

Enhanced Methanogenesis and Shock Resistance in Anaerobic Digestion by Coupling nano- iron biochar with Electrochemistry: Microbial Community Dynamics and Thermodynamic Advantage

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Abstract

To achieve efficient anaerobic digestion of food waste under high organic loading, this study investigated the coupling effects of applied voltage and nano iron-modified biochar on digestion performance and microbial mechanisms. The results showed that within 8–48 gVS/L, the maximum methane yield occurred at 16 gVS/L, and the electro-assisted reactor with nano iron-modified biochar (EAD600) obtained optimal methanogenic performance. Organic loading rate, applied voltage and composite materials jointly shaped microbial communities. *Clostridium* and *Methanothrix* dominated the bacterial and archaeal populations, respectively. Voltage facilitated interspecies electron transfer, while composite materials enriched fermentative bacteria and stabilized methanogens. Their synergy upregulated genes related to organic degradation and methanogenesis, optimized thermodynamic conditions and relieved acidification inhibition induced by VFA accumulation. This coupling strategy provides theoretical support for stable operation of food waste anaerobic digestion at high loading rates.

1.Introduction

Anaerobic Digestion (AD) of organic solid waste for methane production is a key path to achieve waste resource utilization and carbon neutrality goals(Subbarao et al. 2023; Baía et al. 2026). However, traditional anaerobic treatment processes are often limited by the thermodynamic barriers of Interspecies Electron Transfer (IET), resulting in acidification and instability at high loads(Zhao et al. 2024; Othman et al. 2026). Electrochemical Anaerobic Digestion (EAD) can enhance Direct Interspecies Electron Transfer (DIET) through an applied electric field, but it faces high energy consumption and electrode polarization bottlenecks(Obi et al. 2023). The single addition of conductive materials lacks a continuous driving force and has limited shock resistance. Based on this, this paper proposes a strategy of nano iron-modified biochar with EAD, constructing a "material-electric field" dual-driven system. Nano iron-modified biochar have both high conductivity and biological affinity, and can serve as electron conductors and regulate the redox potential; The applied micro electric field further reduces the activation energy of the reaction and provides continuous thermodynamic advantages. In response to the unclear current mechanism of the combined application, this study introduces nano iron-modified biochar, examines the methane production performance of this system under load shock, combines metagenomic sequencing and thermodynamic calculations, analyzes the dynamic evolution rules of the microbial community, and clarifies the intrinsic mechanism of the combined technology in strengthening electron transfer, providing a theoretical basis for the efficient energy utilization of organic solid waste.

2. Materials and Methods

2.1 Inoculum, food waste and nano iron-modified biochar

The inoculum was collected from brewery sludge and pretreated in a water bath at $37 \pm 1^\circ\text{C}$ for one week before the experiment. Food waste was obtained from campus canteens, then degreased with edible alkali, dried, crushed, and sieved for later use. Coffee residues were collected from campus coffee

shops, dried, ground, and sieved as backup materials, while nano-iron powder with a particle size of 50 nm was commercially purchased. The physicochemical properties of the food waste and sludge are detailed in **Supplementary Material Table 1–1**. Coffee residues and nano-iron were mixed at a 1:1 mass ratio, followed by the addition of 25–30 mL of distilled water. After thorough stirring, the mixture was dried and placed in a covered porcelain boat for pyrolysis in a tube furnace (LAB, OTL1200). The heating rate was set at 10°C/min, with pyrolysis temperatures of 600°C, maintained for 2 hours under a nitrogen atmosphere. The resulting materials were designated as Fe@BC600, respectively. After pyrolysis, the materials were cooled to room temperature and stored in sealed bags for subsequent use.

2.2 Analytical methods

The total solids (TS) and volatile solids (VS) content of the sludge were determined using the ignition loss method (HJ 1024–2019). The microstructure of the Fe@BC600 was examined using scanning electron microscopy (SEM), while Fourier-transform infrared spectroscopy (FTIR) was employed to analyze their microstructural characteristics. X-ray diffraction (XRD) was used to determine the functional group distribution, and energy-dispersive spectroscopy (EDS) was applied to assess the elemental composition of the Fe@BC600.

The pH inside the reaction system was measured using a pH meter (Sartorius, PB-30L), and methane content was monitored using a portable biogas analyzer (PNT400-CH₄/O₂/CO₂/H₂S). Samples were collected using syringes and centrifuged at 10,000 rpm for 10 min. The supernatant was then filtered through a 0.45 µm membrane for subsequent measurements, including total alkalinity (TA), chemical oxygen demand (COD), and volatile fatty acids (VFAs) concentration. TA was determined via acid titration (SL 83-1994), COD was measured using spectrophotometry (HJ 924–2017), and VFAs composition was analyzed via gas chromatography (FULI, GC9790Plus).

2.3 Batch anaerobic digestion experiment design

Batch anaerobic digestion experiments were conducted in 800 mL working-volume feeding bottles under a 37 ± 1°C water bath (experimental setup shown in **Supplementary Material Fig. 1–1**). Each bottle was loaded with 400 mL of sludge, and five organic loading were tested sequentially by controlling food waste (FW) dosage at 4, 8, 16, 32, and 48 g VS/L. Experiments proceeded chronologically, with each loading phase terminated when methane content dropped below 1%, lasting 7–11 days depending on the load.

Fe@BC600 were added to the feeding bottles at a dosage of 2 g/L. Two groups are set without voltage, and they are named ADCK and AD600 respectively; Two groups are set with voltage (always providing 0.5V), and they are named EADCK and EAD600 respectively. Triplicate parallel tests were performed for each group to ensure reliability. The naming conventions and experimental design are summarized in **Supplementary Material Table 1–2**. The names and meanings of the samples in the experiment are shown in Table <link rid="tb1">1</link>–1.

Table 1
1 Names and meanings of samples on food waste EAD

Name	Descriptions
L8-ADCK	Control group, with a loading of 8 g VS/L
L8-AD600	Treatment group, Fe@BC600 were separately fed, loading of 8gVS/L
L8-EADCK	Electrochemical control group, with a loading of 8 g VS/L
L8-EAD600	Electrochemical treatment group, Fe@BC600 were separately fed, loading of 8gVS/L
L48-ADCK	Control group, with a loading of 48gVS/L
L48-AD600	Treatment group, Fe@BC600 were separately fed, loading of 48gVS/L
L48-EADCK	Electrochemical control group, with a loading of 48gVS/L
L48-EAD600	Electrochemical treatment group, Fe@BC600 were separately fed, loading of 48gVS/L

2.4 Data analysis

The modified Gompertz equation was applied to model methane production, and the calculation formula was shown in **Supplementary Material**.

Thermodynamic calculations for key VFAs degradation in AD were corrected for actual temperature and concentration to determine ΔG of methane production from propionate, butyrate, and valerate (see formulas in **Supplementary Material**).

All experimental data were processed using Microsoft Excel 2010 for preliminary organization. Graphical representations were generated with Origin 2021 software, while phase composition analysis of samples was performed using MDI Jade 6.

2.5 Microorganisms analysis

The metagenomic sequencing analysis was performed assembly-based experimental protocol, with detailed sequencing procedures provided in the **Supplementary Materials**.

3. Results

3.1 Characters of nano iron-modified biochar

3.1.1 SEM

SEM can observe the apparent properties and microscopic morphology of materials, such as the pore structure and the binding state of Fe@BC600. As shown in Fig. 3 – 1 (a), the biochar prepared by pyrolysis at 600°C has formed layer-like structures of different sizes with corrosion pits at a higher

pyrolysis temperature. The change in structure will affect the loading state of nano-iron. Figure 3 – 1 (b) shows the morphological structure of Fe@BC600. The aggregated spherical particles are the nano-iron particles, indicating that the nano-iron has been successfully loaded. It reveals that the binding between nano-iron and the carbon skeleton becomes increasingly tight, and the aggregated nano-iron almost completely covers the biochar skeleton(He et al. 2024).

Figure 3 – 1 SEM of the biochar (a); iron-modified biochar (b)

3.1.2 EDS

EDS can provide information on the element distribution and composition of the micro-area structure of materials. Figure 3 – 2 shows the EDS image of the Fe@BC600, mainly involving the distribution changes of Fe, C, and O elements. From the figure, it can be seen that the content of C and Fe in the Fe@BC600 is relatively high. C mainly comes from the amorphous carbon generated by the decomposition of cellulose or hemicellulose in coffee residue(Chen et al. 2025). At a thermal decomposition temperature of 600°C, the proportion of hybrid carbon atoms will increase, forming more conjugated π bonds, which can provide free electrons and enhance the conductivity of the material(Wen et al. 2025). The Fe content ranges from 52.36%, combined with the loading state in SEM, indicating a high loading rate of nano-iron. O comes from the oxygen-containing functional groups in coffee residue such as -OH, C = O(Dong et al. 2024). Since the oxygen-containing functional groups can decompose at relatively low temperatures and escape in the form of H₂O and CO₂, the O content of the Fe@C600 is extremely low, only 11.5%.

3.1.2 XRD and FT-IR

XRD can reveal the crystal structure and variation patterns of Fe@C600. In **Fig. 2 (a) of Supplementary Material**, the XRD results detected multiple sharp and distinct diffraction peaks in the Fe@C600, which is a manifestation of high crystallinity. After comparison with the standard PDF cards, Fe₃O₄ and FeO were matched. FeO can provide electron donors and promote direct inter-molecular electron transfer, while Fe₃O₄ enhances conductivity(Xu et al. 2023). The diffraction peak of FeO in the Fe@C600 is weak, and the Fe₃O₄ diffraction peak at around 35° gradually strengthens. This indicates that the graphite-like crystal diffraction peaks shift towards lower angles, meaning that the interlayer spacing increases at high temperatures and the stacking density decreases, resulting in relatively rough pore diameters and Fe₃O₄ small particles adsorbed externally through magnetic attraction(Xie et al. 2024). Peaks that are sharper at 25° and 30° are the diffraction peaks of alkali metal carbonates. Under high-temperature pyrolysis, the carbonates of Fe@C600 may decompose into oxides and release CO₂. At this time, the alkali metal carbonate diffraction peaks disappear.

FT-IR can detect the internal components and functional groups of materials. Figure 2 (b) of **Supplementary Material** shows that in the functional group region of 1300–4000 cm⁻¹, there are stretching vibration peaks such as -OH, -CH₃, -CH₂, -C = C-, -C $\equiv$ C-, etc. With a higher pyrolysis temperature, the peak height of the -OH characteristic peak in the Fe@C600 weakens. This is because -

OH is relatively active and can undergo removal or conversion reactions at relatively lower temperatures. The decomposition of this structure and the synthesis of cyclic rings will promote the formation of biochar pore structure, while high temperatures promote dehydration, cracking and aromatization, which will form more carbon-rich substances. The interaction between carbon and iron may be enhanced (Guo et al. 2024). Finally, carboxyl groups (-COOH) and hydroxyl groups (-OH) can change the electron distribution of the reaction system by releasing or accepting electrons, thereby regulating the oxidation-reduction potential of the system and creating more favorable environmental conditions for anaerobic microorganisms to survive.

3.2 Effect of nano iron-modified biochar on the performance of AD system

3.2.1 Change of methanogenic phase

The accumulated methane production is used to evaluate the methane production potential of anaerobic digestion. Figure <link rid="fig1">3</link> (a) shows that when the organic matter content is low at a 4gVS/L load, the methane production is also relatively low. The peak periods of accumulated methane production in the ADCK and AD600 groups occur at the 8gVS/L load stage, showing a stable trend between 16-32gVS/L, and almost ceases at 48gVS/L. In electrochemical anaerobic digestion, the cumulative methane production of EAD600 is relatively balanced, and there is still methane production potential under the 48gVS/L load shock; however, the EADCK shows poor methane production effect at 8g VS/L.

Figure <link rid="fig1">3</link> (b) shows the kinetic fitting of the cumulative methane production based on the Gompertz equation (Nguyen et al. 2016). The fitting curve can reflect the maximum methane production potential (P_m), maximum methane production rate (R_m), and methane production stagnation period (λ) of the anaerobic digestion system at different times. The lower asymptote line is zero, and the upper asymptote line is close to the maximum value, indicating that the fitting effect of this curve is good, that is, this group has a good methane production potential. The results show that all four groups achieved ideal fitting effects. Comparatively, the EAD600 group had the highest P_m (2290.32 $\text{mL}\cdot\text{g}^{-1}$) and R_m (590.75 $\text{mL}\cdot\text{g}^{-1}$), followed by EADCK and AD600, and ADCK was the lowest.

3.2.2 The decomposition situation of organic substances in the system

COD can represent the concentration of organic matter in the system. The organic matter is first hydrolyzed to produce acids, generating small molecules such as proteins, sugars, and lipids. Then, under the action of acid-producing bacteria, methane is produced. During this process, the COD concentration will change accordingly. As shown in Fig. 3-4 (a), with the increase in load, COD shows a gradually increasing trend. At the end of each load, COD will basically drop to the initial level, indicating that the organic matter has been utilized. When the load is 4gVS/L, the COD concentration is relatively

low, and the COD concentration is within the range of 20000mg/L. The advantage of electrochemical methods is manifested at medium and high loads. From 16 to 48gVS/L, the COD concentration of the electrochemical anaerobic digestion group EADCK and EAD600 is higher than that of the ADCK and AD600 groups, but the overall COD concentration remains within the range of 30000-55000mg/L. Most of the COD in anaerobic digestion comes from various intermediate metabolic products. The high concentration level of COD in the EADCK and EAD600 groups indicates that the electrochemical method promotes the utilization of organic matter and strengthens the metabolism of organic matter. Finally, the excessively high COD level at 48gVS/L indicates that the current effective buffering capacity of the anaerobic digestion system has been impacted. However, the electrochemical group still shows a relatively high COD level and a faster recovery period, indicating that the combination of Fe@C600 and electrochemical methods has to some extent enhanced the system's shock resistance(Huang et al. 2024).

The methanogenesis process cannot do without the production of VFAs. However, the accumulation of VFAs will inhibit the activity of methanogenic bacteria, so the concentration level of VFAs can reflect the activity of methanogenic bacteria and the acidification status of the system. Figure 3–4(b) shows that at the initial stages of each load, the VFAs first increase, then decrease until stabilizing. This is because after the addition of organic matter, the rapid decomposition by hydrolysis-acid-producing bacteria leads to a large amount of VFAs being produced. The methanogenic bacteria will utilize the VFAs to produce methane, and the VFAs level will decrease. When the organic matter is completely consumed, the VFAs will remain at a relatively stable level. The VFAs levels of the EDACK and EAD600 groups at the 32–48 gVS/L load are significantly different from those of the ADCK and AD600 groups, indicating that electrochemical anaerobic digestion has more advantages in dealing with high-load substrate shocks.

pH indicates the acid-base changes within the system and can be used to assist in judging the operation status of anaerobic digestion. A low pH indicates the accumulation of fatty acids, which is not conducive to methane production; a high pH may be due to excessive organic matter load or insufficient methane production capacity of the anaerobic sludge. Figure 3–4 (c) shows that at the initial stages of each load, the pH first decreases, then slowly rises, and finally remains stable. This is because the substrate for hydrolytic bacteria increases at the beginning of the load, and they reproduce and metabolize rapidly, resulting in the presence of a large amount of hydrolysis products such as organic acids, which lowers the pH value(Zhen et al. 2024). As the intermediate metabolic products are utilized and consumed, the pH gradually recovers. At the same time, it can be observed that the pH levels of EADCK and EAD600 are generally higher, ranging from 6.8 to 7.6, possibly due to the presence of electroactive microorganisms, which continuously consume H^+ .

3.2.3 Stability of the anaerobic system

Testing the fermentation components and they has certain indicative significance for the stability of the anaerobic digestion system(Cao et al. 2025). VFAs are organic acids with carbon chains of 1 to 6 carbon atoms, including acetic acid, propionic acid, isobutyric acid, valeric acid, isovaleric acid, butyric acid. The accumulation of propionic acid and acetic acid is the core factor leading to system acidification. Figure 3

of Supplementary Material shows the changes of fermentation components at the beginning and end of the five load stages. At 4-16gVS/L, butyric acid had the highest initial proportion among all loads, and the proportions of each component were relatively balanced at the end of each load. As the load increased, the initial proportion of butyric acid decreased, indicating that the gradient load shock enhanced the buffering capacity of the anaerobic system. At 32 and 48gVS/L, the proportion of ethanol was the highest, indicating that the microbial community in the system was undergoing ethanol-based fermentation. The presence of ethanol can stimulate the production of conductive flagella or cytochromes by electroactive microorganisms (such as *Geobacter*), forming a highly conductive "nanowire" network(Zhou et al. 2023). This enables direct electron transfer (DIET) between ethanol-producing/methanol-producing bacteria and methanogenic bacteria, significantly accelerating the conversion rate of ethanol to methane, thereby improving the gas production efficiency and stability of the entire anaerobic digestion system(Ao et al. 2024). A high proportion of ethanol can also effectively buffer the pH of the system, corresponding to the pH changes at this load, significantly reducing the risk of acidification of the anaerobic digestion system. At the same time, the proportion of EADCK and EAD600 ethanol was over 80%, indicating that the electrochemical anaerobic digestion system successfully initiated the more acid-resistant ethanol-based fermentation pathway, which laid a good foundation for achieving efficient and stable methane production through direct electron transfer (DIET) in the future(Amin et al. 2024).

Total Alkalinity (TA) serves as a key indicator for evaluating the buffering capacity of anaerobic digestion systems, reflecting their ability to resist acidification. As shown in **Fig. 4(a) of Supplementary Material**, the TA of the system generally increased steadily at organic loads of 4–32 g VS/L. Across different phases, TA initially decreased due to consumption, remained at a low level, and then gradually recovered. This trend indicates a succession of the microbial community within the system, where methanogens tolerant to high loads (such as the genus *Methanosarcina*) gradually became dominant. These microbes can more efficiently consume VFAs while producing methane and alkalinity. As VFAs were progressively consumed, the TA values stopped declining and slowly rebounded, allowing the system to regain stability. However, at 48 g VS/L, TA rose rapidly. Coupled with the sharp increase in VFAs, this suggests a significant accumulation of intermediate metabolites, indicating that the system faced a certain risk of acidification(Li et al. 2024a). Nevertheless, the system had developed a degree of self-repair capability by this stage, and the subsequent slow recovery of TA confirmed that the system was successfully acclimated. Compared to single indicators, composite metrics such as the VFA/TA ratio are often used as early warning indicators to monitor the operational status of anaerobic digestion. Figure 4(b) of **Supplementary Material** illustrates that the VFA/TA ratio followed a pattern of “rapid initial increase-unstable maintenance at a high level- recovery to stability.” This occurs because, immediately after the initial substrate loading, the metabolic rate of hydrolytic acidogenic bacteria far exceeds that of methanogens, causing the production rate of VFAs to vastly outpace their consumption. Large amounts of VFAs (particularly propionic and butyric acids) rapidly accumulate in the reactor, continuously consuming the system's alkalinity (TA) and causing the VFA/TA ratio to spike dramatically. At this stage, the abundance of key functional genes involved in the butyric and propionic acid metabolic pathways

may decrease significantly, leading to the massive accumulation of these acids and directly triggering acid inhibition within the system.

3.3 Effects of Fe@C600 on Microbial Community Structure and Function

3.3.1 Community ecological shifts of acidogenic bacteria and methanogenic archaea

Microbial samples from low (8 gVS/L) and high (48 gVS/L) organic loading conditions were selected for sequencing; detailed sample information and naming conventions are provided in Section 2.3.

As shown in Fig. 3–5 (a), the electrochemical environment exerted a more pronounced influence on bacterial distribution than organic loading shock. In the four electrochemical groups, the relative abundances of *Fermentimonas*, *Proteiniphilum*, and *Aminobacterium* increased significantly. These taxa, which are responsible for acidogenesis, protein degradation, and amino acid fermentation, occupied the dominant ecological niches in the electrochemical environment. Their enrichment indicates that the electrochemical system greatly enhanced the hydrolysis and acidification capabilities during the initial stages of anaerobic digestion (Chen et al. 2024).

Furthermore, the continuous current or voltage in the electrochemical environment may have inhibited the growth and metabolism of certain *Clostridium* species (particularly under constant current mode, where excessive voltage can be detrimental to microbial growth). Meanwhile, the electrochemical environment enriched specific electroactive bacteria and hydrogenotrophic methanogens, altering the competitive landscape of the community (Shen et al. 2020). Consequently, the relative abundances of less competitive conventional fermentative bacteria (e.g., *Petrimonas*, *Clostridium*) decreased (Li et al. 2024b). However, the abundances of *Clostridium* in L8-EAD600 and L48-EAD600 were higher than those in the control groups, suggesting that Fe@C600 might mitigate the inhibitory effects of continuous current or voltage on the growth and metabolism of certain *Clostridium* species.

The archaeal communities were predominantly distributed within the phylum Euryarchaeota. At the genus level (Fig. 3–5 (b)), *Methanothrix* emerged as the dominant genus across all groups. *Methanothrix* exhibits an exceptionally high affinity for acetate and demonstrates strong tolerance to high ammonia, high salinity, and pH fluctuations. Additionally, its ability to secrete abundant extracellular polymeric substances (EPS) facilitates the formation of robust filamentous network structures (Feng et al. 2025). Consequently, the relative abundance of *Methanothrix* increased following the rise in organic loading.

In contrast, the relative abundances of *Methanosarcina*, *Methanobacterium*, and *Methanoculleus* all declined. *Methanosarcina* is a typical acetoclastic methanogen that primarily produces methane via acetate cleavage, whereas *Methanobacterium* and *Methanoculleus* are hydrogenotrophic methanogens that utilize H₂ and CO₂. The survival of these taxa relies heavily on syntrophic acidogens/acetogens (e.g., *Syntrophomonas*) for hydrogen supply (Li et al. 2023a). However, these three genera exhibit poor

tolerance to highly acidic, high-VFA environments. Under ultra-high loading conditions, the accumulation of extremely high VFAs inhibits the acetate metabolic pathways of *Methanosarcina* and disrupts the syntrophic relationships required by *Methanobacterium* and *Methanoculleus*, leading to the competitive occupation of dominant ecological niches by *Methanothrix*. Furthermore, the EAD system enriched a notable proportion of *Methanospirillum*. As a typical hydrogenotrophic methanogen, *Methanospirillum* utilizes hydrogen as an electron donor to reduce CO₂ to methane. In the hydrogen-rich environment created by the electrochemical system, *Methanospirillum* gains a distinct competitive advantage, thereby facilitating its proliferation. Besides, **Fig. 5 of Supplementary Material** verified that composite material addition substantially reshapes the microbial community structure of food waste anaerobic digestion systems.

3.4 Analysis of key metabolic pathways and functional gene

3.4.1 fatty acid synthesis and degradation

Given that VFA degradation is tightly coupled with system stability in food waste anaerobic digestion, this section specifically investigates fatty acid metabolism (synthesis and degradation) and the methanogenesis process.

First, the relative abundances of genes encoding the fatty acid biosynthesis (map00061) and fatty acid degradation (map00071) pathways were analyzed. As shown in Fig. 3–6(a), the abundance of genes associated with fatty acid biosynthesis (map00061) was generally higher. Within this synthesis pathway, the EADCKL48 and EAD600L48 groups exhibited the highest gene abundances, indicating that metabolic activity for fatty acid biosynthesis was significantly enhanced under high organic loading conditions. Fatty acid biosynthesis is an energy-consuming process; When sufficient reducing power (NADPH) and structural units (such as two- and three-carbon molecules) are available, anabolic activity accelerates to support microbial growth and storage (Gao et al. 2024). In the fatty acid degradation (map00071) pathway, lower gene abundances were observed in the EAD600L8 and EAD600L48 groups, suggesting that the Fe@C600 prepared at 600°C might influence the fatty acid metabolic processes.

The genes encoding methanogenesis pathways include M00357 (acetoclastic methanogenesis), M00567 (CO₂ methanogenesis), and M00356/M00563 (both methylotrophic methanogenesis). As shown in Fig. 3–6(b), the abundances of genes associated with these methanogenesis pathways were higher in the treatment groups than in the control group. This indicates that Fe@C600 enhanced the methanogenesis process by upregulating the abundance of related functional genes.

In contrast, the abundances of genes across all four methanogenesis pathways declined under high organic loading conditions. It is hypothesized that the proliferation of electroactive microorganisms under high loading promoted VFA production. The subsequent accumulation of VFAs altered the environmental conditions, which was somewhat unfavorable for the proliferation of methanogenic archaea. Meanwhile, the generation of substantial amounts of hydrogen increased the hydrogen partial

pressure, further inhibiting the metabolism of methanogenic archaea. Consequently, the activity of methanogens decreased, as reflected by the reduced gene abundances in the aforementioned methanogenesis pathways. Furthermore, the overall gene abundances in the electrochemical groups were lower than those in the non-electrified anaerobic digestion groups, suggesting that the combination of Fe@C600 and electrochemical intervention exerted a distinct influence on the expression of methanogenesis-related genes.

A further analysis of the key genes involved in fatty acid biosynthesis and degradation, as shown in Fig. 3–7(a), reveals that under the same organic loading rate, the abundance of these key genes is consistently higher in the EAD group. *ACSL* is responsible for fatty acid activation, and *accB* is a rate-limiting enzyme in fatty acid biosynthesis. The upregulation of both genes indicates an enhanced capacity for synthesizing new membrane lipids or storing lipids within the system, which is typically accompanied by an increase in microbial biomass, particularly among methanogens or bacteria (Zhou 2024). Furthermore, *fabH* initiates fatty acid chain elongation, and *lpxC-fabZ* participates in the synthesis of lipopolysaccharides or phospholipids. This suggests that the electrochemical environment may stimulate the construction of microbial cell walls/membranes, thereby facilitating the survival and proliferation of the microbial community under high-load conditions.

Figure 3–7(b) demonstrates that the gene abundance bands in the EAD600 group are generally broader than those in the ADCK group. Notably, *fadA* and *E2.3.1.9* are core enzymes in the β -oxidation pathway, responsible for cleaving long-chain fatty acids to generate acetyl-CoA. The high abundance of these two genes provides direct evidence that electrochemical assistance significantly enhances the system's capacity for fatty acid degradation. Additionally, as the reaction time progresses, the microbial community gradually adapts and enriches functional genes associated with fatty acid degradation (Wu et al. 2024).

3.4.2 methanogenesis process

The genes in the cluster dendrogram correspond to distinct methanogenesis pathways (Fig. 6 of **Supplementary Material**), including CO₂-to-methane (M00567), methylotrophic methanogenesis (M00356, M00563), and acetoclastic methanogenesis (M00357). Given that food waste is rich in lipids and proteins, the high abundance of *mcrA*, *mcrB*, and *mcrG*-which encode methyl-coenzyme M reductase, the most core and iconic enzyme in methanogenesis-directly demonstrates an enhanced potential for hydrogenotrophic methanogenesis. The enzymes encoded by the *hdr* complex are crucial for electron transfer in the final step of methanogenesis; their high expression indicates that the electrochemical system effectively facilitates electron flow toward methanogens. The notably higher abundance of these gene clusters in the EAD600 group suggests that the Fe@C600 significantly boost the metabolic activity and electron transfer efficiency of methanogens within the electrochemical anaerobic system (Zhang et al. 2024).

Additionally, *mtaA*, *mtaB*, and *mtaC* encode methanol-cobalamin methyltransferase, which catalyzes the conversion of methanol to methyl-coenzyme M and participates in electron transfer processes. The marked increase in the abundance of these genes following high-load acclimation indicates an improvement in the microbial community's respiratory and energy metabolism capabilities. Overall, the coupling of Fe@C600 with electrochemistry enhances the acetoclastic methanogenesis pathway. By upregulating key gene expression, it optimizes electron transfer and metabolic pathways, thereby improving the system's methanogenic efficiency and stability(Wang et al. 2021).

3.5 Effects of nano iron modified biochar on the ΔG of VFAs degradation

From a thermodynamic perspective, the degradation of volatile fatty acids (VFAs) is endergonic under standard conditions. The Gibbs free energy change (ΔG) for the degradation of propionate, butyrate, and valerate is positive, meaning these reactions cannot proceed spontaneously. However, methanogenic archaea consume hydrogen to produce methane, which lowers the hydrogen partial pressure(Wu et al. 2022). This shifts the ΔG of VFA methanogenesis to negative values, making the reactions feasible. Figure 3–15 presents the calculated ΔG for methanogenesis from propionate, butyrate, and valerate, respectively.

Figure 3–8(a) shows that as the organic load increased from 4 to 48 gVS/L, the absolute value of ΔG across all groups generally showed an increasing trend. At the same organic load, the AD600 and EAD600 groups exhibited lower ΔG values compared to the ADCK and EAD-CK groups. This indicates that the Fe@C600, acting as conductive media, facilitated Direct Interspecies Electron Transfer (DIET), thereby making propionate oxidation and methanogenesis thermodynamically more favorable(Leng et al. 2018). Under load shock (increasing from 16 to 48 gVS/L), the absolute ΔG values in the ADCK and AD600 groups decreased, suggesting that the thermodynamic advantage of conventional anaerobic digestion weakened under stress, posing a potential risk of acid accumulation. In contrast, the ΔG in the EAD600 system remained consistently low (around -140 kJ/mol) with minimal fluctuation.

Observing Fig. 3–8(b), the impact of organic load on the change in free energy follows a pattern consistent with that of propionate. Under high load shock, the Fe@C600 prove crucial for maintaining a low energy barrier state in the electrochemical system, highlighting the shock-resistance advantage of coupling electrochemistry with Fe@C600. Compared to propionate and butyrate, the ΔG values for valerate degradation in Fig. 3–8(c) are significantly lower (with larger absolute values), ranging from approximately -300 to -420 kJ/mol. The figure reveals that the EAD600 group maintained the lowest free energy change across all loads, particularly under high-load conditions.

4. Discussion

Characters of nano iron-modified biochar indicates the pore structure can adsorb surface-active substances (such as proteins, lipids), improving the utilization rate of organic matter by microorganisms(Liu et al. 2023; Feng et al. 2023); The nano-iron particles provide electron transfer

channels, accelerating the degradation of VFAs, reducing the thermodynamic energy barrier, and maintaining the stability of anaerobic digestion (Shekhurdina et al. 2023).

Change of methanogenic phase reflects that the simple electric field environment promotes the reproduction of acid-producing bacteria, affecting the metabolism of methane-producing bacteria, and the lack of materials leads to the stagnation of the methane production process. At the same time, Fe@C600 is conducive to resisting high-load shock in electrochemical anaerobic digestion (Li et al. 2023b). The combination of Fe@C600 and electrochemistry is to increase the methane production and methane production potential of the system (Pasarari et al. 2021). The rapid recovery of indicators such as VFAs, pH and COD indicates that the material has enhanced the impact resistance of the solar energy system.

At Microbial Community Structure and Function, composite material addition substantially reshapes the microbial community structure of food waste anaerobic digestion systems. Fe@C600 might mitigate the inhibitory effects of continuous current or voltage on the growth and metabolism of certain *Clostridium* species. Further analysis of the genome revealed that Fe@C600 enhanced the methanogenesis process by upregulating the abundance of related functional genes. Fe@C600 also significantly boost the metabolic activity and electron transfer efficiency of methanogens within the electrochemical anaerobic system.

ΔG of VFAs degradation demonstrates the superior resistance of the EAD600 system to shock loads. The combination of electrochemical assistance (EAD) and Fe@C600 foreshadows the potential thermodynamic advantages in treating complex long-chain fatty acids.

5. Conclusion

This study explored the regulatory mechanism of coupling applied voltage and composite materials on food waste anaerobic digestion under different organic loading rates. Within the OLR range of 8–48 gVS/L, the maximum methane yield was achieved at 16 gVS/L, and the electro-assisted system supplemented with composite materials (EAD600) exhibited the best methanogenic performance. Organic loading rate, applied voltage and composite material addition jointly shaped microbial community structure. *Clostridium* was the dominant fermentative bacterium, and acetoclastic methanogen *Methanothrix* prevailed as the core methanogen. Applied voltage facilitated interspecies electron transfer, while composite materials selectively enriched hydrolytic fermentative bacteria and sustained high abundance of methanogens. Their synergy upregulated functional genes related to organic hydrolysis, acetate metabolism and methanogenesis, optimized thermodynamic conditions, and alleviated acidification inhibition caused by VFA accumulation under high loading. In summary, the combined strategy of electro-stimulation and composite material addition effectively enhances substrate degradation and methane production, offering theoretical guidance for stable operation of food waste anaerobic digestion at high organic loadings.

Declarations

Competing Interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Author Contribution

Chuanxing Jia(First Author): Funding acquisition, Investigation; He Zhao: Software, Writing-Original Draft, Writing - Review & Editing; Boxi Li: Resources, Supervision; Haijiao Zhu: Visualization, Investigation; Peike Gao: Validation;

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Footnotes

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