Constructing carbon-based materials loaded with MOFs to realize efficient anaerobic digestion of rural organic waste

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Abstract

The application of anaerobic digestion (AD) technology could convert rural organic waste (ROW) into renewable energy such as methane, which can help to mitigate the scarcity of fossil fuels and positively impact the global environment. However, the inhibition of ammonia nitrogen remains a significant obstacle to the methane production process with high concentrations of AD. Hence, in this study, three adsorption materials for ammonia nitrogen, namely FeMn-MOF, FeMn/MOF-CFs, and FeMn/MOF-CFC, were synthesized through distinct protocols. Their ability to mitigate the effect of ammonia nitrogen inhibition was investigated in a Continuous Stirred-Tank Reactor (CSTR) with TS concentration of 10% under a semi-continuous operation.
of ROW digestion system. The results show that the addition of Metal-Organic Frameworks (MOFs) material substantially mitigated the inhibition of ammonia nitrogen and enhanced methane production. Compared with the control group, FeMn/MOF-CFC exhibited the best performance, with a 40.21% decrease in ammonia nitrogen concentration and 66.96 L/L-reactor cumulative methane production. Furthermore, the potential mechanisms underlying microbial community characteristics were explored, indicating that the addition of FeMn/MOF-CFC to AD provides the optimal enhancement of methane production. The addition of FeMn/MOF-CFC can enrich *Methanosarcina*, enhance the acetoclastic pathway for methane production, and increase the relative activity of coenzyme F₄₂₀, achieving a 193.68% increase.

**Keywords:** Anaerobic digestion, Metal-organic frameworks, Ammonia nitrogen inhibition, CSTR, Rural organic waste

1. Introduction

The shortage of energy remains a critical global issue, and the development of renewable energy is a viable solution to this problem. Rural organic waste (ROW) is an essential but underutilized resource, which can be efficiently transformed into renewable energy such as methane using anaerobic digestion (AD) technology. This strategy is particularly useful in rural regions of developing countries. In China, the rural population accounts for 65% of the total population and generates an annual average of around 294 million tons of ROW [1]. A large amount of unused waste has not been properly treated, leading to serious environmental problems and negative
impact on the health of local residents. Despite efforts to recycle ROW, landfilling is still still be the major content of ROW disposal in the foreseeable future [2]. ROW is generally characterized by scattered distribution and high content of degradable organic matter, which is regarded as a reusable biomass resource [3]. AD technology is widely recognized as a promising option for handling small to medium-sized organic waste in rural areas [4]. Because it can not only transform ROW into clean energy in presence of fermenting bacteria, specialized acidogenic syndromes and various methanogenic archaeas, but also reduce greenhouse gas emissions and obtain considerable social and economic benefits [5].

However, ROW is rich in compounds such as protein and amino acids, which are usually degraded to generate a large amount of ammonia nitrogen, leading to an increased risk of ammonia nitrogen inhibition in AD [6]. Although the degradation of proteins is slow, released ammonia nitrogen often accumulates under anaerobic conditions [7]. Excessive ammonia nitrogen can interfere with the metabolic enzymes of microorganisms and ultimately lead to a decrease in methane production efficiency [8]. Lay et al., (1998) reported that the activity of methanogens will decrease by 10% when the ammonia nitrogen concentration exceeds 3720 mg N/L at medium temperature [9]. Liu et al., (2020) reported that when the ammonia nitrogen concentration in the AD system reaches 6000 mg N/L, the AD reactor will completely stop producing methane [10]. Therefore, the high concentration of ammonia nitrogen is still a great threat to the stability of AD system. Researchers have proposed and evaluated various strategies to avoid the impact of ammonia nitrogen inhibition on AD
performance. Commonly reported strategies include microbial community
domestication, anaerobic ammonia oxidation, ammonia stripping and microwave.
Despite the demonstrated effectiveness of nearly all these methods in removing
excessive ammonia nitrogen in laboratory-scale experiments, some limitations remain,
such as long ammonia removal cycles and high investment costs. Krakat et al., (2017)
reported that anaerobic bacteria can produce methane successfully through
domestication or long-term adaptation, but the adaptation process may require more
time [11]. Bousek et al., (2016) indicated that ammonia stripping could be a promising
tool to overcome the inhibition of ammonia nitrogen. But this approach may have
adverse effects on microbial communities due to the inhibition of AD process through
oxygen exposure during operation [12]. Therefore, a simple operation and strong
ammonia nitrogen removal capacity strategy is needed to improve the effectiveness of
AD.
Nowadays, the application of adsorption materials has shown its potential for
mitigating ammonia nitrogen inhibition. Metal-organic frameworks (MOFs) is a
promising high-capacity adsorbent which is self-assembled by metal clusters as nodes
and organic ligands as connectors [13]. Glomb et al. (2017) reported a maximum
ammonia uptake of 302.43 mg/g for UiO-66 modified with urea-functionalized
dicarboxylic acid [14]. In another study, Godfrey et al., (2018) synthesized MFM-
300(Al) with an ammonia adsorption capacity of 266.75 mg/g, which exhibited
reversible adsorption for over 50 cycles [15]. However, their performance studies were
mostly conducted under dry conditions, and it is unclear if there are significant
performance advantages under humid AD conditions. In our previous research, we successfully alleviated the inhibition of ammonia nitrogen in a high-concentration AD system by applying a MOFs-derived porous metal oxide/graphene nanocomposite (FeMn-MOF/G), which has an ammonia nitrogen adsorption capacity of 102.68 mg/g. However, the magnetic recovery efficiency of powdered FeMn-MOF/G appears to be unsatisfactory, and the recovery rate is only 43.04%. In order to give consideration to high adsorption capacity and high recovery performance, suitable ammonia nitrogen capture materials need to be designed. A feasible technology to achieve this goal is to load MOFs onto special substrates.

Carbon-based materials are suitable substrates for the loading of MOFs due to their large surface area, high porosity, fast adsorption rate, and low cost [16]. Lee et al., (2019) loaded Cu–TCPP MOFs onto fibers and found that the ammonia adsorption capacity increased threefold compared to the original MOF powder, and the material exhibited better structural stability [17]. Additionally, carbon-based materials are also considered as an efficient adsorbent for removing ammonia nitrogen. Sasaki et al., (2011) found that adding CFC (Carbon Fiber Cloth) can prevent ammonia inhibition (including 6000 mg/L ammonia nitrogen) in the thermophilic reactor, which makes acetic methanogens proliferate stably and realizes efficient digestion of garbage slurries [18]. Therefore, we chose two types of carbon-based materials, CFs (carbon felts) and CFC, as substrates for loading MOFs materials to prepare new materials with unprecedented properties. The low synthesis cost can provide the possibility for the industrial application of materials. Hence we improved the formula for preparing
FeMn-MOF/G, replacing the original expensive graphene with low-cost activated carbon. Through this strategy, we achieved the uniform growth of MOF precursors on CFs and CFC, resulting in two novel composite materials, FeMn/MOF-CFs, and FeMn/MOF-CFC. To the best of our knowledge, there have not been any reports on the use of FeMn/MOF-CFs and FeMn/MOF-CFC to mitigate ammonia nitrogen inhibition during AD. It is worth mentioning that most previous AD experiments using adsorption materials to alleviate ammonia nitrogen inhibition were conducted in batch mode. While batch data can offer guidance, it is critical to evaluate the advantages of adsorption materials in a continuous stirred tank reactor (CSTR) to further investigate the effectiveness of composite materials in relieving ammonia nitrogen inhibition.

The aim of this study is to assess the impact of incorporating FeMn/MOF-CFs and FeMn/MOF-CFC in the performance of a semi-continuous CSTR anaerobic digester. We prepared and characterized the FeMn/MOF-CFs and FeMn/MOF-CFC, and monitored the concentrations of ammonia nitrogen and volatile fatty acids (VFAs) in the effluent to demonstrate the effectiveness of the adsorption materials in mitigating inhibition. Additionally, we evaluated the biogas quality, methane production, and degradation of total solids (TS) and volatile solids (VS) during the AD process of ROW. To study the impact of the adsorbent on the microbial community of the AD reactor, we investigated the changes in the community structure and metabolic mechanisms through Illumina MiSeq sequencing technology. Finally, the adsorption material was recovered and regenerated, and its recycling performance was studied.

2. Materials and methods
2.1. Collection and treatment of digestion substrates and inoculums

The rural organic domestic garbage used in the semi-continuous AD experiment was provided by the garbage sorting center of Datun Street, Peixian County, Xuzhou City, Jiangsu Province, China. The primary constituents of ROW include decomposed fruits and vegetables, rice, pasta, along with a small proportion of bones and fish bones. The bones and plastics, amassed from the waste, were manually separated, followed by grinding into fine particles having a diameter of no more than 4mm using a pulverizer. The crushed samples that resulted were frozen at -20°C for future use, with TS and VS of 16.60% and 81.03% respectively. The inoculum was collected from the AD reactor of Liuhe Animal Science Base, Academy of Agricultural Sciences, Liuhe District, Nanjing, Jiangsu Province. By adopting the AD reaction mode of sequencing batch, the retrieved inoculum was placed in a water bath at 37±1°C for domestication and culture with rural organic domestic garbage. The inoculum culture experiment was conducted at a TS concentration of 5% and was left to digest stationary until no gas was produced. The purpose of cultivating inoculum at 5% TS concentration is to accelerate the stability of methane production and ensure the consistency of reaction matrix in semi-continuous digestion stage. The physical and chemical properties of the substrate and inoculum used during the AD experiment can be found in Table 1.

2.2. Preparation of FeMn/MOF-CFs and FeMn/MOF-CFC

2.2.1 Activation of carbon-based materials

Firstly, the green CFs (CFC) was immersed in a mixture of concentrated sulfuric acid and concentrated nitric acid in a volume ratio of 3:1, placed in a beaker, and soaked
for 12 hours. Then, it was washed three times with deionized water and ultrasonically oscillated for 20 minutes to fully remove the sulfuric acid and nitric acid from the CFs (CFC). Next, the green CFs (CFC) is soaked in a round bottom flask filled with concentrated nitric acid and refluxed at 100°C for 2 h. After that, it is washed to neutrality by distilled water and absolute ethanol to remove impurities on the CFs (CFC) and improve the hydrophilicity of the CFs (CFC). Finally, a total of 5.7090 g of ferric chloride and 1.9030 g of sodium sulfate were weighed and dissolved in a 35.00 mL aqueous solution with a 1:1 molar ratio, and the resulting solution was labeled as solution A. Put CFs (CFC) in solution A and transfer it to a high-pressure reaction kettle at 120°C for 6 hours. Following this, the CFC was washed several times with deionized water and ethanol before being dried at 60°C. The primary objective of this step is to react the ferric chloride with sodium sulfate under high temperature and high pressure to produce ferric hydroxide, which is then deposited onto the CFs (CFC), allowing for the FeMn-MOF nano-array to be uniformly loaded onto the CFs (CFC).

2.2.2 preparation of FeMn/MOF

Add 6.0990 g FeSO$_4$·7H$_2$O, 1.1700 g KMnO$_4$, 1.1362 g activated carbon and 4.0935 g 3,5-pyrazole dicarboxylic acid into a solution of 144 g N,N-dimethylformamide (DMF) and H$_2$O (with a mass ratio of 7:3). Keep mechanical stirring to obtain solution B. Then transfer solution B to the reaction kettle and incubate it at a constant temperature of 100°C for 48 h in a hot air circulating oven, with a heating rate of 5°C per minute. The reaction kettle was taken out and aged for 12 h after maintaining the constant temperature. Finally, it was filtered at room temperature and
naturally dried to obtain FeMn/MOF.

2.2.3 The load of FeMn/MOF on carbon-based materials

The dried CFs (CFC) was put into solution B and and sealed in a 200.00 mL PTFE-lined stainless steel autoclave. The mixture was then self-pressure heated at 100°C for 48 h before being naturally cooled to room temperature. Afterwards, the CFC were repeatedly washed with DMF and ethanol before being dried to obtain FeMn-MOF nano-arrays loaded onto the CFC, which were respectively labeled FeMn/MOF-CFs and FeMn/MOF-CFC.

2.3. Simulation experiment on ammonia nitrogen adsorption and cycling performance of FeMn/MOF-CFs and FeMn/MOF-CFC

The adsorption effects of three different MOFs materials were studied under different ammonia nitrogen concentrations. The simulated ammonia nitrogen wastewater was prepared with dried ammonium chloride, with concentrations of 1000.00 mg/L, 2000.00 mg/L, 3000.00 mg/L, and 4000.00 mg/L. Add 1.00 g of FeMn/MOF, FeMn/MOF-CFs and FeMn/MOF-CFC into the 100.00 mL triangular flask containing simulated wastewater of different ammonia nitrogen concentrations and fully mix them. Each group of experiments was repeated three times. Adjust the pH value of the simulated wastewater to 7.50 with 4.00 mol/L NaOH solution, and place the triangular flask in a constant temperature water bath at 37°C to simulate the AD environment. The sample is taken with a pipette every hour and filtered through a 0.45 μm filter after which the filtrate is centrifuged at 5000 rpm for 3 minutes and the supernatant is diluted 1000 times. The ammonia-nitrogen concentration of the sample...
is determined using the Nessler’s reagent spectrophotometric method, and the observed data is recorded as the average of three repeated experiments. Sampling continues until the ammonia-nitrogen concentration in the simulated experiment no longer changes. The data calculated by the formula studied by predecessors are as follows:

$$R = \frac{(C_{\text{initial}} - C_{\text{final}}) \times V}{W}$$  \hspace{1cm} (1)$$

Among them, $R$ (mg/g) is the adsorption capacity of the three MOFs materials, $W$ (g) is the added mass of the three MOFs materials, $C_{\text{initial}}$ and $C_{\text{final}}$ (mg/L) represent the ammonia nitrogen concentration of the simulated wastewater at the beginning and end of the experiment, respectively, and $V$(L) is the simulated ammonia nitrogen wastewater volume.

After the adsorption experiment, three MOFs materials were retrieved from the solution. These MOFs were then regenerated using a mixed solution of NaCl/NaOH, and dried in an oven at 50°C. At last, the ammonia nitrogen adsorption capacity of three kinds of MOFs materials after regeneration was tested by repeating the above experimental steps.

2.4. Design of Semi-continuous anaerobic digestion experiment

Four semi-continuous digesters with a working volume of 3.5 L each were utilized and maintained at a medium temperature of (37±1°C) by circulating water in the water jacket. The stirring frequency was once every half hour, with a stirring time of 3 minutes and a rotating speed of 20 rpm. Throughout the experiment, rural household garbage was used as the substrate, with an initial concentration of TS kept at 10% and a
hydraulic retention time (HRT) of 25 days. To achieve steady-state conditions, the
digestion substrate was maintained in the CSTR for more than three HRTs. Steady-state
conditions referred to a constant loading rate and gas production. At the start of the
experiment, 1133.20 g of ROW and 2366.80 g of inoculum were thoroughly mixed and
added to the semi-continuous digester based on its effective volume and TS
concentration. The initial pH was adjusted to 7.50, and the experiment commenced after
establishing anaerobic conditions through N₂ gas purification.

Feeding and discharging of the digester were regularly initiated once the methane
content in the biogas reached a stable 60%. A designated volume of the digested sludge
from the reactor was extracted daily using a syringe, and the same volume of substrate
was promptly added through the same procedure to maintain a constant volume
configuration for digestion. Four groups of laboratory AD experiments were set up in
this study, with one control experiment labeled CK, which did not use MOFs materials.

The other three groups of experiments used FeMn/MOF, FeMn/MOF-CFs and
FeMn/MOF-CFC, which were labeled as MOF-1, MOF-2 and MOF-3 respectively.
Four groups of experiments were conducted simultaneously, and each group had three
replicates. When discharging the digestate, ammonia nitrogen adsorption materials may
be discharged along with it. Therefore, it is necessary to regularly replenish ammonia
nitrogen adsorption materials by adding 5g of FeMn/MOF, FeMn/MOF-CFs, and
FeMn/MOF-CFC at the beginning of each HRT cycle for MOF-1, MOF-2, and MOF-
3, respectively. During the testing process, the machine automatically recorded
methane production in the AD process. Methane content was sampled and measured

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daily, and discharged material was sampled every 7 days to measure the TS and VS content, pH, ammonia nitrogen concentration, and VFAs concentration. On the 5th day after the experiment started, a 10.00 mL syringe was used to take samples directly from the digestion bottle through the sampling port, and the sampling time was approximately 10 seconds. The collected samples were analyzed for the activities of acid phosphatase (ACP), alkaline phosphatase (ALP), and α-glucosidase. Samples were also collected after the experiment to determine the activity of F_{420} enzyme and microbial community composition.

### 2.5. Analytical method

The growth state of MOF was monitored by EVO-LS10 field emission scanning electron microscope (SEM). The machine runs at 10 KV, and the probe current ranges from 0.5 PA-5 μA. X-ray diffraction (XRD) (PANalytical B.V, Empyean, Netherlands) provides images of the structure and purity of the materials. The testing machine utilizes a copper target to analyze the MOFs materials at a scanning speed of 2°/min, specifically examining the range between 10°-90°. According to the standard method, the TS and VS of the CSTR experiment were measured at temperatures of 105°C and 550°C, respectively. The digital pH meter was calibrated by 5-point method in acid-base buffer, and then used to determine the pH value (HZP-L502, USA). The total carbon in the digested matrix was determined by TOC analyzer (Multi 3100, Germany), and the NPOC method was adopted, which was set to automatic sampling. The dried samples after grinding and sieving with a 100-mesh screen were placed in a digester tube, and the data for total nitrogen was obtained by Kjeldahl nitrogen determinator.
The biogas components were detected using a chromatographic detector (Renhua, GC-9890B/T, China) with hydrogen as the carrier gas and the gas flow rate is set to 50 mL/min. Using the external standard method, each gas sample had a gas intake of 0.70 mL. Before the operation of the machine, the standard mixture of \( \text{N}_2 \) with concentration of 32.8%, \( \text{CH}_4 \) with concentration of 38.4% and \( \text{CO}_2 \) with concentration of 28.8% should be used for calibration. VFAs data were obtained by liquid chromatograph (GC-2030, Shimadzu, Japan), and the samples were acidified with 34% phosphoric acid before determination. The concentrations of coenzyme F\(_{420} \) were quantitatively determined by ultraviolet spectrophotometer (UV-4802, USA) at the absorbance of 420 nm. On the 5th day after the AD experiment, extracellular hydrolase activity was evaluated for ACP, ALP, and \( \alpha \)-glucosidase using an upgraded methodology described in previous literature [19]. The calculation formula of relative enzyme activity is:

\[
\text{Relative enzyme activity (\%)} = \frac{A_{\text{MOF}}}{A_{\text{control}}} \times 100\%
\]

(2)

Among them, \( A_{\text{MOF}} \) corresponds to the absorbance of each experimental group at a specific wavelength, \( A_{\text{control}} \) corresponds to the absorbance of the control group and the corresponding experimental group at the same wavelength.

Complete the DNA extraction and PCR amplification of the sample based on the standard protocol using 338F_806R as the bacterial amplification primer and 524F10extF_Arch958RmodR as the archaea amplification primer. Subsequently, following the preliminary quantitative results of electrophoresis, determine the quantity
of PCR products through fluorescence analysis. After the construction of Illumina library, high-throughput sequencing was carried out using the Illumina NovaSeq 6000 platform. Samples were then centrifuged at 7000 rpm for 5 minutes using the centrifuge (Centrifuge 5810 R, Eppendorf, Germany) followed by ammonia nitrogen estimation using a fast ammonia nitrogen analyzer (5B-6D (V8), Lianhua, China). TS and VS of effluent during CSTR operation are calculated, which is helpful to analyze the information of biodegradation degree and biodegradation rate of digested substrate for monitoring the stability of reactor operation. The formula involved in the equation is:

\[
\text{TS degradation efficiency (\%)} = \frac{\text{TS}_{\text{in}} - \text{TS}_{\text{out}}}{\text{TS}_{\text{in}}} \times 100\%
\]  
(3)

\[
\text{VS degradation efficiency (\%)} = \frac{\text{VS}_{\text{in}} - \text{VS}_{\text{out}}}{\text{VS}_{\text{in}}} \times 100\%
\]  
(4)

Among them, TS\textsubscript{in} and VS\textsubscript{in} correspond to TS and VS of influent(\%), TS\textsubscript{out} and VS\textsubscript{out} correspond to TS and VS of effluent from CSTR experiment (\%).

2.6. Data analysis

All the data recorded during the experiment need to be imported into Microsoft Excel 2016 (Microsoft, USA) for descriptive statistics and calculation of mean and standard deviation. The charts appearing in the manuscript are drawn by OriginPro 2021 (OriginLab, USA) software. The original SEM image should be processed by ImageJ (National Institute of Mental Health) software to increase the scale. Data obtained from XRD analysis are read into MDI Jade 6 (Materials Data, USA) to generate images.
3. Results and discussion

3.1. Physical and chemical properties of FeMn/MOF, FeMn/MOF-CFs and FeMn/MOF-CFC

Fig. 1a to 1d presents the SEM spectrum of the bare CFS, bare CFC, FeMn/MOF-CFs and FeMn/MOF-CFC grown by FeMn/MOF. It can be seen from Fig. 1a that for untreated CFs, a smooth surface was observed from SEM. And the shape is straight and arranged regularly. As shown in Fig. 1b, compared with smooth original CFs, the surfaces of treated CFs (FeMn/MOF-CFs) became rough, forming a uniform and compact MOF coating. Fig. 1c shows the electron microscope image of the untreated original CFC. Similar to the original CFs, the appearance of the original CFC is smooth but staggered. Fig. 1d shows a CFC loaded with FeMn/MOF, which is attached with many surface-bonded regular cubic nanocrystals. SEM images showed that both CFs and CFC have successfully loaded MOFs materials. Fig. 1e records the diffraction spectra of three materials within the 2θ range of 20° to 85°. The original CFC exhibited a typical broad peak at 26°, corresponding to the (002) plane of graphite carbon, which was in line with the expected spectrum of graphite materials. No other impurity peaks were observed in the XRD pattern, which indicated that the impurities in the original material had been completely removed after activation treatment [20]. However, the characteristic diffraction peak of graphite material is missing in the XRD pattern of CFs, possibly due to an excessive amount of amorphous components in the original carbon felt. In the XRD pattern of FeMn/MOF-CFC, two characteristic peaks belonging to FeMn/MOF were observed at 31° and 35° respectively. Similarly, the diffraction peak
was also observed at 31° in the XRD pattern of FeMn/MOF-CFs, which indicated that FeMn/MOF was successfully loaded on CFC and CFs.

### 3.2 Simulated ammonia nitrogen adsorption effect and cycle performance

This study aimed to investigate the impact of varying concentrations of ammonia nitrogen on the adsorption properties of MOFs materials. The results of simulated ammonia nitrogen adsorption experiments show that the adsorption equilibrium time of three different MOF materials is the same at 25°C, which is 3 h. The simulation experiment observed the adsorption capacity of MOF materials at varying concentrations of ammonia nitrogen. It can be seen from Fig. 2 that FeMn/MOF, FeMn/MOF-CFs, and FeMn/MOF-CFC had the greatest ammonia nitrogen adsorption effects at a simulated concentration of 1000.00 mg/L, reaching 281.44 mg/g, 269.46 mg/g, and 239.52 mg/g, respectively. In contrast, conventional natural adsorbents like natural zeolite and biochar typically exhibit an adsorption range of 0.75-6.00 mg/g. Even after modification, the adsorption range is only increased to 11.00-35.00 mg/g [21]. MOFs composites, in comparison to conventional adsorbents, have an ammonia nitrogen adsorption capacity that is over ten times greater. The AD reactor with a 10% TS concentration had an initial ammonia nitrogen concentration of around 3500.00 mg/L. Based on the correlation between MOFs’ adsorption capacity and ammonia nitrogen concentration, 5 g of MOFs material addition can be selected for each HRT within the experimental group. Finally, the FeMn-MOF, FeMn/MOF-CFs and FeMn/MOF-CFC were retrieved from the triangular flask for re-testing of their ammonia nitrogen adsorption capacity in a simulation wastewater with a simulated
ammonia nitrogen concentration of 1000.00 mg/L. The data analysis shows that the recovery rates of FeMn-MOF, FeMn/MOF-CFs and FeMn/MOF-CFC are 33.28%, 95.14% and 92.63% respectively. It can be seen that the recovery rate of adsorbent is greatly improved after loading FeMn-MOF on CFs and CFC. After regeneration, the ammonia nitrogen adsorption capacities of the three kinds of MOFs materials are 218.37 mg/L, 224.54 mg/L and 192.29 mg/L, which are 77.59%, 83.33% and 80.28% of the initial ones, respectively. All three kinds of MOFs materials have good recycling performance, which makes them have broad application prospects in practice.

3.3 Methane production performance and organic solid degradation of CSTR experiment with different MOFs materials

The CSTR experiment recorded the daily methane production of ROW anaerobic digestion for 95 days. As can be seen from Fig. 3a, the methane content began to increase rapidly during the start-up of the control group. It reached the maximum value of 74.30% after 14 days and then entered a stable period. Upon regular feed and discharge being initiated on the 17th day, the daily methane production remained stable at about 0.25 L/L·d, with a maximum value of 0.54 L/L·d observed on the 28th day (Fig. 3b). During the following 15 days, methane content varied between 50.00% and 70.00%, until the daily methane production significantly decreased to 0.04 L/L·d on the 33rd day. By the 46th day, the daily methane production further decreased to 0.006 L/L·d, indicating that the experimental process of the control group was inhibited, leading to a complete failure of the reactor on the 48th day. The experiment had to be terminated for the control group. On the other hand, as can be seen from Fig. 3a and 3b,
the semi-continuous digestion of all experimental groups can be classified into three stages: start-up period, first stable period and second stable period. During the start-up stage, the change trend is similar to that of the control group, with daily methane production and methane content increasing rapidly. The MOF-2 reactor in the experimental group exhibited the best performance, with the daily methane production reaching the maximum of 1.30 L/L·d on the 13th day and the methane content reaching the maximum of 77.55% on the 11th day. Afterward, daily methane production slightly decreased, entering the first stable period, and the methane content remained between 55% and 65% (Fig. 3b). Steady state was determined as ten consecutive days of operation with less than 10% fluctuation in methane production [22]. All three reactors are in a stable state, and the methane output and rate of CSTR reactor are evaluated as the average methane output at different stages. The average daily methane production of all three experimental groups during the first stable period was 0.53 L/L·d, 0.67 L/L·d and 0.75 L/L·d respectively. In this work, the digestive substrate is fed regularly once a day. At the beginning of feeding, the decrease of methane production per day was first observed, with MOF-3 decreasing to 0.22 L/L·d on the 23rd day. The daily methane production fluctuation is potentially due to newly added digestive substrates, which can impact the microbial population dynamics in the anaerobic process, requiring time for the anaerobic microbial community to adjust to the new feed. Additionally, fresh air is also input during the feeding and discharging process, which may disturb the strict anaerobic environment employed for the growth of methanogens, leading to a decrease in daily methane production. Until the 50th day, the reactor established a new
steady state and entered the second stable period, with the average daily methane production of all three groups being 0.39 L/L-d, 0.54 L/L-d, and 0.72 L/L-d, respectively. Two methane production peaks were observed in MOF-2 and MOF-3 in the second stable period, which were 2.09 L/L-d and 2.15 L/L-d respectively. The surge in methane production could be linked to the increase of organic matter in AD system caused by the residue of indigestible substrate in the previous stage, which is then rapidly decomposed with the continued operation of the reactor. It was also observed that the average daily methane production of all three reactors in the second stable period was lower than that in the first stable period during the subsequent experiments. The reason for the difference could be due to the loss of more organic solids with the effluent. The decrease in organic matter reduced the TS concentration in the AD reactor, ultimately resulting in the decrease of methane production in all three experimental groups during the second stable period.

Severe inhibition may have occurred during the AD period, as evidenced by the cumulative methane production of 7.98 L/L-reactor in the control group. However, the addition of FeMn/MOF, FeMn/MOF-CFs, and FeMn/MOF-CFC significantly increased the cumulative methane production. Fig. 3c illustrates a 4.27 times, 6.09 times, and 7.39 times increase in the cumulative methane production for the three experimental groups, reaching 42.08 L/L-reactor, 56.59 L/L-reactor and 66.96 L/L-reactor, respectively, compared to the control group. Overall, all three adsorbents effectively improved methane production, with MOF-3 having the best effect and MOF-1 having the least effect. The improvement effects of MOF-2 and MOF-3 were
similar but slightly less effective than MOF-3. The reason for this could be that CFs and CFC, as carbon-based materials, also have certain AD improvement ability. When they are used as supporting materials and compounded with FeMn/MOF, the properties of the original MOF materials are improved, resulting in the production of MOF-2 and MOF-3 with enhanced adsorption capabilities. To assess the degree of substrate biodegradation, important parameters such as TS and VS were characterized. These parameters were consistent with the changing trend of methane production. Figs. 3d and 3e reflect the degradation degree of organic solids in all four groups of experiments. At the end of the experiment, the TS and VS in the control group decreased by 39.50% and 12.66%, respectively, indicating unsuccessful methane production. The maximum degradation range of TS and VS was consistent with the highest methane production, observed in the experimental group with MOF-3. The maximum degradation rates of TS and VS also appeared in the MOF-3 group, which were 84.40% and 61.15%, respectively. The final experimental results show that all three MOFs material can effectively improve the AD process, which may be related to their capability to adsorb ammonia nitrogen. The adsorbent addition relieved the inhibition of ammonia nitrogen in CSTR experiment, which finally showed the improvement of methane production and high degradation rate of organic solids.

3.4 Effects of different MOFs materials on the changes of volatile fatty acids and pH value

The consumption of VFAs may be helpful to accelerate the production of methane during digestion. Changes in VFAs are often accompanied by changes in pH in the
AD system, which can affect the overall methane production performance of semi-
continuous AD. This is because pH is a critical factor that affects enzyme activity
during the AD process, with enzyme activity typically being greatest within a specific
and narrow pH range [22]. Fig. 4a reflects the changing trend of pH and VFAs during
the CSTR experiment. During the initial phase of the experiment, all four groups
experienced rapid VFA accumulation, primarily consisting of acetic acid and propionic
acid. The initial total VFAs concentrations were 10945.05 mg/L, 13155.84 mg/L,
11499.89 mg/L and 10431.12 mg/L, respectively. Although more VFAs were produced
in the initial phase of the AD experiment, the pH value rose instead of falling and
fluctuated in the range of 7.05-8.35. This is likely because ammonia nitrogen released
from deamination reaction brings buffer capacity to AD, but the pH level stayed within
the acceptable range of a semi-continuous AD process, commonly ranging between
6.50 and 8.40 [23]. The production of methane usually corresponds to the rapid
consumption of VFAs. From the 14th day onwards, VFAs in the three experimental
groups began to degrade rapidly. The previous reaction mainly degraded butyric acid
into acetic acid and H\textsubscript{2} provided nutrients for methanogens, which kept acetic acid in a
dynamic balance for a certain period of time. A significant amount of acetic acid began
to be consumed after 28 days and eventually decreased to 2000.00 mg/L. Meanwhile,
isobutyric acid, valeric acid, and isovaleric acid gradually degraded, whereas propionic
acid remained in large quantities due to thermodynamic limitations. Additionally, Fig.
4b illustrates that the pH value began to increase on the 28th day and then remained
stable at approximately 7.84 ±0.67 until the end of the experiment. This stability
demonstrates that all experimental groups have successfully reached the stage of methane production [24]. Moreover, the pH data recorded in this study are within the suitable growth range of methanogenic bacteria, indicating no excessive accumulation of VFAs throughout the CSTR experiment. According to Giwa et al. (2019), a low concentration of VFAs can increase the biological activities of acid production and methane production in the AD reactor [25]. However, on the 35th day, the methane production in the control group had ceased, leading to an inability to further destroy VFAs. As a result, acetic acid and propionic acid concentrations drastically increased to 7385.01 mg/L and 2833.57 mg/L, respectively, destroying the buffer capacity of the digester. Consequently, the pH dropped below 6.50. The findings reveal that acid inhibition was severe in the control group, resulting in decreased methane production. The lower methane production may be linked to the higher concentration of ammonia nitrogen in the control group, leading to acid inhibition.

3.5 Analysis of ammonia nitrogen concentration fluctuation in CSTR experiment

The concentration of ammonia nitrogen is a critical parameter in controlling ammonium removal for most materials that adsorb ammonia nitrogen because it affects both the adsorption performance and the material itself. Typically, concentrations of around 3500 mg/L are considered inhibitory for the AD process [26]. The study observed that only the ammonia nitrogen concentration in the control group had a significant inhibitory effect on methane production, whereas the ammonia nitrogen levels in all the experimental groups were within the acceptable range previously reported for AD. As depicted in Figure 5, the ammonia nitrogen concentration in each
group exhibited an upward trend during the initial phase of the experiment. By the end of the CSTR experiment, the control group’s ammonia nitrogen concentration had soared to 3432.00 mg/L, demonstrating an apparent inhibition. In contrast, in the experimental groups that had MOF-1, MOF-2, and MOF-3 as adsorbents, the concentrations of ammonia nitrogen were 2366.00 mg/L, 2328.00 mg/L, and 2052.00 mg/L, respectively, which were 31.06%, 32.17%, and 40.21% lower than those in the control group. This suggests that the use of adsorbents can efficiently mitigate ammonia nitrogen-induced inhibition and stimulate methane production. Accumulation of VFAs is a symptom of methane-producing microorganism inhibition caused by high ammonia nitrogen concentration [27]. As previously stated, VFAs began to accumulate during the final stage of the control group, and it can be deduced that the high concentration of ammonia nitrogen in this group had a significant inhibitory impact on the microorganisms responsible for methane production. Finally, The ammonia nitrogen adsorption capacity of three kinds of MOFs materials in CSTR was calculated according to formula (1), resulting in ammonia nitrogen adsorption capacities of 226.73 mg/g, 339.73 mg/g, and 355.13 mg/g for FeMn-MOF, FeMn/MOF-CFs, and FeMn/MOF-CFC, respectively. Compared with the maximum adsorption capacity of three kinds of MOFs in simulated ammonia nitrogen adsorption experiment, the adsorption capacity of FeMn-MOF decreased by 19.58%, while the adsorption capacities of FeMn/MOF-CFs and FeMn/MOF-CFC increased by 20.68% and 32.55% respectively. This result is due to the decreasing adsorption capacity of FeMn-MOF as the concentration of ammonia nitrogen increases, while FeMn/MOF-CFs and
FeMn/MOF-CFC can still maintain high adsorption capacity at high ammonia nitrogen concentration above 3000.00 mg/L.

**3.6 Variations of enzyme and microbial physiological activities**

In order to clarify the changes of microbial metabolic activity in the presence of adsorbents, the activities of key enzymes in AD system were determined at the beginning and end of CSTR experiment, as well as sequenced the 16S rRNA gene of total bacteria and archaea by extracting DNA. Although many enzymes are involved in the transformation of ROW into CH$_4$, ACP, ALP, α-glucosidase and coenzyme F$_{420}$ were selected as representatives to analyze their activities in this study. ACP and ALP are important extracellular hydrolases involved in the process of AD, while α-glucosidase is also vital for the degradation of carbohydrates. Three enzyme activities were measured on the 5th day of the CSTR experiment, as indicated in Figure 6a. The enzyme activity in each experimental group exceeded that of the control group. The enzyme activity of MOF-3 was the highest among the experimental groups, and the relative enzyme activities of ACP, ALP, and α-glucosidase reached 142.62%, 151.97%, and 136.04%, respectively. These results indicate that adding MOFs material can improve the performance of hydrolase, thereby expediting the hydrolysis and acidification process of AD, which is consistent with the conclusion that the experimental group has accumulated more VFAs than the control group in the early stage. Coenzyme F$_{420}$ is regarded as a crucial electron carrier enzyme for reducing carbon to methane [28]. Previous studies have shown that coenzyme F$_{420}$ can be utilized as an indicator for monitoring methanogenic activity. Figure 6a illustrates the variations
of coenzyme F$_{420}$ for different groups during AD. The relative enzyme activities of coenzyme F$_{420}$ of MOF-1, MOF-2 and MOF-3 reached 151.58%, 188.42% and 193.68% respectively, by the experiment’s end. Obviously, three different MOFs materials significantly enhanced the activity of F$_{420}$, with MOF-3 exhibiting the highest relative enzyme activity and MOF-1 displaying the lowest, which corresponded precisely with the cumulative methane production of each experimental group. The changing trend of F$_{420}$ enzyme concentration indicates a correlation between its activity and methane production. An increase in enzyme activity can generally induce higher methane production levels.

Using Illumina MiSeq sequencing of 16S rRNA gene, the members of bacterial communities in all four groups of experimental AD were identified. The alpha diversity index provides a detailed depiction of the species richness and evenness in a microbial community. The bacterial diversity index of four groups of CSTR experiments was analyzed in this study and the specific test data are shown in Table 2. The Coverage index is greater than 0.99, indicating the accuracy of sequencing results. At the experiment’s conclusion, the bacterial community in all reactors had similar diversity index values, although the Shannon index for all experimental groups was higher than that of the control group. The low Shannon even and Simpson even indexes of MOF-3 may result from the functional microorganisms' enrichment in the reactor. Simultaneously, Ace and Chao1 index showed that the microbial community in the experimental group had very high species richness, 1.65-2.27 times greater than that of the control group. The results indicated that the control group may have experienced
inhibition caused by ammonia nitrogen, resulting in a decrease in bacterial species richness and diversity.

The influence of three different MOFs materials on methane production in CSTR experiment was revealed by identifying bacterial community composition. Figure 6b depicts the bacterial microbial community composition at the phylum level. At the end of CSTR experiment, Bacteroidota, Firmicutes and Spirochaetota were the dominant bacteria in all reactors. These three bacteria are also common hydrolytic fermentation bacteria in AD process, especially Bacteroidota and Firmicutes, which can degrade organic matter and convert it into VFAs [29]. Firmicutes is considered to be a kind of hydrolytic bacteria that participate in the degradation of protein, fat and cellulose in various substrates into VFAs [30]. The relative abundance of Firmicutes of MOF-1 and MOF-2 in the experimental group increased from 20.18% in the control group to 31.31% and 44.33% respectively, but the Bacteroidota decreased from 60.26% in the control group to 48.54% and 41.00%, and the Spirochaetota decreased from 17.15% in the control group to 10.37% and 2.28%. In the experimental group MOF-3, the Bacteroidota was dominant, and its relative abundance increased to 70.85%.

Bacteroidota can greatly promote the transformation of protein into VFAs and ammonia nitrogen. The results of bacterial community analysis at the door level show that different MOFs materials will enrich different bacteria. At the same time, compared with the control group, the bacterial diversity in all experimental groups has been significantly increased. For example, the relative abundance of Cloacimonadota in MOF-1, MOF-2 and MOF-3 in experimental groups has increased from 0.04% in the
control group to 2.02%, 5.94% and 6.68% respectively.

Further comparison of the dominant phyla down to the genus level was performed to reveal the effects of adding different MOFs on microbial communities. Fig. 6c illustrates that the most prevalent bacteria in the control group are *Rikenellaceae_RC9_gut_group* (phylum Bacteroidota) and *Prevotella* (phylum Bacteroidota), accounting for 26.00% and 20.00% of the total sequencing reads respectively. These bacteria are common in the case of severe acid inhibition in AD system. According to Liang et al., (2022), the bacterial community structure undergoes a shift due to the acidic environment created by VFAs during AD, with *Prevotella*, *Rikenellaceae_RC9_gut_group*, *Ruminococcus* and *Succiniclasticum* being the dominant bacteria [31]. Yu et al., (2023) also revealed that the increase in abundance of *Fibrobacterota*, *Rikenellaceae_RC9_gut_group* and *Butyrivibrio* may result in an increase in VFAs concentration during AD [32]. The change in bacterial community structure in the control group can explain the reason for acid inhibition, which is consistent with the previous conclusion that higher VFAs concentrations were detected in the control group. The dominant bacteria in MOF-1 and MOF-2 are *Proteiniphilum* (phylum Bacteroidota), accounting for 27.00% and 17.00% of the total sequencing reads, respectively. *Norank_f_ rikenellaceae*, the dominant bacterium in MOF-3, is an unclassified member with a relative abundance of 53.00%. The genus in the *Rikenellaceae* family is composed of fermentation bacteria that can convert carbohydrates or protein into VFAs [33].

As shown in Fig. 6d, the changes of archaea community during methane
production were analyzed at the genus level. *Methanosarcina*, *Methanoculleus*, *Methanomassiliicoccus* and *Methanobrevibacter* were recognized as the prominent archaeal groups across all four CSTR experiments, although their relative abundance varied. *Methanosarcina* is dominant in the control group, with a relative abundance of 73.70%. This acetotrophic methanogen can synthesize methane via acetate, methanol, and H$_2$/CO$_2$ generated by diverse substrates [34]. Followed by *Methanoculleus* and *Methanomassiliicoccus*, with relative abundance of 13.12% and 3.42%, both of which are key members of hydrogen trophic methanogens. Compared with the control group, the relative abundance of *Methanosarcina* in MOF-1 decreased to 67.84%, while that of *Methanoculleus* increased to 30.90%. This implies that the addition of FeMn-MOF could inhibit the metabolic pathway of acetate decomposition to CH$_4$, and archaea that uses hydrogen to reduce methane began to play an important role in AD. However, the archaea diversity in MOF-1 decreased significantly, and *Methanosarcina* and *Methanoculleus* accounted for 98.74% of the total sequencing reads. This might explain why the methane production of MOF-1 is less than that of other experimental groups.

MOF-2 and MOF-3 exhibit a similar archaeal community composition, which may account for why the cumulative methane production of the two experimental groups is relatively similar. In experimental groups MOF-2 and MOF-3, the relative abundance of *Methanosarcina* is 87.96% and 88.76%, and that of *Methanoculleus* is 7.59% and 5.76%, respectively. *Methanosarcina* can not only crack methane by acetoacetic acid, but also hydrolyze water-soluble methane. According to Zhang et al., (2019), the presence of *Methanosarcina* can effectively prevent the negative consequences caused
by VFAs accumulation and allow effective methane production [35]. Therefore, the above results show that different MOFs materials have different mechanisms to affect the methane production process. In the experimental group MOF-1, the addition of FeMn-MOF will gradually change the metabolic pathway from acetic acid fermentation to hydrogen trophic methane production, but it may also inhibit the growth of *Methanosarcina* and other hydrogen-loving methanogens, resulting in the decline of archaea diversity and weak methane production. In experimental groups MOF-2 and MOF-3, the addition of FeMn/MOF-CFs and FeMn/MOF-CFC will not change the pathway of methane production, but can significantly increase the relative abundance of other methanogenic bacteria, especially *Methanosarcina*. The co-existence of various methane production pathways could be one of the significant contributing factors towards the enhancement of methane production.

4. Conclusion

This experiment investigated the effects of FeMn-MOF, FeMn/MOF-CFs, and FeMn/MOF-CFC on the methane production process in a CSTR reactor. The results indicated that the control group without the addition of MOFs materials suffered significant inhibition, with ammonia nitrogen concentration and VFAs concentration reaching 3432.00 mg/L and 16221.21 mg/L, respectively, and pH value dropping below 6.50. In the experimental group, MOF-3 recorded the highest cumulative methane production of 66.96 L/L-reactor. The ammonia nitrogen concentrations of MOF-1, MOF-2, and MOF-3 were 2366.00 mg/L, 2328.00 mg/L, and 2052.00 mg/L, respectively, which were 31.06%, 32.17%, and 40.21% lower than the control group at
the end of the experiment. This suggests that the addition of three kinds of MOFs materials effectively alleviated the inhibition of ammonia nitrogen and increased the methane production. The potential mechanism was explored by the determination of enzyme relative activity and microbial community characterization. The enzyme activity of MOF-3 in the experimental group was the highest, and the relative enzyme activities of ACP, ALP, α-glucosidase and coenzyme F420 reached 142.62%, 151.97%, 136.04% and 193.68% respectively. The relative abundance of *Metanosarcina* in MOF-1 decreased to 67.84%, while the relative abundance of *Metanoculleus* increased to 30.90% compared to the control group. This discovery suggests that the addition of FeMn-MOF may enhance the hydrogen trophic methane production pathway. The relative abundance of *Methanosarcina* in MOF-2 and MOF-3 increased to 87.96% and 88.76% respectively, compared with the control group. The addition of FeMn/MOF-CFs and FeMn/MOF-CFC will not change the pathway of methane production, but can significantly increase the relative abundance of methanogenic bacteria, thus obtaining higher methane production.

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Constructing carbon-based materials loaded with MOFs to realize
efficient anaerobic digestion of rural organic waste

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