Enhanced Anaerobic Digestion of Kitchen Waste with Activated Carbon: Performance and Mechanisms under Varying Total Solids and Organic Loading Rates

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Abstract. Anaerobic digestion (AD) is a key technology for kitchen waste (KW) treatment in China. To enhance AD efficiency under the current carbon neutrality goals, this study investigated the effects and mechanisms of activated carbon addition under varying total solids (TS) contents and organic loading rates (OLRs). The results showed that activated carbon improved system buffering capacity, increased methane yield by up to 37.72%, and allowed stable operation at high OLRs (up to 13.5 gVS/(L·d)), with volatile solids (VS)degradation rates exceeding 85%. Functional groups and metal elements on the activated carbon surface enhanced alkalinity and electron transfer, accelerating volatile fatty acid (VFA) degradation and methane production. Microbial analysis revealed that activated carbon enriched syntrophic VFA-degrading bacteria and methanogens, particularly promoting the acetoclastic pathway through Methanosaeta. Moreover, the establishment of direct interspecies electron transfer (DIET) among Anaerolinea, Bacteroides, Streptoides, and methanogens further improved electron transfer efficiency. Overall, activated carbon effectively enhances the stability and performance of high-TS AD systems treating KW.

Keywords: Kitchen waste; activated carbon; dry anaerobic digestion; direct interspecies electron transfer.

1. Introduction

Despite China's rich biomass resources, increasing production and consumption have led to substantial waste generation. Kitchen waste (KW), a major component of municipal solid waste, accounted for approximately 30%-50% (72.44-127.4 million tons) of the 254.79 million tons generated in 2020 [1, 2]. To meet national carbon peaking and neutrality targets of China, effective resource utilization of KW is urgent. Anaerobic digestion (AD) is widely used for this purpose, providing a sustainable means for resource utilization, reduction, and harmless treatment of KW [3, 4]. Depending on the total solid (TS) content of the feedstock, anaerobic digestion can be divided into two types: wet type (TS content <15%) and dry type (TS content 15%~20%) [5]. The TS content of the feedstock and the organic load are important factors that affect methane production capacity and system stability [6]. Due to KW's low moisture and high salt content, dry AD is more appropriate, as it requires less water and retains more nutrients [7]. Wet AD, by contrast, often requires extensive dewatering, which leads to higher energy consumption, reduced efficiency, and biomass energy loss [8].

However, KW has a high organic content which can lead to the accumulation of organic acids during anaerobic digestion, causing the breakdown of the reaction system [9]. To address this, pretreatment, co-digestion, and the addition of conductive materials are commonly applied [10, 11]. The addition of conductive materials such as bentonite, biochar, activated carbon, carbon cloth, and iron oxide is a promising strategy to enhance anaerobic digestion [12]. Activated carbon is a highly promising material due to its simple production process and abundant raw material reserves [13]. It improves buffering capacity, stabilizes pH, enhances VFA degradation, and boosts methane production [14, 15]. Sunyoto et al. [16] demonstrated that activated carbon shortens the methane

production lag phase and accelerates VFA breakdown. There are also studies indicating that activated carbon made from fir wood can increase methane production by 48.3% compared to the blank control group [10].

With the recent implementation of garbage classification in cities like Beijing and Shanghai of China, the physicochemical properties of KW have changed significantly, reinforcing anaerobic digestion as a key technology for future development. This study investigates the effects of activated carbon, applied as an exogenous additive, on anaerobic digestion under varying total solids (TS) contents and organic loading rates. Key indicators such as pH, VFAs, and ammonia nitrogen are analyzed to elucidate the enhancement mechanisms. The study also assesses how activated carbon influences microbial activity across different TS and loading conditions, providing insights into system stability and digestion maturity.

2. Materials and methods

2.1. Substrate and inoculum

KW samples were collected from five residential waste separation points in Haidian District, Beijing. Following the "Sampling and Analysis Methods for Domestic Waste" (CJ/T 313-2009), samples were taken every 3 days at fixed locations. After thorough mixing, a quadratic sampling method was used to select representative samples. The KW was then crushed to < 2 mm particle size for property analysis and stored at 4°C. The inoculum was sourced from the anaerobic digestion unit of a sewage treatment plant in Hebei province. The sludge had TS and VS contents of 7.20% and 5.17%, respectively, a carbon-to-nitrogen ratio of 8.58, and a pH of 6.59. It was pre-incubated at 35 ± 0.5°C for 7 days, then left to stand until gas production ceased, after which it was used as the experimental inoculum. The activated carbon used was coconut shell activated carbon with a black amorphous appearance, purchased from Henan Lizhe Environmental Technology Co., Ltd. The particle size ranged from 10 to 40 mesh, with well-developed porosity, strong adsorption capacity, good mechanical strength, and conductivity between 30 and 350 μS/cm. Prior to use, it was soaked in deionized water for 12 hours, dried at 105°C, and stored in a dryer.

2.2. Experimental setup design and operation

A semi-continuous anaerobic digestion system was used to simulate the single-phase dry Dranco process for high-solids food waste. The reactor consisted of a 500 mL bottle with a modified 50 mL syringe at the center, connected to a hose extending into the reactor for feeding. Material was added by removing the syringe piston. The right port was linked to a gas bag for biogas collection, while the left port was connected to a discharge hose reaching the bottom of the reactor (Fig.1). The reactors were maintained in a constant-temperature water bath at 37 ± 1°C. Each experimental run lasted 23 days, ensuring operation exceeded the hydraulic retention time.

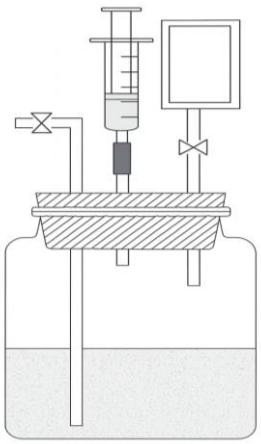

Fig 1. Schematic diagram of dry anaerobic digestion experimental device.

2.3. Analysis methods

TS and VS contents were determined by the gravimetric difference method. pH was measured using a glass electrode. Volatile fatty acids (VFAs) were analyzed via high-performance liquid chromatography (HPLC, Waters e2695, USA). Ammonia nitrogen (NH_4^+-N) was determined by using Nessler's reagent spectrophotometry (HJ 535-2009). Biogas volume was measured by the water displacement method, and gas composition was analyzed using gas chromatography (GC, Agilent 7890A) with a flame ionization detector.

2.4. Microbial community analysis

At the end of the AD process, digestate samples were collected for 16S rDNA high-throughput sequencing (Shanghai Paisennuo Biotechnology Co., Ltd.). The bacterial 16S rRNA V3–V4 region (338F/806R) and archaeal V4–V5 region (524F/958R) were sequenced using the Illumina MiSeq platform. Raw reads were assembled with FLASH software and taxonomically classified using QIIME2 with the Silva database.

2.5. Calculation methods

In a continuously stable operating system, the VS degradation rate can be calculated using Eq 1.

$$VS\ degradation\ rate = \left[1 - \frac{\omega \times (100-\eta)}{\eta \times (100-\omega)}\right] \times 100 \quad (1)$$

Where ω represents the VS content in the sludge, %; η represents the VS content in the input material, %.

The modified Gompertz equation was used to analyze and describe the experimentally observed cumulative methane production in each reactor, as shown in Eq 2.

$$P = P_m \times \exp\left\{-\exp\left[\frac{R_m \times e}{P_m} \times (\lambda - t) + 1\right]\right\} \quad (2)$$

Where P is methane production, L/g; P_m is maximum methane production, L/g; R_m is the maximum methane production rate, L/(g·d); λ is the lag time, d; t is digestion time and e are Euler's number,2.718.

3. Results and discussion

3.1. Effect of activated carbon on the performance of anaerobic digestion with different feed TS contents

3.1.1. Biogas and methane production

The biogas and methane production are the most intuitive indicator to evaluate the anaerobic system. As shown in Fig. 2, the addition of activated carbon led to varying degrees of increase in gas and methane yields at all TS levels. The cumulative methane production of the anaerobic system increased by 13.35%, 36.92%, 30.65%, 32.28%, 37.72%, and 32.53% respectively. The relative increase was the most significant at 28% TS, while the system with 25% TS achieved the highest absolute gas and methane yields. After carbon addition, cumulative gas production at 25% TS rose from 29.24 L to 37.44 L, and methane production from 16.81 L to 22.23 L, confirming that 25% TS provided the best overall gas production.

To further analyze methane production kinetics, the modified Gompertz equation was used to fit cumulative methane yield per unit VS under different TS levels (Fig.3), and the corresponding parameters are listed in Table 1. All fitted curves showed high goodness-of-fit ($R^2 > 0.995$). Compared to the non-carbon group, both the maximum methane yield per unit VS and the peak production rate increased significantly ($P < 0.05$), with the highest values being 12.24 L/g and 0.49 L/(g·d), respectively, observed at 12% TS. The methane lag phase significantly increased ($P<0.05$), with the shortest in the system with a feedstock TS content of 33%, being 0.76 d. Similar

to the non-carbon group, increasing TS content enhanced both the maximum methane yield per unit VS and the peak production rate, but also prolonged the lag phase. With activated carbon addition, the maximum unit VS methane yield and the highest methane production rate in all systems were higher than those in the systems without added charcoal, with average increases of 41.70% and 22.38%, respectively.

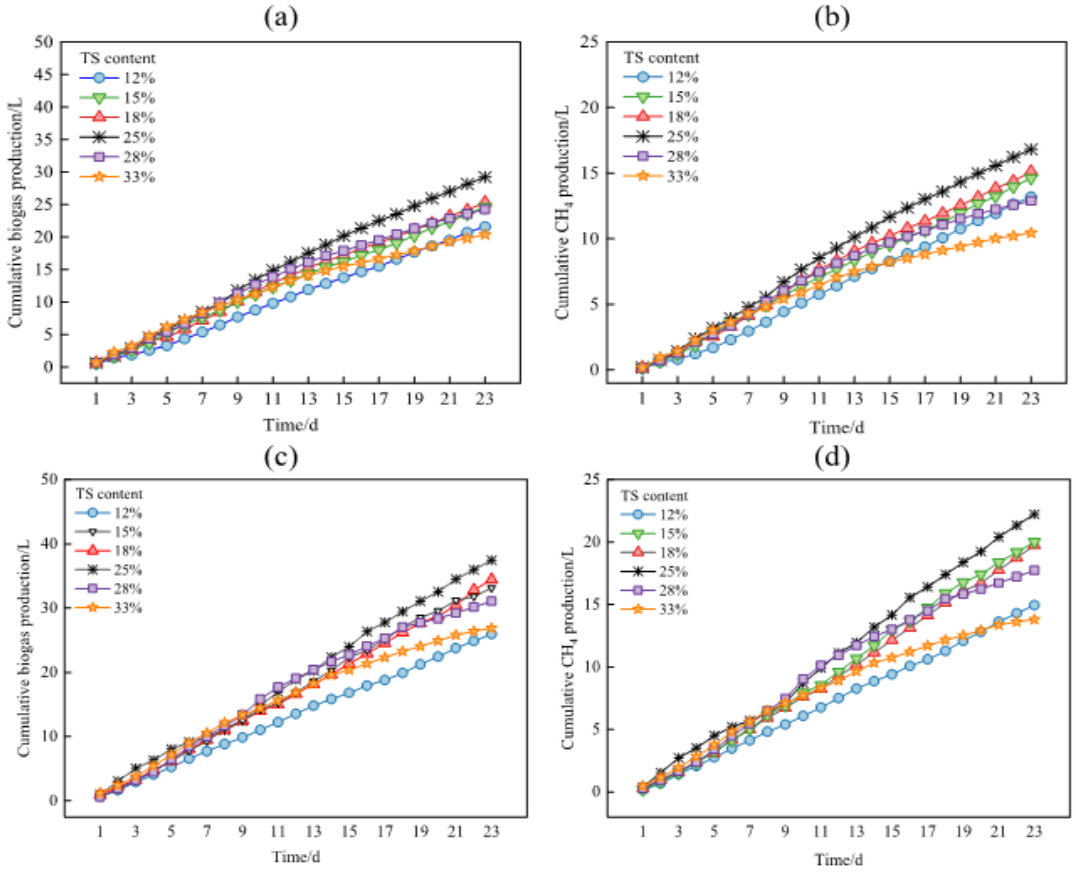

Fig 2. Biogas and methane production under different TS conditions. (a-b) Without activated carbon; (c-d) with activated carbon.

Activated carbon addition enhanced both the maximum methane yield per unit VS and the peak production rate. In terms of gas output, 25% TS remained optimal, yielding the highest cumulative gas and methane production. The Gompertz model fitting further supports that carbon addition improves system kinetics without excessively extending the lag phase.

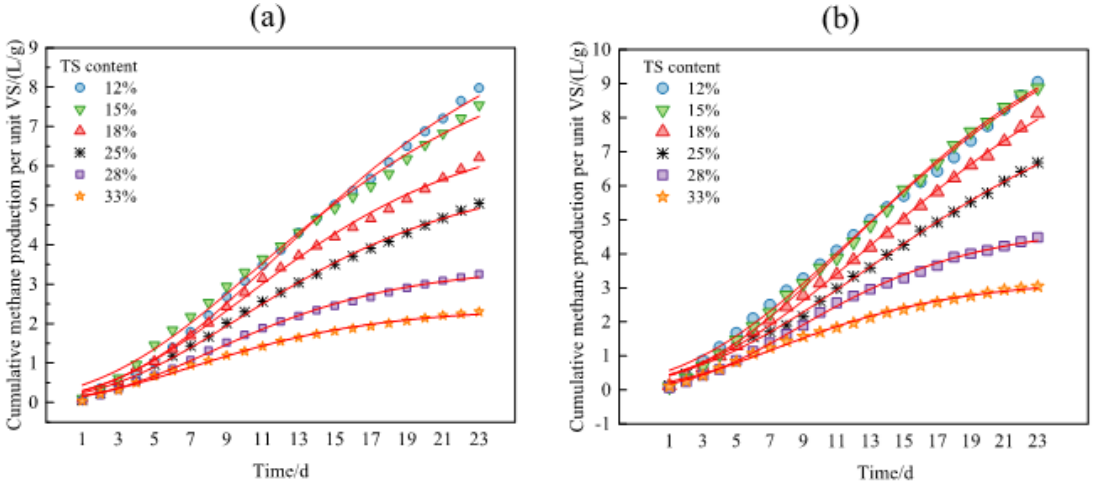

Fig 3. Cumulative methane production per unit VS under different TS conditions. (a) Without activated carbon; (b) with activated carbon.

Table 1. Kinetic parameters for methane production at different TS levels (Gompertz model).

TS content (%)	P_m(L/g)		R_m(L/g·d)		λ(d)		R^2	
	None	activated carbon	None	activated carbon	None	activated carbon	None	activated carbon
12	10.28±0.38	12.24±0.74	0.44±0.01	0.49±0.01	3.31±0.17	3.01±0.25	0.998	0.995
15	9.23±0.47	11.84±0.42	0.39±0.01	0.46±0.01	2.37±0.27	2.86±0.16	0.995	0.998
18	7.16±0.23	11.62±0.58	0.34±0.01	0.42±0.01	2.23±0.21	2.84±0.18	0.997	0.998
25	5.87±0.15	9.70±0.47	0.28±0.01	0.34±0.01	2.17±0.18	2.34±0.18	0.998	0.997
28	3.48±0.06	4.86±0.07	0.21±0.01	0.28±0.01	1.86±0.16	2.20±0.14	0.998	0.998
33	2.41±0.05	3.27±0.07	0.15±0.01	0.19±0.01	0.83±0.23	0.76±0.23	0.996	0.996

3.1.2. VS removal

Fig.4 shows the VS degradation rates under varying TS conditions. At the TS content of the feedstock being 25%, the addition of activated carbon achieved the highest degradation rate, reaching 83.61%, an increase of 11.33% compared to the situation without activated carbon. For TS contents of 12%, 15%, 18%, 28%, and 33%, average VS degradation rates with carbon addition were 79.89%, 78.25%, 76.55%, 75.88%, and 69.87%, improving by 24.58%, 20.72%, 10.71%, 0.83%, and 3.96% respectively compared to the non-carbon groups. These results demonstrate that activated carbon significantly enhances VS degradation, especially under lower TS conditions. For higher TS (28% and 33%), the improvement was marginal, aligning with previous findings that excessive solids content can limit substrate accessibility and microbial activity.

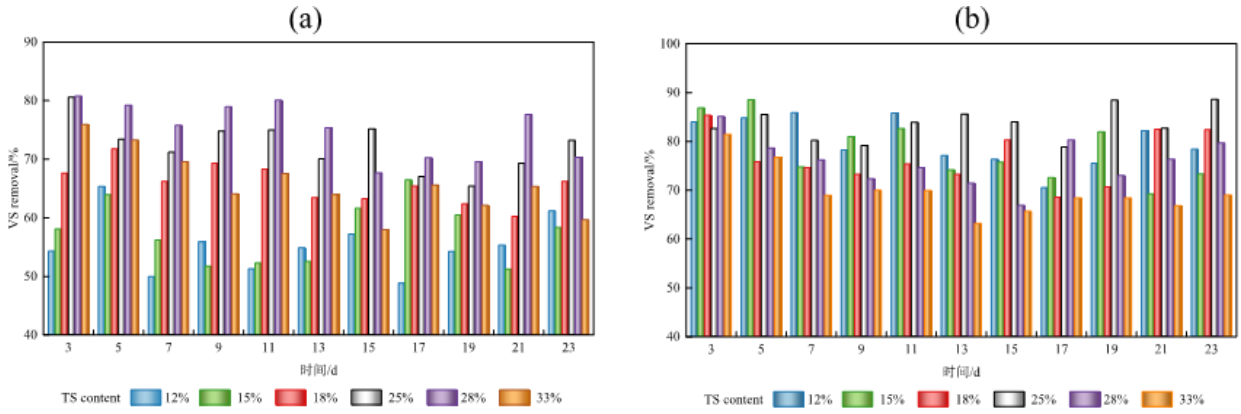

Fig 4. VS removal of different TS contents.
(a) Without activated carbon; (b) with activated carbon.

3.1.3. pH, VFAs and NH4+-N

pH, VFAs, and NH_4^+-N are important indicators for evaluating anaerobic systems. During the initial stages, acetic acid accumulation leads to a drop in pH, which, if too low, can inhibit methanogenic activity and destabilize the system. Due to the volatilization of ash content and acidic functional groups, the pH of activated carbon is generally within the alkaline range, which provides a good buffer for the accumulation of organic acids. As shown in Fig.5, pH values in the control group ranged from 7.2 to 8.0, while those with activated carbon were more stable, maintaining a range of 7.6 to 8.3. After five days, all carbon-amended systems stabilized around pH 8.0, clearly outperforming the control. This buffering effect aligns with Ren et al. (2020a), who reported that activated carbon enhances methane production even under acidic conditions (pH = 5.3), particularly at high organic loads.

Changes in pH are closely linked to VFA dynamics. During acidogenesis, VFAs accumulate when not efficiently converted into methane, leading to pH decline. These acids permeate microbial membranes and dissociate intracellularly, disrupting metabolic processes. As shown in Fig.6, total VFA (TVFA) concentrations rise sharply in the early digestion phase across all TS levels, with higher TS contents showing greater VFA accumulation due to accelerated hydrolysis and acidification. At

33% TS, the final TVFA concentration reached 11,874 mg/L-substantially higher than other groups. Among the VFAs, propionic and acetic acids dominated, with propionic acid consistently more abundant, indicating its slower conversion rate compared to acetic acid.

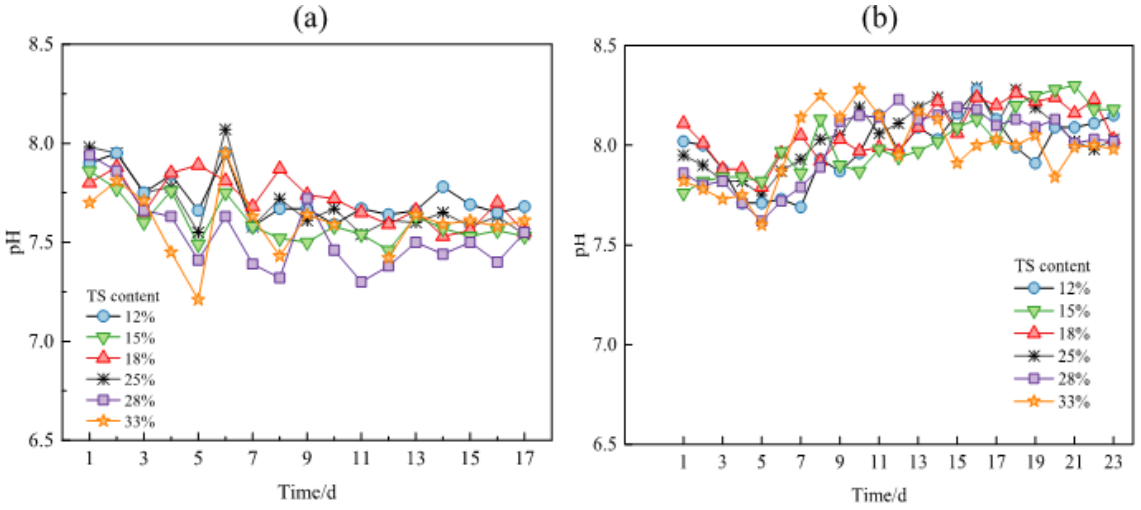

Fig 5. pH trends under varying TS contents.
(a) Without activated carbon; (b) with activated carbon.

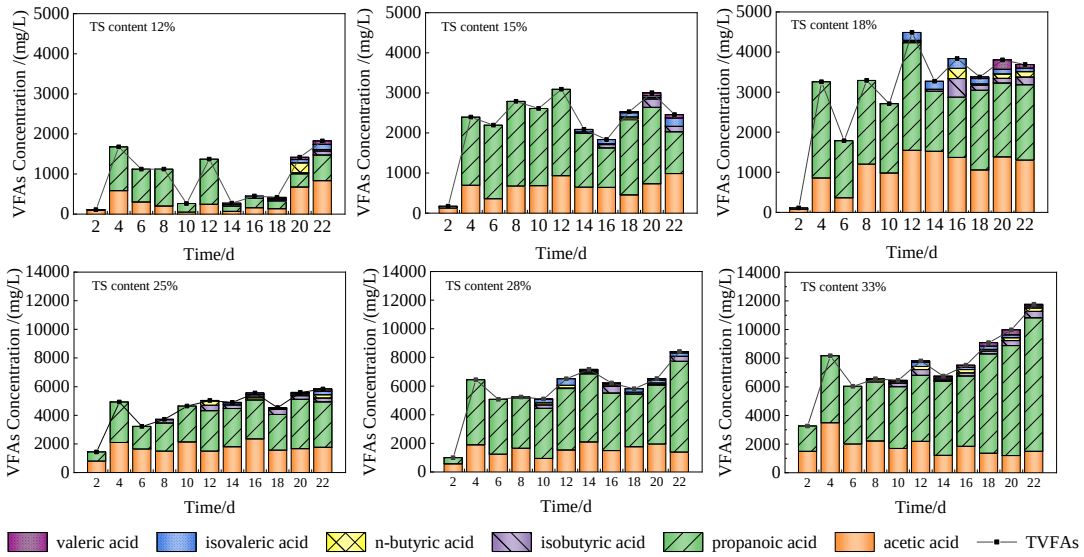

Fig 6. Different concentrations of TVFAs and VFAs components in different TS systems without the addition of activated carbon.

As shown in Fig.7, activated carbon addition significantly reduced organic acid accumulation and delayed the rise of VFAs. Before carbon addition, VFAs were primarily composed of acetic and propionic acids, with the latter prone to accumulation due to its thermodynamically unfavorable conversion to acetic acid under standard conditions ($\Delta G > 0$) [17]. After carbon addition, propionic acid levels declined markedly while acetic acid increased, indicating improved hydrolysis efficiency and more effective conversion of propionate to acetate. This may be attributed to stimulated direct interspecies electron transfer (DIET), which promotes hydrogen consumption during propionate metabolism, thereby shifting the thermodynamic balance and facilitating the degradation of both propionic and butyric acids.

As shown in Fig.8, the addition of activated carbon had no significant effect on NH_4^+-N concentrations. This suggests that the presence of activated carbon did not play a significant role in the adsorption of NH_4^+-N and the degradation of nitrogen-containing substances during anaerobic digestion of KW. Therefore, the improved methane production is not attributable to the alleviation of ammonia inhibition.

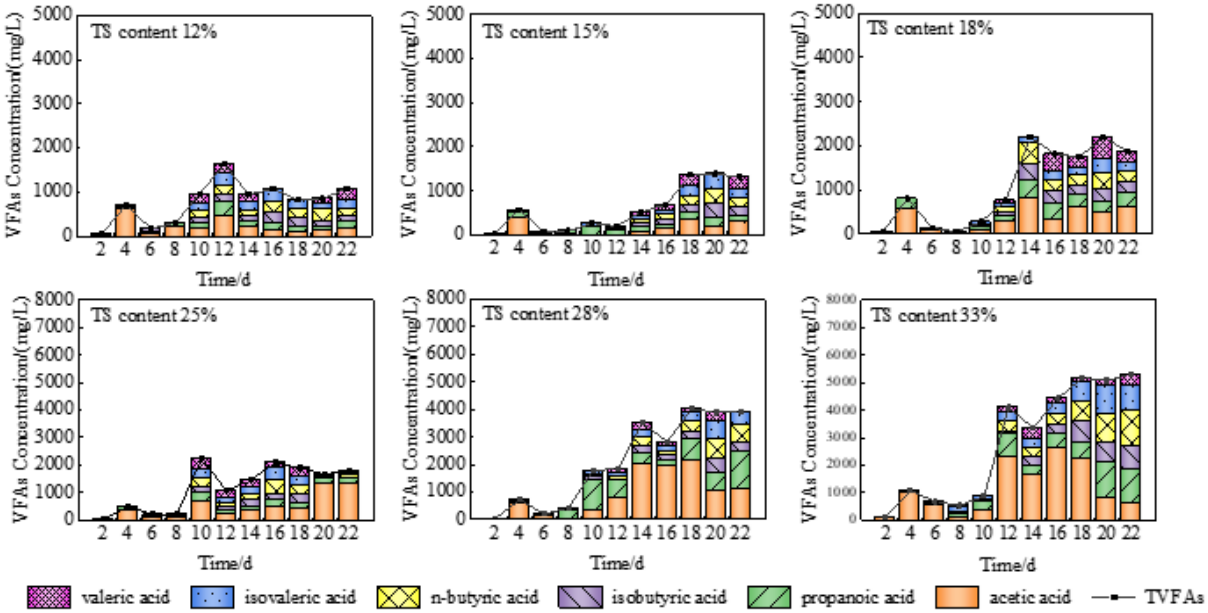

Fig 7. Different concentrations of TVFAs and VFAs components in different TS systems with the addition of activated carbon.

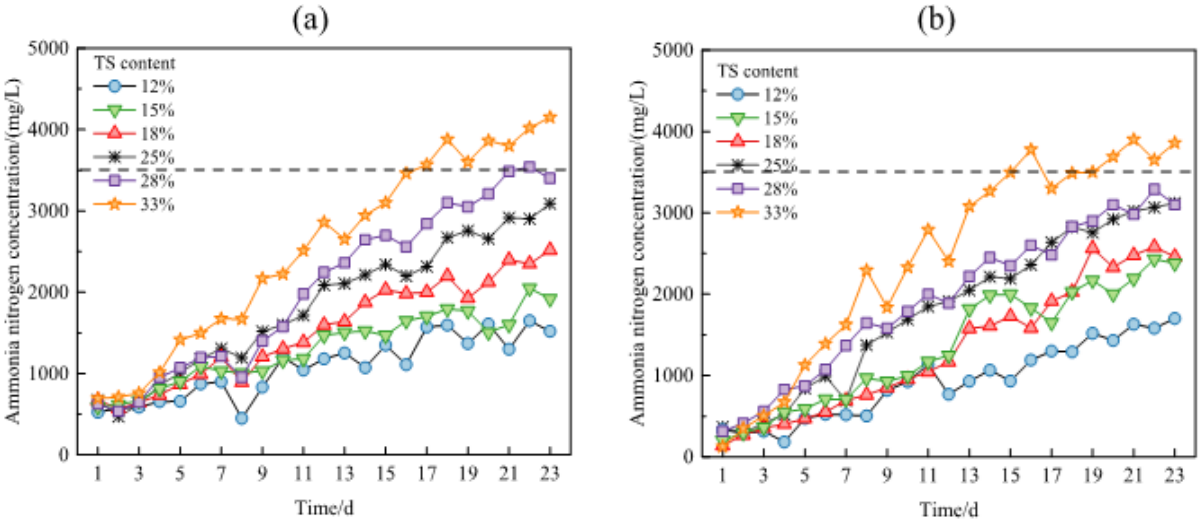

Fig 8. Different concentrations of ammonia nitrogen in different TS systems with the addition of activated carbon.

3.2. Effect of activated carbon on the performance of anaerobic digestion under different organic loads

The system with 25% TS content exhibited optimal biogas production, efficient organic matter degradation, and stable operation. Based on this, experiments with varying organic loads were conducted to assess methane production and determine the system's maximum load tolerance.

3.2.1. Biogas and methane production

Changes in cumulative biogas and methane production over time (Fig.9a, b) show that systems with organic loads of 8.5 and 10.5 g/(L·d) exhibited stable growth in both biogas and methane production during the 23-day anaerobic digestion period. This suggests a balanced dynamic between hydrolysis acidification and methane production, ensuring good efficiency. Increasing the organic load from 8.5 to 10.5 g/(L·d) resulted in the highest cumulative production, with biogas rising by 18.77% and methane by 16.76%. However, at an organic load of 13.5 g/(L·d), excessive VFAs accumulation inhibited methane-producing bacteria, leading to minimal methane production from day 12 onward, with a final production of only 24.48 L of gas and 10.67 L of methane.

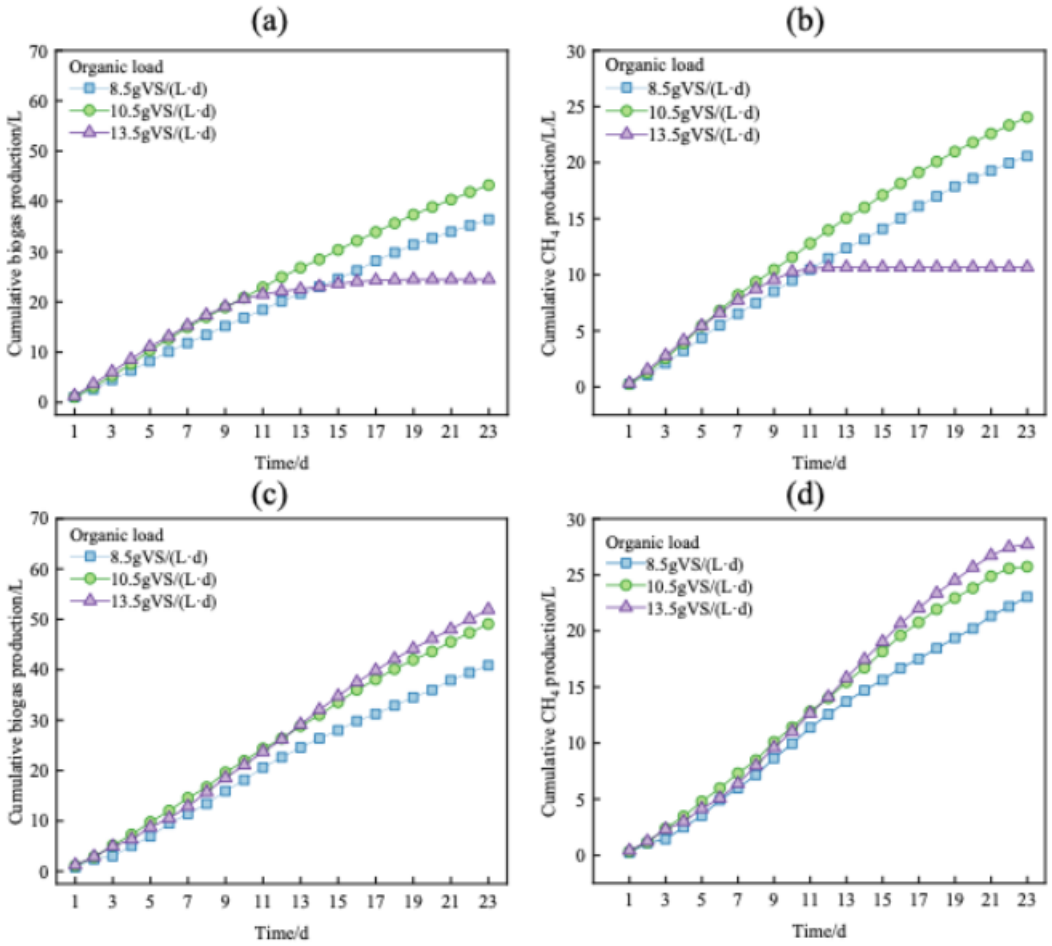

Fig 9. Biogas and methane production under different organic loads. (a-b) Without activated carbon; (c-d) with activated carbon.

Fig.9c, d shows the changes in cumulative biogas and methane production over time at different organic loads with activated carbon addition. During the 23-day anaerobic digestion period, systems with organic loads of 8.5, 10.5, and 13.5 g/(L·d) exhibited a steady increase in gas and methane production. Compared to the system without activated carbon, the addition of activated carbon enhanced both gas and methane production. At an organic load of 8.5 g/(L·d), biogas and methane production increased by 12.38% and 11.88%, respectively. For the system with a load of 10.5 g/(L·d), biogas and methane production rose by 13.57% and 6.99%, respectively. In the system without activated carbon, when the load increased to 13.5 g/(L·d), the acid production rate exceeded the methane production rate due to the high load, resulting in a large accumulation of VFAs and the cessation of anaerobic digestion. The maximum cumulative biogas and methane production reached 51.93 L and 27.71 L, respectively, representing increases of 112.08% and 159.61% compared to the system without activated carbon. These results indicate that activated carbon enables the system to handle higher organic loads, maximizing economic benefits in practical applications.

After adding activated carbon, the unit VS cumulative methane production and corresponding kinetic parameters under different organic loads are shown in Fig.10 and Table 2. The results indicate that the modified Gompertz equation provides a good fit ($R^2 > 0.995$) for methane production during anaerobic digestion with carbon addition at various organic loads. As the organic load increased, the maximum methane production and production rate per unit VS decreased, while the methane lag phase shortened. At an organic load of 13.5 g/(L·d), the maximum methane production and production rate were 7.15 L/g and 0.31 L/(g·d), respectively, with a lag phase of 3.42 days. Compared to the system without activated carbon, methane production and production rate per unit VS increased by 255.72% and 68.97%, respectively. At an organic load of 10.5 g/(L·d), methane production per unit VS increased by 9.33% compared to the system without activated carbon.

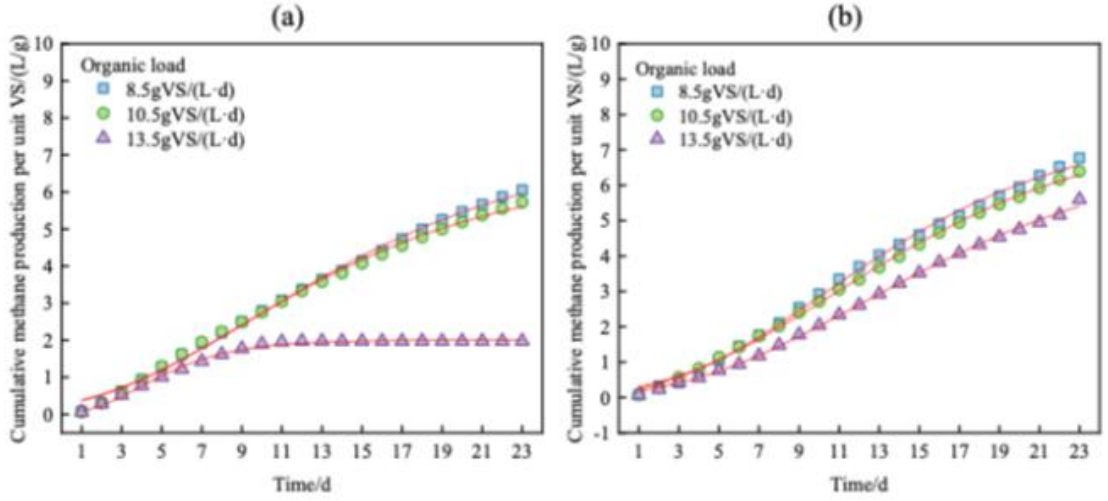

Fig 10. Cumulative methane production per unit VS for different organic loads before and after activated carbon addition.

Table 2. Kinetic characteristics of methanogenesis fitted by Gompertz model under different organic loads.

Organic loads gVS/(L·d)	P_m(L/g)		R_m(L/g·d)		λ(d)		R^2	
	None	activated carbon	None	activated carbon	None	activated carbon	None	activated carbon
8.5	7.29±0.26	7.83±0.20	0.33±0.01	0.39±0.01	1.65±0.23	2.71±0.17	0.996	0.998
10.5	6.54±0.21	7.85±0.19	0.32±0.01	0.35±0.01	1.46±0.24	2.46±0.14	0.996	0.998
13.5	2.01±0.01	7.15±0.18	0.29±0.01	0.31±0.01	1.36±0.13	3.42±0.11	0.996	0.999

3.2.2. VS removal

During the operation of systems with organic loads of 8.5 gVS/(L·d) and 10.5 gVS/(L·d), the VS degradation rate remained relatively stable, with average values of 78.31% and 79.64%, respectively (Fig.11). For the system with an organic load of 13.5 gVS/(L·d), the VS degradation rate exceeded 50% in the early stage, but decreased in the later stage due to system acidification and inhibited microbial activity. The addition of activated carbon improved VS degradation in all systems, with average rates exceeding 85%. Under the high load of 13.5 gVS/(L·d), the degradation rate improved most significantly, reaching 86.63%, a 29.14% increase compared to the system without carbon. In systems with lower organic loads of 8.5 gVS/(L·d) and 10.5 gVS/(L·d), the degradation rates increased from 78.30% to 87.01% and from 79.64% to 85.61%, respectively.

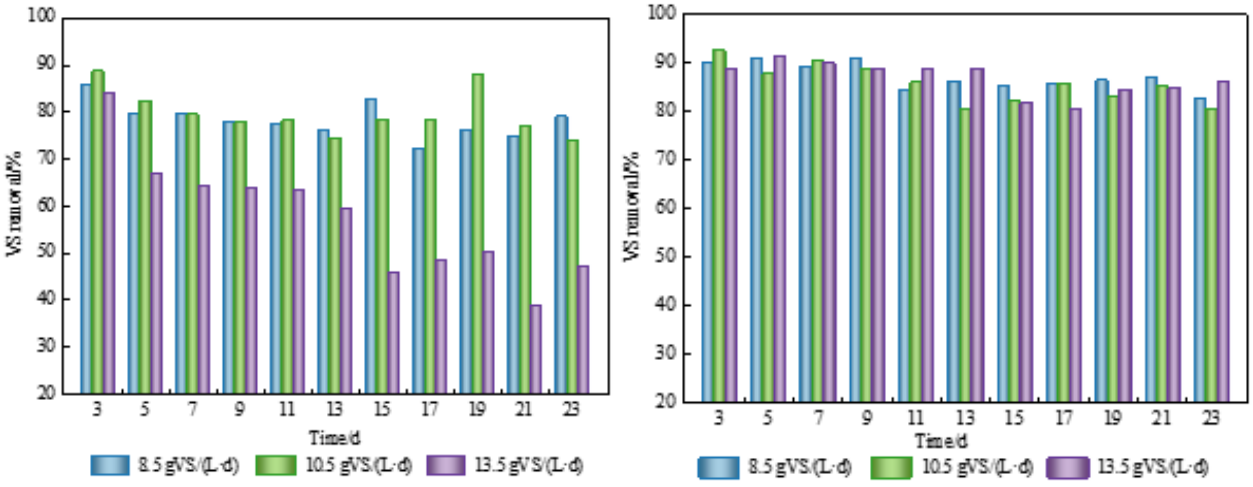

Fig 11. VS removal at different organic loads before and after activated carbon addition.

3.2.3. pH, VFAs and NH4+-N

Research shows that methanogenic bacteria are inhibited when pH drops below 6, and anaerobic digestion halts when TVFA exceeds 13,000 mg/L. As shown in Fig.12 and Fig.13, under organic loads of 8.5 gVS/(L·d) and 10.5 gVS/(L·d), TVFA concentrations remained below 6000 mg/L and 7000 mg/L, respectively, and the pH fluctuated slightly between 7.37 and 8.03, indicating system stability. However, with an organic load of 13.5 gVS/(L·d), the pH dropped significantly from 7.40 to 6.12 by day 11, leading to acidification and system instability. TVFA concentrations exceeded 21,000 mg/L at this point. After adding activated carbon, the rapid pH decrease was alleviated, and higher alkalinity was maintained throughout the digestion process. Even under high loading conditions, the pH remained stable between 7.5 and 8.5. Without activated carbon, the anaerobic digestion system with an organic load of 13.5 gVS/(L·d) experienced acidification, with butyric and propionic acid concentrations increasing rapidly, leading to significant acid accumulation. The concentration of TVFA reached 29,500 mg/L by day 12, and methane production almost ceased. After adding activated carbon, TVFA concentrations in all systems decreased. For the 8.5 gVS/(L·d) and 10.5 gVS/(L·d) loads, TVFA levels reduced from 5629 mg/L to 1905 mg/L and from 6984 mg/L to 2267 mg/L, respectively. This indicates that activated carbon significantly accelerates VFA consumption. In the system with an organic load of 13.5 gVS/(L·d), TVFA concentrations peaked at 42,478 mg/L without activated carbon, causing system failure. However, with activated carbon, the highest TVFA concentration was only 5220 mg/L, and cumulative methane production exceeded that of the other two systems.

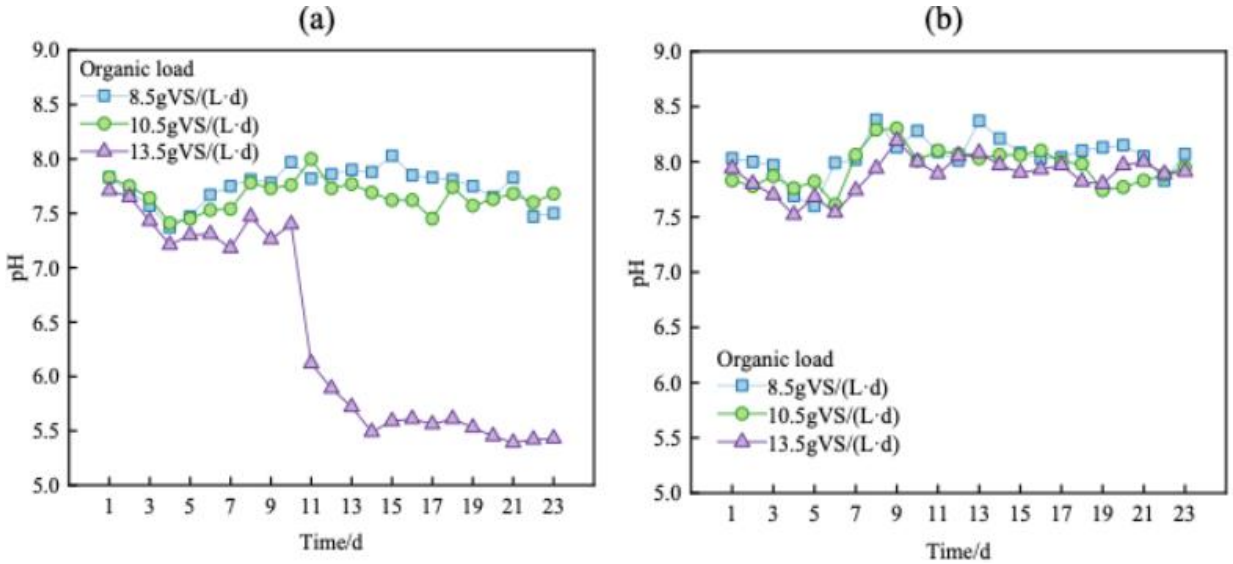

Fig 12. pH variation at different organic loads before and after activated carbon addition.

The ammonia nitrogen concentration in each experimental group increased over time, reaching 3020, 3250, and 3390 mg/L, respectively. At an organic load of 13.5 gVS/(L·d), there was no significant change in ammonia nitrogen compared to the 10.5 gVS/(L·d) system. This could be due to the shorter hydraulic retention time, leading to incomplete degradation of nitrogen-containing organic matter despite the higher organic load. Although none of the systems reached the ammonia nitrogen inhibition threshold, prolonged operation could lead to ammonia inhibition. In practice, further measures to control ammonia nitrogen should be implemented, with regular monitoring. Fig.14 shows the ammonia nitrogen concentrations during the addition of activated carbon. At the end of the anaerobic digestion process, the ammonia nitrogen concentrations were 2780, 3300, and 3538 mg/L for organic loads of 8.5, 10.5, and 13.5 gVS/(L·d), respectively. Compared to the system without activated carbon, there was no significant difference in ammonia nitrogen concentrations.

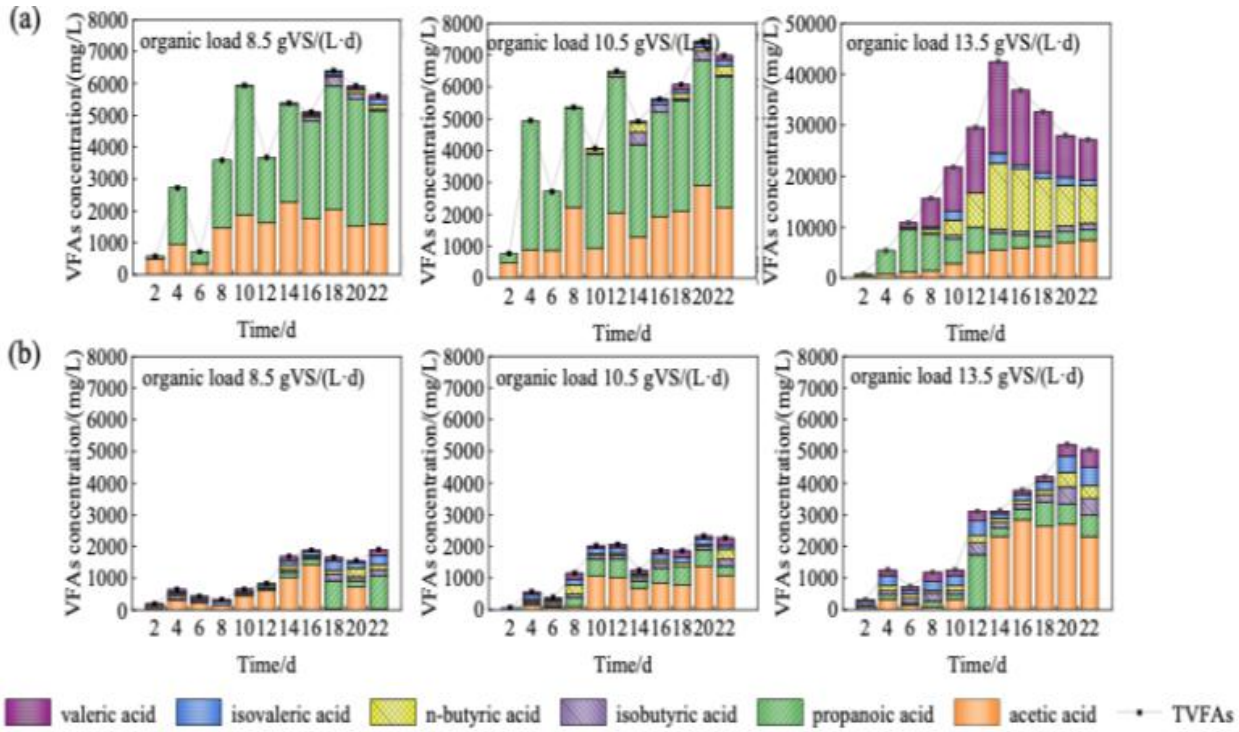

Fig 13. Changes of VFAs and TVFA with time under different organic loads. (a) Without activated carbon; (b) with activated carbon.

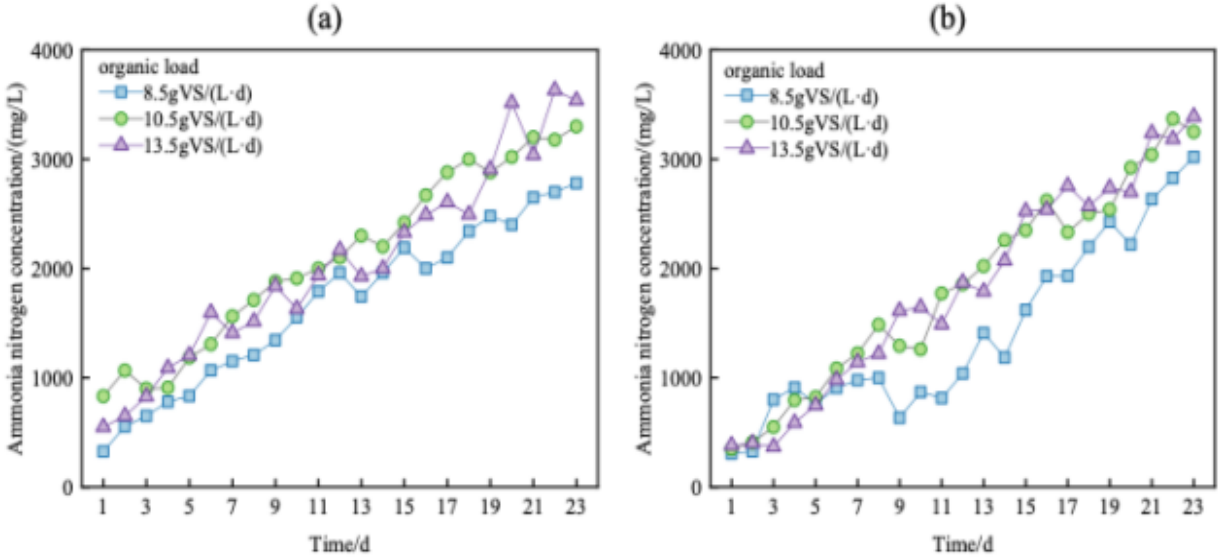

Fig 14. Ammonia nitrogen concentration for different organic loads before and after activated carbon addition.

3.3. Effect of activated carbon addition on microorganisms

3.3.1. Microbial diversity and PCA analysis

Microbial diversity analysis (Table 3) showed that activated carbon had limited impact on bacterial richness but reduced community evenness, leading to lower Shannon indices. For archaea, both OTU number and diversity decreased after carbon addition. This suggests that activated carbon promoted the enrichment of specific functional methanogens while reducing overall diversity-a trend further supported by PCA and taxonomic composition analyses (Fig.15).

Table 3. OTU value and Shannon index of bacteria and archaea under different conditions.

	Bacteria			Archaea		
	OTU	Shannon	Coverage	OTU	Shannon	Coverage
M1	863	6.95	1.00	1320	1.21	1.00
M2	859	7.02	1.00	1515	1.32	1.00
M3	751	7.58	1.00	1932	1.37	1.00
M4	749	7.29	1.00	1688	1.40	1.00
M5	748	6.94	1.00	1745	1.38	1.00
M6	827	7.04	1.00	2046	1.94	1.00
L1	813	7.28	1.00	1789	2.06	1.00
L2	884	7.21	1.00	1852	3.04	1.00
L3	858	7.06	1.00	1558	2.08	1.00
ACM1	712	6.63	1.00	850	1.11	1.00
ACM2	803	6.63	1.00	840	1.03	1.00
ACM3	796	6.77	1.00	1350	1.26	1.00
ACM4	796	6.77	1.00	1150	1.14	1.00
ACM5	724	6.68	1.00	1400	1.06	1.00
ACM6	857	6.84	1.00	750	0.99	1.00
ACL1	885	7.08	1.00	940	1.13	1.00
ACL2	741	6.62	1.00	900	1.24	1.00
ACL3	745	6.46	1.00	1000	2.17	1.00

*M1-M6 are the groups without activated carbon, ACM1-ACM6 are the groups with activated carbon added. The feedstock TS content is 12%, 15%, 18%, 25%, 28%, and 33% respectively. L1-L3 are the groups without added charcoal, ACL1-ACL3 are the groups with activated carbon added. The organic loading rates of the feedstock are 8.5, 9.5, and 13.5 gVS/(L·d) respectively.

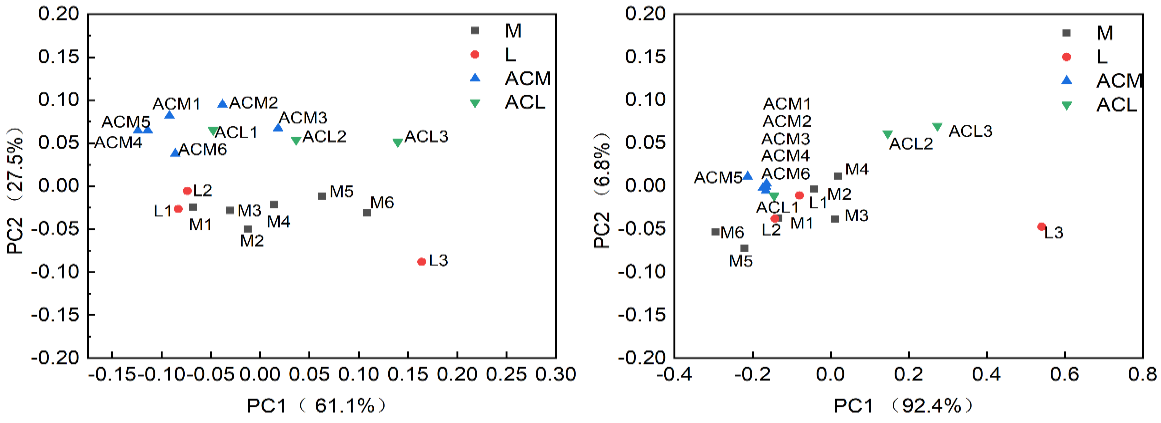

Fig 15. Bacteria and archaea PCA distribution map.

3.3.2. Microbial community structure

The bacterial community composition at the phylum level is shown in Fig.16. In samples without activated carbon, the dominant phyla are Firmicutes, Chloroflexi, Proteobacteria, Actinobacteria, and Synergistetes, accounting for the majority of the community. Except for reactor L3, bacterial compositions across reactors are similar, with Firmicutes and Bacteroidetes being the most abundant, followed by Chloroflexi and Proteobacteria. Under high organic load (13.5 g/(L·d)), the system without activated carbon (L3) becomes unstable, and the bacterial structure shifts notably. The proportion of Actinobacteria increases significantly, consistent with previous findings in acidified systems [18]. Firmicutes also rises sharply. Together, these two acid-producing phyla dominate the community, indicating that excessive organic load promotes acid accumulation and favors the growth of acidogenic bacteria, ultimately leading to system acidification.

After the addition of activated carbon, the dominant phyla became Chloroflexi, Firmicutes, Bacteroidetes, Proteobacteria, and Synergistetes, together accounting for the vast majority of the

community. Actinobacteria decreased significantly and was no longer dominant, while Synergistetes showed a slight decline. In contrast, the relative abundances of Firmicutes, Chloroflexi, Proteobacteria, and Bacteroidetes increased. Notably, reactor L3 exhibited a composition similar to the other reactors, indicating that activated carbon mitigated the structural shifts caused by high organic load. Chloroflexi increased from 7.63%-20.57% to 8.59%-37.14%, contributing to the breakdown of complex carbohydrates and organic matter [19, 20]. Proteobacteria rose from 3.93%-17.50% to 12.43%-25.27%, enhancing protein and carbohydrate degradation [21], while Bacteroidetes also increased (from 1.70%-26.97% to 11.60%-32.07%), promoting acetate production during acidogenesis. Although Firmicutes remained active in hydrolysis [22], their relative abundance did not increase significantly under high load. These changes suggest that activated carbon supports the enrichment of key degraders, improving digestion performance under high-strength conditions.

The composition of the bacterial community structure at the genus level is shown in Fig.17. However, to date, the only bacteria with direct and sufficient evidence of DIET function are *Geobacterspp* and *Syntrophus*,but there are also several lines of evidence suggesting that strains from *Anaerolinea*, *Bacteroides*, *Syntrophomonas* and *Streptoides* can also be used as electron-donating microorganisms for DIET. Two genera, *Anaerolinea* and *Bacteroides*, were abundantly enriched in the reactor, and their percentages increased from 0.40%-2.78% and non-detectable to 7.51%-18.82% and 6.64%-16.09%, respectively, and became the dominant genera. *Anaerolinea* possesses the pilA gene responsible for the formation of pili, which is a prerequisite for the formation of DIET. *Anaerolinea* is capable of transferring electrons to iron during anaerobic digestion's synthetic metabolism [23]. *Bacteroides* is a common hydrolytic fermenting bacterium capable of transferring the produced electrons to extracellularly reduce Fe^{3+} through the metabolism of organic compounds such as glucose and sodium lactate [24].

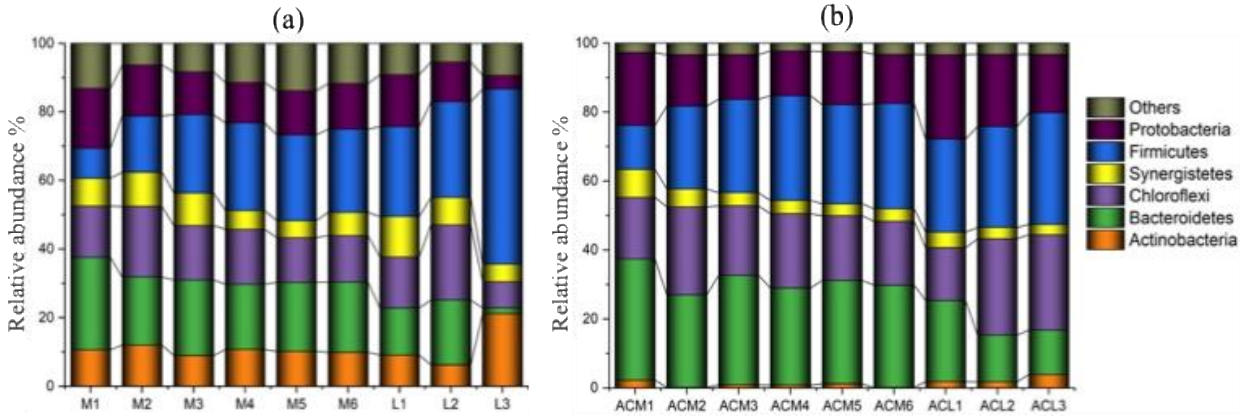

Fig 16. Relative abundance of bacteria at the phylum level (a) without activated carbon; (b) with activated carbon.

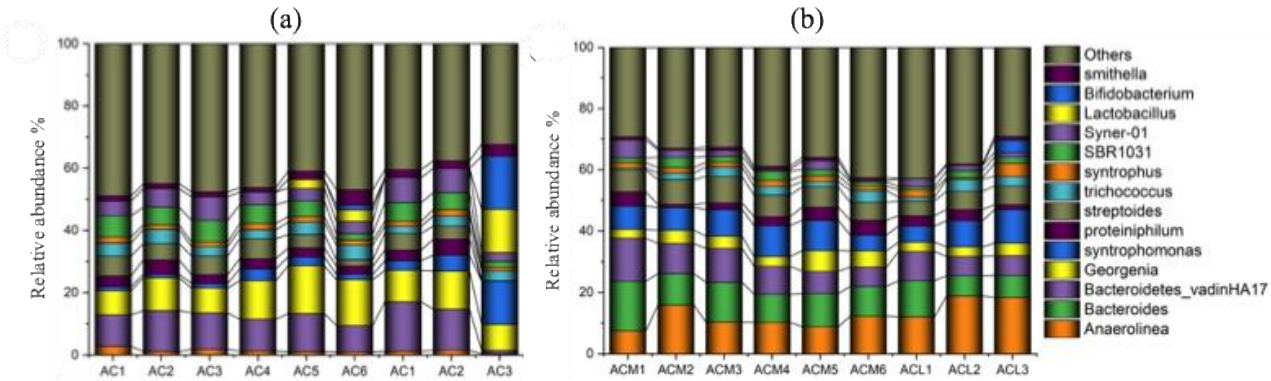

Fig 17. Relative abundance of bacteria at the genus level (a) without activated carbon; (b) with activated carbon.

Activated carbon also promoted the enrichment of Syntrophomonas and Streptoides. Syntrophomonas, known for degrading fatty acids, can transfer electrons extracellularly and may act as an electron donor in DIET [25]. In contrast, although Syntrophus has the potential to participate in DIET via conductive filament secretion [26], its abundance changed only slightly with activated carbon addition, suggesting no significant enrichment.

As shown in Fig.18, Methanosaeta, Methanobacterium, and Methanolinea were the dominant archaeal genera in reactors M1–M4 without activated carbon, together accounting for over 75% of the total abundance. Methanosaeta is an obligate acetoclastic methane-producing bacterium that can only produce methane through the acetate degradation pathway [27]. In the early stage of digestion, it utilizes readily available acetate, while in the later stage, it relies on acetate produced by syntrophic propionate-oxidizing bacteria. The hydrogen generated from propionate degradation promotes the growth of hydrogenotrophic methanogens such as Methanolinea and Methanobacterium, which convert H_2 and CO_2 into methane in cooperation with Methanosaeta [28].

In reactors M5 and M6, although the overall archaeal community structure remained similar to that in M1-M4, the relative abundance of Methanosaeta declined. This may be due to the increased concentration of ammonia nitrogen, as Methanosaeta is known to be less tolerant to ammonia inhibition [29, 30]. These findings suggest that elevated ammonia levels can suppress Methanosaeta, potentially shifting the dominant methanogenic pathway from acetoclastic to hydrogenotrophic. The relative abundance of Methanosarcina was higher in reactors M5 and M6, making it a dominant genus alongside Methanosaeta, Methanobacterium, and Methanolinea. Methanosarcina is a versatile methanogen capable of acetoclastic, hydrogenotrophic, and methylotrophic pathways [31], but it primarily uses acetate under typical conditions. Its ability to form dense cell aggregates with intact membrane structures enhances its resistance to toxic compounds, such as high levels of organic acids and ammonia. Experimental results confirmed that in reactors M5 and M6, where organic acids and ammonia nitrogen exceeded 3500 mg/L in the later stages, Methanosaeta activity was inhibited. Consequently, Methanosarcina increased in abundance and maintained methane production via the acetate pathway by utilizing the accumulated acetate.

In reactors L1 and L2, the dominant archaeal genera were Methanosaeta, Methanobacterium, and Methanolinea, with acetoclastic methanogens making up a higher proportion than hydrogenotrophic ones. However, at a higher organic loading rate of 13.5 g/(L·d) in reactor L3, Methanolinea and Methanobacterium became more dominant, while Methanosaeta dropped to 20.91%, indicating a shift in the methanogenic pathway from acetate-based to H_2/CO_2-based metabolism. However, the reliance on freely diffused H_2-limited by its low solubility and slow diffusion in water—may reduce the methane production rate. Compared to L1 and L2, the relative abundance of Methanosaeta in L3 decreased by over 24%, suggesting that acidification inhibits its growth and metabolic activity. This suppression not only lowers methane yield but also favors the proliferation of hydrogenotrophic methanogens such as Methanolinea and Methanobacterium. In contrast, L2 showed better methane production performance, implying that maintaining acetate-driven methanogenesis is more effective for stable and efficient biogas output.

After the addition of activated carbon, the relative abundance of Methanosaeta increased under all TS conditions, from 20.92%-50.69% to 54.43%-64.14%. Methanosaeta has been shown to participate in DIET by directly accepting electrons to reduce CO_2 to methane [32], and its enrichment is often promoted by activated carbon. This may also be linked to the increased abundance of Proteiniphilum, a proteolytic bacterium that accelerates hydrolysis and acetate production, creating favorable conditions for acetoclastic methanogens like Methanosaeta. Methanosarcina, another genus capable of DIET, also increased significantly in groups ACM1–ACM4 with carbon addition, suggesting a similar enrichment effect. However, in reactors M5 and M6, this enrichment was not observed, possibly because high levels of acids and ammonia in the absence of activated carbon had already promoted Methanosarcina, masking any further effect from carbon addition.

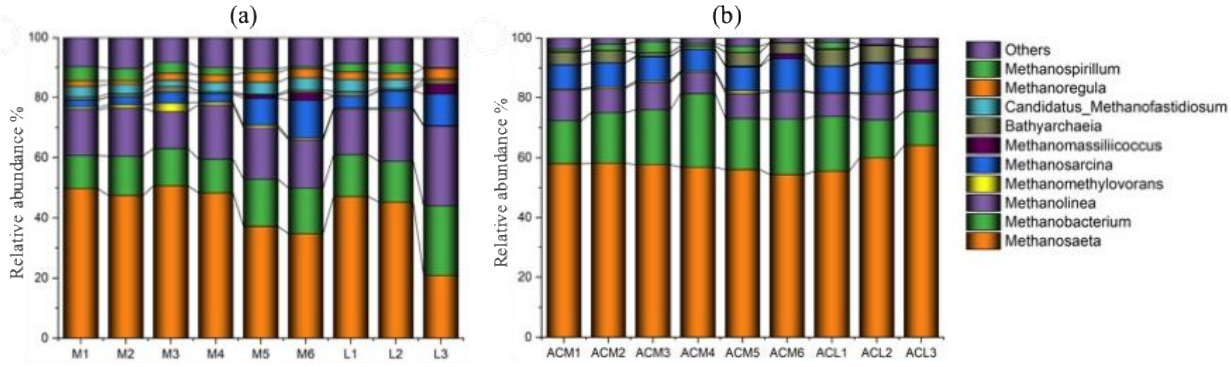

Fig 18. Relative abundance of archaea in anaerobic reactors without activated carbon (a) and with activated carbon (b).

4. Conclusion

This study systematically investigated the effects of activated carbon addition on dry anaerobic digestion systems treating KW under varying TS contents and OLR. The results showed that activated carbon significantly improved biogas production performance, methane yield, and organic matter degradation. The system exhibited optimal performance at a TS content of 25% and an OLR of 10.5 gVS/(L·d). Furthermore, activated carbon effectively buffered pH fluctuations caused by acid accumulation, mitigated VFA build-up, and enhanced system stability.

Microbial community analysis further revealed that activated carbon addition facilitated the hydrolysis and acidogenesis stages of anaerobic digestion, promoted syntrophic interactions between VFA-oxidizing bacteria and methanogenic archaea, and enhanced DIET during syntrophic metabolism. This DIET-mediated pathway significantly increased electron transfer efficiency and accelerated methane production rates.

In summary, activated carbon, as a simple and efficient additive, demonstrates significant potential for enhancing the performance of high-TS anaerobic digestion systems, increasing their tolerance to organic loading, and optimizing the microbial community structure.

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