally, a weak galvanic cell can be established in the reaction system due to the potential difference between the components of ZVI/PAC. In the ZVI/PAC-galvanic cell, ZVI as the anode released electrons and PAC on the cathode gained electrons, leading to an electrochemical reaction under acidic anaerobic conditions. The Fe^{2+} generated by the anode can be used to synthesize a variety of redox enzymes in microorganisms, enhancing their metabolic capacity (Zandvoort *et al.* 2006). The H_2, generated at the cathode was one of the important pathways to produce methane, which was important for improving the redox potential and pH of the system (Ray *et al.* 2023). During the hydrolysis and acidification phase, most of the macromolecular organic matter in KW was converted to dissolved VFAs. The utilization of the iron-carbon micro-electrolysis technology was beneficial in promoting the conversion of VFAs, raising the pH of the system, increasing the activity of methanogens, and providing a basis for the subsequent increase in methane production (Huang *et al.* 2016). In summary, the addition of ZVI/PAC to the AD of KW was a more effective method to resist acidification.

Microbial Community Analysis

The analysis of bacterial structure

Insight into the interactions between key microorganisms is crucial to understanding the mechanism of AD in KW. As shown in Fig. 5(a), *Firmicutes* (37.1% to 65.6%), *Chloroflexi* (2.84% to 20.9%), *Bacteroidota* (7.07% to 18.5%) *and Synergistota* (6.4% to 14.3%), were the predominant bacterial phyla in all groups.

Firmicutes can greatly promote the degradation of organic matter by proteases, cellulases, and other extracellular enzymes (Jin *et al.* 2019). *Bacteroidota* is a kind of acid-producing bacteria, which can convert monosaccharides, oligosaccharides, and other substances into HAc, HBu, IVa, H_2 and CO_2 (Shao *et al.* 2023). *Synergistota* plays an important role in the production of HAc (Si *et al.* 2016). Moreover, the *Chloroflexi* can degrade complex organic compounds (Ariesyady *et al.* 2007). The relative abundance of these phyla in KdS, AdS, Fe/C-4, and TE9 were 86.3%, 81.4%, 86.9%, and 87.1% respectively. In addition, the relative abundance of *Proteobacteria* and *Actinobacteriota* in KdS were markedly higher than that of the control group. *Proteobacteria* can participate in the acidification of organic matter and convert organic matter into HAc and H_2, and *Actinobacteriota* can secrete extracellular enzymes to degrade macromolecular organic matter to VFAs (Zhang *et al.* 2023).

The abundances of *Chloroflexi* and *Thermotogota* in AdS increased from 8.3% to 20.9% and 4.8% to 7.4%. *Thermotogota* had a diverse set of hydrolase genes that were highly thermally stable and capable of producing H_2 through the consumption of polysaccharides and monosaccharides (Conners *et al.* 2006). The abundance of *Firmicutes, Bacteroidota,* and *Proteobacteria* in Fe/C-4 increased from 48.9%, 11.8%, and 1.1% to 59.6%, 14.6%, and 3.6%, respectively. In addition, the abundances of *Bacteroidota, Chloroflexi* and *Cloacimonadota* in TE9 increased markedly from 11.8%, 8.3%, and 0.20% to 18.5%, 16.8%, and 2.6%. *Cloacimonadota* was a typical acidogenic bacterium involved in the degradation of organic matter and the production of VFAs during AD (Johnson and Hug 2021).

The bacterial community is closely connected with the metabolites formation (VFAs), which can further affect the methanogenesis process. At genus level (Fig. 5(b)), the predominant bacterium was different in each group. The dominant genera in the inoculum were *JGI-D21* (11.9%), *Mesotoga* (9.47%), and *Bacteroidetes_vadinHA17* (6.65%). There was a reduction in the abundance of these bacteria, and an increase in the abundance of *Proteiniphilum* (5.24%), *Sporanaerobacter* (23.2%) and *Aminobacterium* (5.25%). The abundance of *DTU014* (phylum *Firmicutes*) and *Syntrophomonas* (phylum *Proteobacteria*) were markedly increased in KdS, Fe/C-4 and TE9 compared to control. *Syntrophomonas* was the major butyrate-oxidizing bacterium and oxidized HBu mainly through β-oxidation (Meng *et al.* 2022b). In addition, the abundance of *DMER64* (phylum *Bacteroidetes*) in TE9 and Fe/C-4 increased markedly, by 6.79% and 11.11%, respectively. An active member of the bacterial community in AD systems was *DMER64*, which may be beneficial for interspecies hydrogen transfer (Meng *et al.* 2022b). As shown in the archaeal community, the abundance of the hydrogenotrophic *Methanobacterium* also increased, which can form a syntrophic relationship. In addition, the abundance of *Izemoplasmatales* in Fe/C-4 increased markedly. Previous research indicated that *Izemoplasmatales* encoded multiple extracellular nucleases and extracellular nucleotidases for decomposition of DNA polymers outside the cell, and then it used the liberated nucleosides as nutrient and energy source (Wasmund *et al.* 2021). Currently, there has been limited research on the effect of *Izemoplasmatales* on AD. The dominant genera in AdS were *JGI-D21* (7.18%), *Sporanaerobacter* (5.79%), *Aminobacterium* (5.62%), and *Mesotoga* (6.88%), and the difference in dominant bacteria was not significant compared to control. These results indicated that there was little impact on the bacterial community while the sludge was domesticated by HAc.

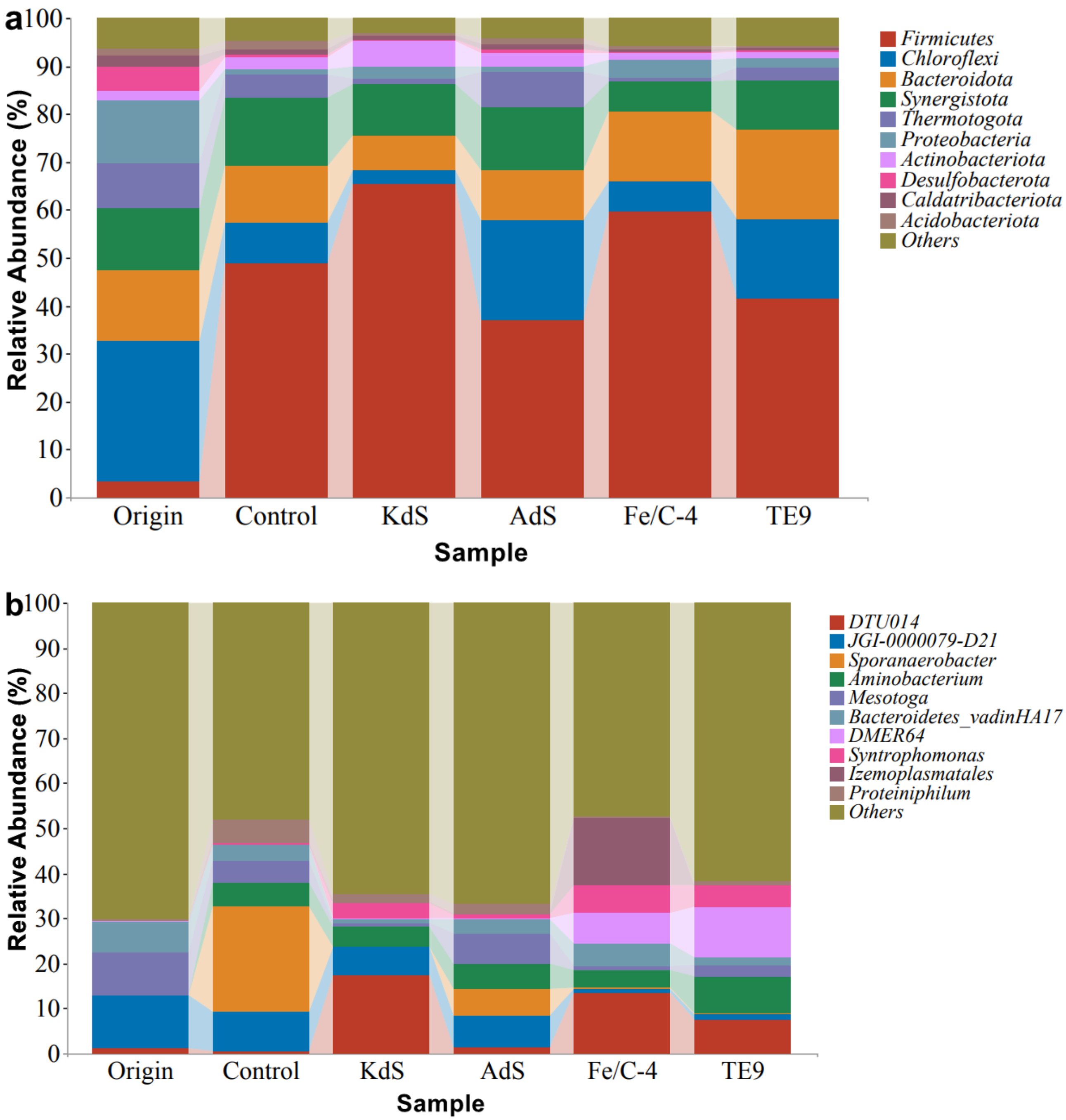

Fig. 5. The analysis of bacterial structures of Origin, Control, KdS, AdS, Fe/C-4 and TE9. Phylum level (a), Genus level (b)

The analysis of archaeal community

The relative abundances of archaea at phylum and genus levels in each group are shown in Fig. 6(a). At phylum level, *Halobacterota* and *Euryarchaeota* were absolutely dominant (94.14% to 99.33%). Most methanogens in the *Halobacterota* could accept both HAc, H_2, and methanol as electron donors (Zanaroli *et al.* 2012). Most methanogens in the *Euryarchaeota* were strictly anaerobic, capable of fixing nitrogen, reducing nitrate, metabolizing sulfur and iron, and producing methane (Usman *et al.* 2021). The abundance of *Euryarchaeota* in KdS was the highest, at 31.1%. At the genus level (Fig. 6(b)), the dominant methanogens in the inoculum were acetoclastic *Methanosaeta* (86.3%) and hydrogenotrophic methanogenic *Methanobacterium* (10.1%). The abundance of *Methanobacterium* increased to 19.8%, while the abundance of *Methanosaeta* decreased to 75.7% after AD. The dominant methanogens in KdS were *Methanosaeta* (39.3%), *Methanobacterium* (30.8%) and *Methanosarcina* (23.0%). *Methanosarcina* was the most metabolically and physiologically versatile methanogen. It can convert different substrates, such as HAc, H_2, and methyl compounds to methane (Yin *et al.* 2018). The abundance of *Methanosarcina* and hydrogenotrophic *Methanobacterium* were significantly increased in the archaeal community of KdS. This indicated that the application of domesticated sludge by KW could improve the structure of the microbial community and induce microorganisms with greater acclimatization to KW. In Fe/C-4, AdS and TE9, *Methanosaeta* and *Methanobacterium* were the dominant methanogens. The relative abundances of *Methanosaeta* were 76.4%, 71.5%, and 63.4%, respectively, and the relative abundances of *Methanobacterium* were 14.7%, 16.2%, and 23.8%, respectively. In addition, the abundance of *Methanosarcina* in Fe/C-4 and TE9 with 6.88% and 8.03% was markedly higher compared to control. The increase in the abundance of *Methanosarcina* also indicated that the addition of TEs and ZVI/PAC can improve the capacity for substrate utilization and enhance the stability of the AD system, thereby increasing methane yield. The relative abundance of *Methanomassiliicoccus* and *Methanolinea* in AdS increased markedly compared to control. *Methanomassiliicoccus* can utilize a wide range of substrates such as formic acid, methanol, and HAc to produce methane (Dridi *et al.* 2012). Moreover, *Methanolinea* was a typical hydrogenotrophic methanogen.

Compared to the control, the diversity indices (Simpson, Shannon, and Chao1) of bacterial and archaeal communities exhibited an increasing trend in the KdS, Fe/C-4, and TE9. This indicated that the addition of TEs, ZVI/PAC and the application of domesticated sludge by KW could enhance the diversity of the bacterial and archaeal communities, which was consistent with the observation reported previously (Cho *et al.* 2013). Furthermore, the Chao1 indices of bacteria and archaea in AdS were markedly increased, while the Shannon and Simpson indices had no obvious differences. This indicated that the complexity of the substrate was crucial for the microbial community during domestication and that complex substrates could promote the growth of more relevant functional microorganisms. In addition, according to the analysis of PCoA, the overall differences of bacteria and the archaea were shown in Fig. S2. It could be found that there were obvious differences between the microbial communities of KdS, Fe/C-4, TE9, AdS, and the control. Therefore, the addition of TEs, ZVI/PAC and the application of domesticated sludge can increase the diversity of microbial communities and thus improve the methane yield.

Table 2. Alpha Diversity Indexes of Archaea and Bacteria (The Microbial Community of Varying Acidification-Resistant Methods)

Sample	Bacteria				Archaea			
	Chao1	Shannon	Simpson	OTUs	Chao1	Shannon	Simpson	OTUs
Control	1096.25	6.23	0.95	1058	424.24	3.75	0.85	390
KdS	1867.81	7.23	0.98	1844	528.78	5.20	0.93	490
AdS	1365.79	6.17	0.94	1305	549.06	4.15	0.86	510
Fe/C-4	2139.14	7.25	0.97	2080	536.78	4.34	0.87	503
TE9	1513.99	7.14	0.98	1452	477.71	4.51	0.88	448

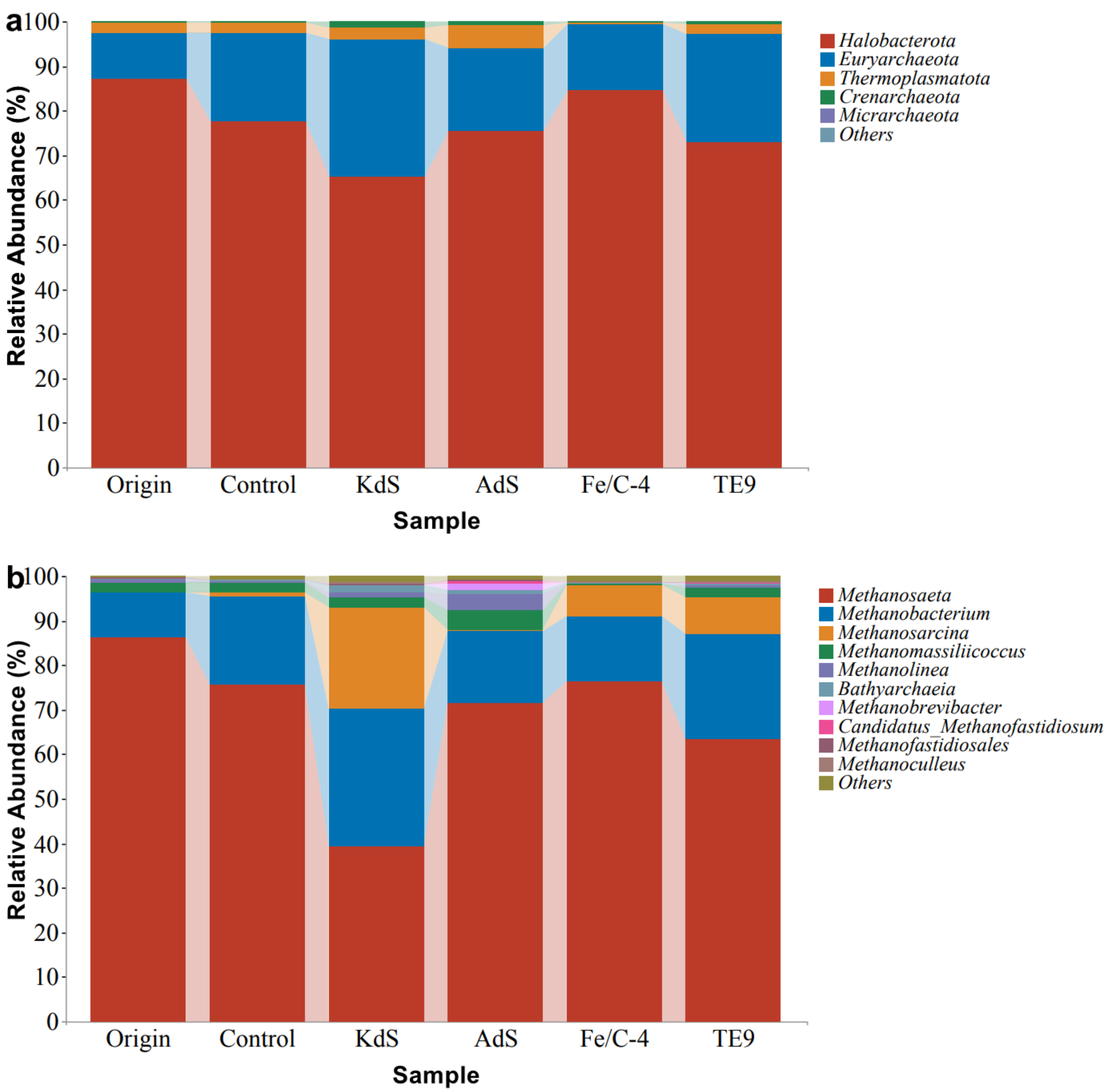

Fig. 6. The analysis of archaeal structures of Origin, Control, KdS, AdS, Fe/C-4 and TE9. Phylum level (a), Genus level (b)

CONCLUSIONS

1. The study systematically investigated the effect of the addition of different functional additives (trace elements (TEs) and zero-valent iron/powdered activated carbon (ZVI/PAC)) and the application of sludge domesticated by kitchen waste (KW) and acetic acid (HAc) as inoculum to enhance the acidification-resisting capacity and methane production during the anaerobic digestion (AD) of KW.

2. The results showed that the optimal doses for TEs were Fe^{3+} 125 mg/L, Co^{2+} 1.5 mg/L and Ni^{2+} 5.0 mg/L, respectively and for ZVI/PAC were ZVI 5.0 g/L and GAC 7.5 g/L, respectively.
3. The addition of TEs, ZVI/PAC and the application of sludge domesticated by KW and HAc as inoculum improved methane yield by 288%, 311%, 267%, and 170% respectively, markedly shortened the lag phase of methane production and increased pH buffering capacity.
4. Microbial community analysis revealed that the abundance of *Methanosarcina* and hydrogenotrophic *Methanobacterium* were significantly increased in the archaeal community of kitchen waste-domesticated sludge bacteria (KdS).
5. *Methanosarcina* was enriched by adding TEs and ZVI/PAC, along with the application of sludge domesticated by KW. This approach improved the substrate utilization capacity and stability of the AD system, ultimately leading to an increase in methane yield.
6. Thus, the addition of TEs, ZVI/PAC, and the application of domesticated sludge by KW and HAc as inoculum were the promising methods to increase both the capacity of acidification-resisting and whole AD process for higher efficiency. The addition of ZVI/PAC was shown to be the most effective method in this study.

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APPENDIX

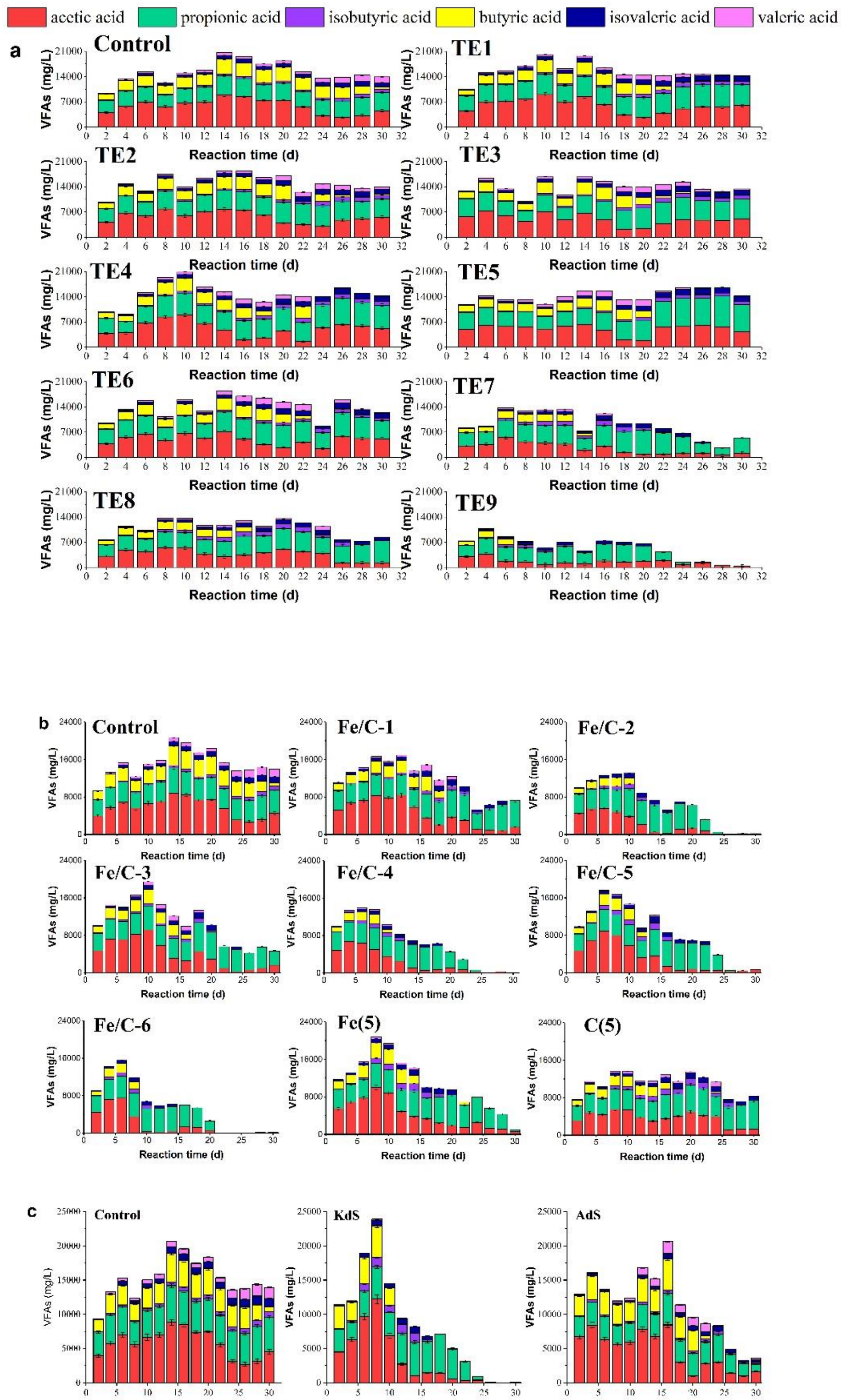

Fig. S1. The concentration and composition of VFAs in varying acidification-resisting methods. The addition of TEs (A), the addition of ZVI/PAC (B), the application of the sludge domesticated by HAc and KW as inoculum (C)

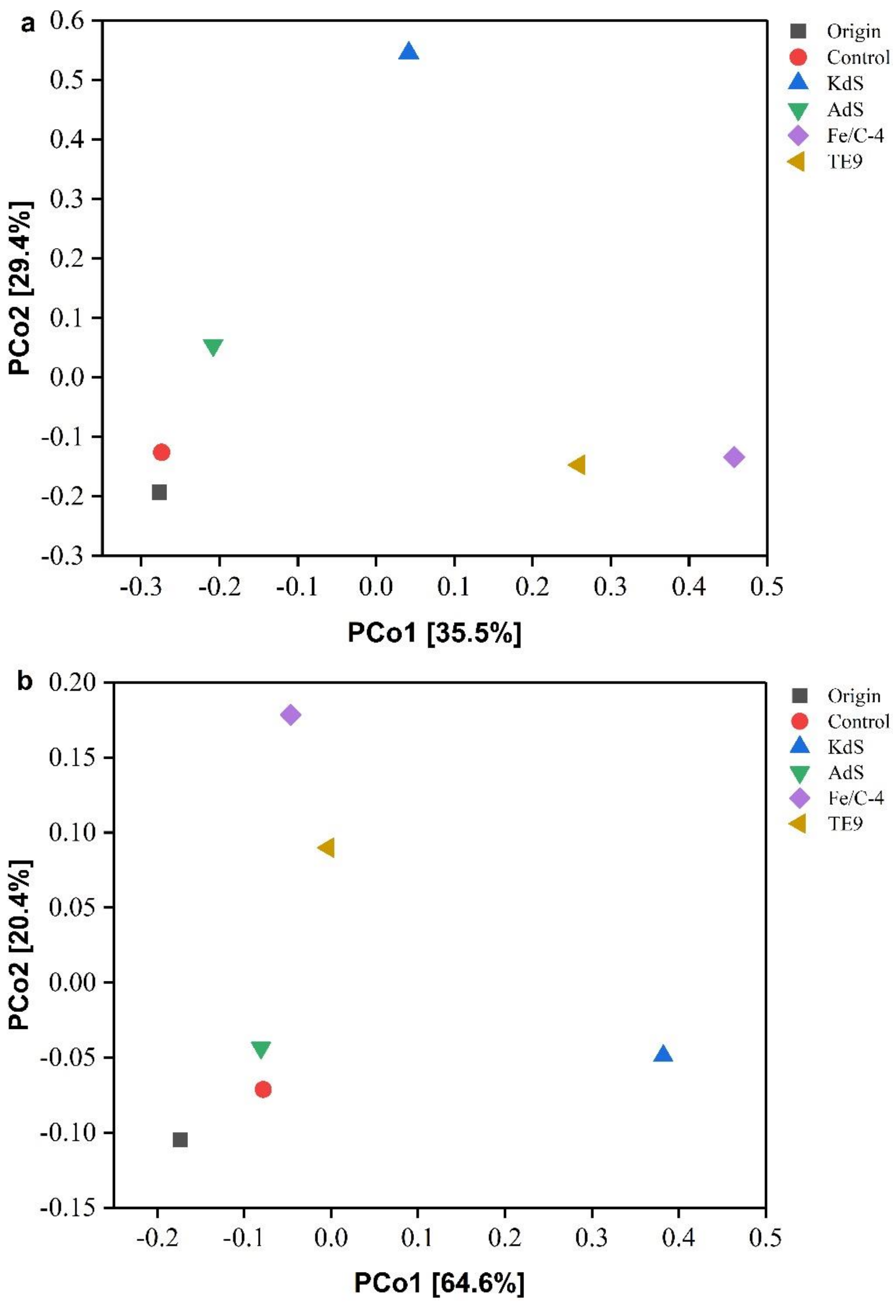

Fig. S2. Bacterial (A) and archaeal (B) community dissimilarity of inoculum and biomass samples from digestions applying the analysis of PCoA.