Biogas and volatile fatty acid production during anaerobic digestion of straw, cellulose, and hemicellulose with analysis of microbial communities and functions

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Abstract

Background

Anaerobic digestion (AD) is a promising method for straw treatment, but the complex composition and structure of straw limit AD efficiency and methane production. The main biodegradable components of straw are cellulose and hemicellulose. Because of the different chemical structures and physicochemical properties, the performance of AD of cellulose and hemicellulose is different, thus it's also different from that of straw. Research on the similarities and differences of AD of straw, cellulose and hemicellulose is helpful to clarify the law of anaerobic digestion of straw and provide theoretical basis for further improving the efficiency of anaerobic digestion. However, there are very few studies on AD using cellulose and hemicellulose as raw materials.

Results

Rice straw (RS), cellulose, and hemicellulose were used as raw materials to study biogas production performance and changes in the volatile fatty acids (VFAs). Further, microbial communities and genetic functions were analyzed separately for each material. The biogas production potential of RS, cellulose, and hemicellulose was different, with cumulative biogas production of 620.64, 412.50, and 283.75 mL/g·VS⁻¹, respectively. The methane content of the biogas produced from cellulose and hemicellulose was approximately 10% higher than that produced from RS after the methane content stabilized. Biogas production and the methane content of RS stabilized more quickly than that of cellulose and hemicellulose. The accumulation of VFAs occurred in the early stage of anaerobic digestion in all the three materials, and the main volatile fatty acid component of RS was acetic acid, whereas that of cellulose and hemicellulose was propionic acid. The cumulative amount of VFAs in both cellulose and hemicellulose was relatively higher than that in RS, and the accumulation time was 12 and 14 days longer, respectively. When anaerobic digestion progressed to a stable stage, *Clostridium* was the dominant bacterial genus in all three AD systems, and the abundance of *Ruminofilibacter* was higher during anaerobic digestion of RS. Genetically, AD of all the three materials proceeded mainly via acetoclastic methanogenesis, with similar functional components.

Conclusion

The biogas and VFAs production during AD of RS, cellulose, and hemicellulose showed marked differences. But when the AD progressed to the stable stage, there was no significant difference in microbial community and genetic function. Specifically, the biogas production potential of cellulose and hemicellulose was greater than that of RS. The accumulation of VFAs in the three AD systems occurred in the early stages. The main component of VFA that accumulated in RS was acetic acid, while the major component of VFAs accumulated in cellulose and hemicellulose digestions was propionic acid. At the stable stage, *Clostridium* was the dominant bacterial genus in all three AD systems. The AD of all the

three materials proceeded mainly via acetoclastic methanogenesis, with similar components of gene functions.

Background

Straw is a lignocellulose agricultural waste that is produced in large quantities^[1]. Therefore, it may cause severe environmental pollution and threaten human health and ecosystems if utilized improperly^[2]. However, straw is an excellent source of biomass with significant utilization and development potential. Enabling the utilization of straw as energy is an effective way, not only, to potentially solve the problem of environmental pollution caused by straw, but also to ease the world energy crisis. As anaerobic digestion (AD) can degrade the organic components in biomass under mild conditions and produce a large amount of biogas, with methane as the main component, AD is considered to be one of the most suitable and promising biomass energy utilization technologies^[3].

The main components of lignocellulose materials, such as straw, are cellulose, hemicellulose, and lignin, with a small amount of gum, fructose, protein, and ash^[4]. Typically, the contents of cellulose, hemicellulose, and lignin are 40–50%, 25–30%, and 15–20%, respectively. Cellulose, hemicellulose, and lignin have different chemical structures and physicochemical properties^[5]. Cellulose is a linear compound composed of D-glucopyranose units connected by β -1,4 glycosidic bonds, and there are crystalline and amorphous regions inside the cellulose molecule. It is stable in nature, and hydrogen bonding significantly reduces the accessibility and reactivity of cellulose to degradable cellulase and anaerobic microorganisms^[6]. The other reason that cellulose is resistant to microorganisms is the complex components of crystalline build-up, which are an antidegradation barrier. Hemicellulose is composed of a variety of sugar aldehyde carboxyl groups, sugar groups, and acetyl groups^[7] and is a general term for complex glycans with branched chains in their molecular structure. These branched structures form an amorphous polysaccharide with reduced crystallinity. Hence, hemicellulose is more readily hydrolyzed than cellulose. However, the more complex structure of hemicellulose than cellulose requires multiple enzymes for its hydrolysis. Lignin is an amorphous high-molecular-weight polymer, and its three-dimensional structure comprises C6-C3 units in a nonlinear and random manner through the action of C-H and C-C bonds^[8]. It is also only degraded by microorganisms with great difficulty. Thus, biogas produced during AD of straw is derived from the degradation of cellulose and hemicellulose, rather than lignin.

To enhance the anaerobic degradation efficiency of straw, extensive studies have been dedicated to destabilizing lignin, cellulose, and hemicellulose to increase microbial accessibility to cellulose and hemicellulose^[9]. From the perspective of the physicochemical properties of straw raw materials, many scholars have utilized straw pretreatment technology^[10, 11], parameters of AD^[12, 13], and microbial communities in the AD system^[14, 15]. Although many studies on this topic have produced many fruitful results, there are still challenges, including a low AD efficiency and low methane content in biogas during anaerobic processes. Therefore, further studies on the mechanisms of AD are needed.

A routine study of the primary components of straw is necessary to elucidate the AD patterns of straw. Under conditions of extreme pretreatment, when the accessibility of cellulose and hemicellulose becomes 100%, the principles of their AD are hypothesized to be comparable with that of straw and provide a basis for studying the principles of the AD of straw. Li et al. [16] used cellulose, hemicellulose, and lignin as their mixtures of raw materials to study AD characteristics, and their results showed that hemicellulose is hydrolyzed more easily and acidified more quickly than cellulose. Therefore, the biomethane potential of cellulose was higher than that of hemicellulose. *Clostridium sensu stricto*, *Lutaonella*, *Cloacibacillus*, and *Christensenella* are the microorganism of high abundance in the AD system of cellulose, while *Saccharofermentans*, *Petrimonas*, and *Levilinea* are more abundant in the AD system of hemicellulose. However, there are very few studies on AD using cellulose and hemicellulose as raw materials. Thus, the available information on this is also very limited.

In this study, rice straw (RS), cellulose, and hemicellulose were used as raw materials for biogas production, and volatile fatty acids (VFAs) were investigated under identical digestion conditions. Moreover, the composition of microorganisms was determined, and genetic function analyses were performed to provide some reference for further clarifying the principle of the AD of RS.

Results And Discussion

Biogas production performance

The AD performances of RS, cellulose, and hemicellulose are shown in Figure 1. The daily biogas production of RS and its main components contained two daily gas production peaks during the entire AD process (Figure 1A). The intensity of the first peak was lower than that of the second peak for RS digestion. In contrast, the first gas production peaks from cellulose and hemicellulose digestion were higher than the second. Among them, production peaks of RS digestion occurred on the first and sixth days, and biogas production on these days was 14.19 and 34.78 mL/g·VS⁻¹, respectively. The two biogas production peaks of cellulose appeared on the third and twelfth days, and biogas production on these days was 85.36 and 35.64 mL/g·VS⁻¹, respectively. The first biogas production peak of cellulose was 501.55% higher than that of RS. The second biogas production peak of cellulose was similar to the second gas production peak of RS. The two biogas production peaks of hemicellulose appeared on the fourth and fourteenth days, with production on these days of 45.00 and 30.00 mL/g·VS⁻¹, respectively. The first biogas production peak was 217.12% higher than that of RS digestion, and the second biogas production peak was 15.71% lower. The peaks of biogas production of RS occurred significantly earlier than those of cellulose and hemicellulose. This may be because RS contains fructose and other substances, in addition to cellulose and hemicellulose, which are more anaerobically digestible^[4], leading to the early start of the AD process.

For cumulative biogas production, cellulose had the highest at 620.64 mL/g·VS⁻¹, followed by hemicellulose at 412.50 mL/g·VS⁻¹, and RS at 283.75 mL/g·VS⁻¹ (Figure 2B). The cumulative gas production of cellulose and hemicellulose was 118.72% and 45.37% higher than that of RS, respectively.

These results are consistent with those of Li et al.^[1, 16]. This is because the VS of RS is lower than that of cellulose and hemicellulose, which also contain lignin. Studies have shown that lignin is composed of three main monomers: coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These basic monomers constitute the basic structural unit of lignocellulose: syringyl phenylpropane (S), guaiacyl phenylpropane (G), and p-hydroxyphenylpropane (H)^[20]. Lignin is challenging to biodegrade under anaerobic conditions^[21, 22]. Indeed, in straw, cellulose, hemicellulose, and lignin form structures that are difficult to biodegrade^[8, 9]. In addition, some cellulose and hemicellulose cannot be used by microorganisms in RS, so its cumulative gas production is significantly lower than that of cellulose and hemicellulose. Inhibition mechanisms may also contribute to the difficulty of biodegrading enzymes to adsorb to hydrophobic lignin^[23].

For the methane content during AD, RS first showed a slow increase, reaching a maximum of 72.39% on the fifth day, and then gradually decreased to 61.44% on the eighth day, finally stabilizing at around 60% (Figure 1C). The trends for cellulose and hemicellulose were similar: both showed a slow increase in the early stage of DA and stabilized at about 70% after day 14. Before day 12, the methane content of RS was higher than that of cellulose and hemicellulose. After 12 days, the methane contents of cellulose and hemicellulose were higher than that of RS. After the methane content stabilized, the methane content of the biogas produced from cellulose and hemicellulose were approximately 10% higher than that produced from RS.

The pH level, ammonia nitrogen content, and total alkalinity are important indices that can be used to evaluate the stability of anaerobic digestive systems and microbial metabolism. The pH levels of the digestion sludge of RS, cellulose and hemicellulose were 7.33, 7.29, and 7.25, respectively, and the alkalinity levels were 4500, 4200, and 4100 mg/L, respectively. The values for each of these parameters were within the normal range: pH > 6.8 and alkalinity > 2000 mg/L^[24]. In addition, the ammonia nitrogen contents of RS, cellulose, and hemicellulose were 710, 720, and 735 mg/L, respectively, which did not exceed the tolerance range of anaerobic microorganisms, which is 2 g/L. The methane production process was not suppressed^[25], and the system reached a stable status after AD.

The modified Gompertz model was used to fit the dynamics of AD of RS, cellulose, and hemicellulose to the dynamics of biogas production. The correlation coefficient, R^2 , of the experimental value and the fitted value was above 0.98 (Figure 1D; Table 2). The maximum biogas production of the fitted value was very similar to the cumulative biogas production. The biogas production rates R_m of cellulose and hemicellulose were 266.79% and 144.09% higher than the R_m of RS, respectively, and hemicellulose showed the longest lag time.

Volatile fatty acids

VFAs are important intermediate metabolites in the process of AD and are one of the indicators that characterize the performance of AD^[26]. Changes in VFAs during AD of RS, cellulose, and hemicellulose are shown in Figure 2. The accumulation of VFAs occurred in the early stages of AD of all three raw

materials (Figure 2A). As AD proceeded, the accumulated VFAs were consumed. The accumulation stages of VFAs during AD of RS, cellulose, and hemicellulose were 4, 16, and 18 days, respectively. The concentration of VFAs from RS digestion was highest on day one (740.12 mg/L), while VFA concentration from cellulose reached its maximum on day seven at 2414.29 mg/L and was 3.26 times the highest concentration of RS. The maximum concentration of VFAs from hemicellulose was reached on day nine, which was 2234.58 mg/L and was 3.01 times the highest concentration of RS. The VFA concentration of RS stabilized below 50 mg/L from day five, and cellulose and hemicellulose stabilized below 50 mg/L from days 16 and 18, respectively. According to Lee et al.^[27], VFA concentrations above 6000 mg/L suppress AD. As VFA concentrations in this study did not exceed 2500 mg/L, expectations were met in that no suppressive effects were seen. It has been reported that xylan in lignocellulose promotes the swelling of cellulose, thereby increasing its enzymatic hydrolysis^[28], which may be the reason why RS enters a hydrolytic acidification condition earlier than cellulose and hemicellulose.

Studies have shown that acetic acid is the main carbon source for the AD of methane production, and other VFAs, including propionic acid, are also mostly converted into acetic acid to be inserted into methane metabolism by microorganisms^[29]. The composition of VFAs is closely related to the performance of AD^[30]. For the composition of VFAs produced during AD of the three raw materials (Figure 2B, C, D), the main component of the cumulative stage from day one to day four of RS was acetic acid (88.38%–91.88%). For cellulose, the acetic acid content was highest on day one (79.08%), and the main component for days 2–16 was propionic at 65.70%–98.42%. Similar results were observed for hemicellulose for days 1–18). Acetic acid (52.60%) dominated the first day, and propionic acid was the main component for days 2–18 at 81.57%–97.19%. Studies have shown that VFAs with even- and odd-numbered carbon are beneficial for the synthesis of 3-hydroxybutyrate and 3-hydroxyvalerate^[31], respectively, while 3-hydroxybutyrate is more beneficial for the methanation process. The accumulation of substantial amounts of propionic acid could inhibit the AD process^[31]. Studies by Pullammanappallil et al.^[32] showed that the maximum accumulation of propionic acid was 2750 mg/L, which did not inhibit AD. In this study, the maximum cumulative concentrations of propionic acid of cellulose and hemicellulose were 2149.01 and 2148.60 mg/L, respectively, neither of which exceeded 2750 mg/L. Inhibition of digestion is thus not likely. When the AD progressed into the stable biogas production stage, and the methane content remained stable, the accumulated propionic acid was quickly consumed.

In this study, the concentration of VFA produced from the AD of RS reached a maximum value before cellulose and hemicellulose, but its maximum concentration was lower than that of the other two components. This finding is consistent with the law of biogas production that discussed before. Similar to VFAs, the biogas yield of RS was lower than that of cellulose and hemicellulose. The main component of VFAs that accumulated during the AD of RS was acetic acid, which was quickly consumed. The major component of VFAs that accumulated during the AD of cellulose and hemicellulose was propionic acid, which accumulated over an extended period. This finding is consistent with the law of methane content during the AD of these three materials, and it shows that the production of VFAs had a dual effect on the efficiency and biogas production performance of AD. On the one hand, VFAs are substrates for

methanogenesis. Within a certain concentration range, the accumulation of VFAs is positively correlated with biogas production. On the other hand, the excessive accumulation of VFAs will also lead to the inhibition of AD.

Microbial community

After splicing all the sequences, quality control, and filtering out the low-quality sequences, a total of 175,423 bacterial sequences and 132,506 archaea sequences were obtained. The obtained bacterial and archaea sequences were clustered using the UPARSE algorithm, and a total of 534 bacterial OTUs and 21 archaea OTUs were obtained. These 34 bacterial OTUs belong to 31 different phyla, 60 different classes, 94 different orders, 142 different families, and 243 different genera. The 21 archaea OTUs obtained belonged to four different phyla, six different classes, seven different orders, 11 different families, and 15 different genera.

The composition of microbial communities at the genus level is shown in Figure 3. In the bacterial Venn diagram (Figure 3A), the data show that most of the bacteria in the three raw materials were the same, with 181 genera accounting for 74.49% of the total bacterial genera. The number of bacterial genera unique to the AD systems of RS, cellulose, and hemicellulose were 13, 9, 6, respectively. In the archaeal Venn diagram (Figure 3B), the data show that the archaeal genus in the AD systems of the three raw materials was the same.

As shown in the ternary phase diagram of the bacteria (Figure 3C), *Clostridium* is the dominant genus in the AD systems of RS, cellulose, and hemicellulose, with relative abundances of 38.44%, 43.14%, and 40.62%, respectively. The second most abundant genus was *Terrisporobacter* with relative abundances of 12.29%, 12.42%, and 11.65% for the AD systems of RS, cellulose, and hemicellulose, respectively. In addition, the abundances of *Sedimentibacter* and *Romboutsia* in the AD systems of the three raw materials are also very similar. *Ruminofilibacter* showed a higher abundance in RS AD and a lower abundance in cellulose and hemicellulose ADs. *Bacteroidetes* vadinHA17 and vadinBC27 wastewater sludge groups were present in the cellulose and hemicellulose AD systems in higher abundances than in the RS system. The abundance of *Bacteroidetes* vadinHA17 in the cellulose AD system was higher than in the hemicellulose AD system, and the abundance of the vadinBC27 wastewater sludge group in the hemicellulose AD system was significantly higher than in the cellulose AD system.

In the process of anaerobic acidification, carbohydrates are hydrolyzed into ethanol, lactic acid, acetic acid, and butyric acid by monosaccharide fermentation acid-producing bacteria, and lactic acid is further reduced to propionic acid. At the same time, VFAs are mutually converted by redox reactions under the action of different microorganisms. *Clostridium* plays an important role in degrading organic components and generating VFAs^[33]. For example, Merlino et al.^[34] found that *Clostridium* can degrade wood fibers and vegetarian raw materials. *Terrisporobacter* is a strictly anaerobic bacterium that can degrade carbohydrate raw materials into acetic acid^[35]. In this study, the abundances of *Clostridium* and *Terrisporobacter* were relatively high, which provided a suitable environment for the smooth progress of

the hydrolysis and acidification process in the AD system. It has been reported that the vadin BC27 wastewater sludge group produced small molecular fatty acids from fermentable amino acids, cysteine, leucine, methionine, serine, tryptophan, and valine^[36], and was well adapted to ammonia nitrogen concentrations^[37].

We found that the substrates available for methane production in our study were very limited. This is consistent with *Methanosaeta*, which can only use acetic acid as a substrate for AD ^[38] to produce CH₄. Whereas, *Methanobacterium* produces methane through CO₂ reduction with hydrogen^[39], and *Methanomassiliicoccus* produces methane by reducing methanol^[40]. From the ternary phase diagram of the archaea (Figure 3D), it is evident that *Methanosaeta* is the dominant bacterium in the AD systems of the three raw materials, with similar abundances of 81.66%, 79.24%, and 70.22% for the AD systems of RS, cellulose, and hemicellulose, respectively. *Methanobacterium* abundances were 5.39%, 4.23%, and 3.96%, and *Methanomassiliicoccus* abundances were 1.30%, 1.52%, and 1.74% for the AD systems of RS, cellulose, and hemicellulose, respectively, indicating that the AD of the three raw materials was based primarily on the reduction of acetic acid.

Functional Analysis

A total of 35.66 GB of data was obtained via high-throughput sequencing. Based on the original sequencing data, the low-quality and N-reads in the data were eliminated, and 331,859,324 high-quality sequences, required for subsequent analysis were obtained. 4,533,413 gene sequences were predicted using ORF prediction. When our results were compared with the KEGG database to obtain the functional annotations of each sample. The principles of the three raw materials at levels one, two, and three were found to be similar. At level one, metabolism was the most abundant pathway with relative abundances of 68.46%, 68.62%, and 68.56% for the AD systems of RS, cellulose, and hemicellulose, respectively. At level two, carbohydrate metabolism was the most abundant pathway, with relative abundances of 15.42%, 15.35%, and 15.29% for the AD systems of RS, cellulose, and hemicellulose, respectively. At level three, the most abundant metabolic pathway was biosynthesis of amino acids with relative abundances of 5.22%, 5.15%, and 5.15% for the AD systems of RS, cellulose, and hemicellulose, respectively, followed by carbon metabolism, with relative abundances of 5.04%, 5.11%, and 5.03 for the AD systems of RS, cellulose, and hemicellulose, respectively. The relative abundances of methane metabolism were 2.26%, 2.41%, and 2.29% for the AD systems of RS, cellulose, and hemicellulose, respectively. This is consistent with the findings of Li et al.^[41] and the chemical composition of the raw materials.

At the module level, a total of four modules were related to methane production: M00357, M00567, M00356, and M00563. Of these, M00357 uses acetate as a substrate, M00567 uses H₂-CO₂, M00356 uses methanol, and M00563 is based on methylamine-dimethylamine-trimethylamine. The abundances of these four modules are shown in Table 3. M00357 accounted for the highest proportion of modules, with relative abundances of 47.10%, 47.62%, and 47.15% for the AD systems of RS, cellulose, and hemicellulose, respectively, followed by M00567 with relative abundances of 28.40%, 28.65%, and 28.98%

for the AD systems of RS, cellulose, and hemicellulose, respectively (Table 3). The next was M00356, whose relative abundances were 23.07%, 22.51%, and 22.65% for the AD systems of RS, cellulose, and hemicellulose, respectively, and M00563 had the lowest relative abundances, which were 1.43%, 1.22%, and 1.21% for the AD systems of RS, cellulose, and hemicellulose, respectively. These results indicate that the methanogenic process in this study was acetoclastic methanogenesis, which is also consistent with the analysis results of microbial communities.

The functional composition at the enzyme level is shown in Figure 4A; it was compared with methane metabolism metabolic pathway map00680 to depict dominant pathways in this study (Figure 4B). The results in Figure 4 show that the most abundant pathway in this study is that acetic acid first produces acetyl-CoA, then undergoes a series of reactions to produce 5-methyl-5,6,7,8-tetrahydromethane, then biosynthesizes methyl-CoM, and finally produces methane.

In the methanogenesis pathway, the methyl coenzymes methyl-CoM and acetyl-CoA were important intermediate products. Five pathways can produce methyl-CoM. In this study, 5-Methyl-THM (S) PT produced the highest abundance of methyl-CoM through the action of enzyme EC 2.1.1.86, with relative abundances of 83.19%, 86.22%, and 86.14% for the AD systems of RS, cellulose, and hemicellulose, respectively.

Fisher's exact test analysis in the STAMP difference analysis was used to compare the abundance of functions between two samples. Through this analysis, significant difference functions could be obtained. The gene set was constructed and annotated with methane metabolism and acetyl-CoA-related genes, and the abundance was calculated using the RPKM method. At the module level, the function of microorganisms in the AD systems of RS, cellulose, and hemicellulose were analyzed using STEMP. As shown in Figure 5, there is a significant difference between RS and cellulose in the acetyl-CoA related genes (Figure 5A). The results indicate that there are two deviations exceeding 1: the highest deviation is M00036, followed by M00088, with the deviations are +3.55 and -3.30, respectively. The difference between RS and hemicellulose in the acetyl-CoA-related genes (Figure 5B) was the same as that for cellulose. The two modules with the highest deviations were +3.33 and -2.76, respectively. The function of M00036 is to convert leucine to acetoacetate and acetyl-CoA, and the function of M00088 is to convert acetyl-CoA to acetoacetate, 3-hydroxybutyrate, or acetone. In the significant difference analysis of RS and cellulose in methane metabolism (Figure 5C), it was evident that the highest deviation was M00001, which is +0.50. Its function is to convert glucose to pyruvate from straw to hemicellulose. The significant difference analysis of RS and hemicellulose in methane metabolism (Figure 5D) showed that one of the absolute values of deviation was greater than 1 – M00001, the same as cellulose, with a deviation of +1.07. The results show that the AD process of RS, cellulose, and hemicellulose had little difference in the function of microorganisms during the methanogenesis stage, while the functions of microorganisms related to acetyl-CoA were quite different.

Conclusions

The biogas and VFAs production during AD of RS, cellulose, and hemicellulose showed marked differences. The biogas production potential of cellulose and hemicellulose was greater than that of RS. The accumulation of VFAs in the three AD systems occurred in the early stages. The main component of VFA that accumulated in RS was acetic acid, which was quickly consumed after a short period of accumulation, while the major component of VFAs accumulated in cellulose and hemicellulose digestions was propionic acid, which had a higher cumulative amount and a substantially longer period of production than in the AD of RS. When the AD progressed to the stable stage, there was no significant difference in microbial community and genetic function. Specifically, *Clostridium* was the dominant bacterial genus in all three AD systems. *Ruminofilibacter* had a higher abundance in the AD system of RS than in the other two AD systems. From the perspective of genetic function, the AD of all three raw materials proceeded mainly via aceticlastic methanogenesis, with similar components of gene functions.

Methods

Feedstock and Inoculum

Experimental RS was obtained from Jixian County, Tianjin. After being naturally air-dried, the whole straw was cut into small pieces of 3–4 cm using a trowel, and these pieces were pulverized with a YSW-180 type grinder and passed through a 20-mm mesh sieve. The materials were then placed in a dry and ventilated area, ready for use. Cellulose and hemicellulose were both purchased from Beijing Jianqiang Weiye Technology Co., Ltd. They were derived from RS and had a purity of 99% and 97%, respectively. The digestion sludge of pig manure, which was taken from Shunyi District, Beijing, was used as the inoculum in the experiment. The physicochemical properties of raw materials and inoculum are provided in Table 1.

Experimental equipment and methods

The RS, cellulose, and hemicellulose were separately loaded into a 5 L reaction device, and then the inoculum was added. The organic load, which was based on total solids (TS), of raw materials was 50 g/L, and the organic load of inoculum was 20 g/L. Pure water was added to the organic load to adjust the effective volume of the AD reactor to 4 L, and the initial pH in the reaction device was adjusted to 6.8–7.2 by adding $\text{Ca}(\text{OH})_2$. The AD reaction device was placed in a $35^\circ\text{C} \pm 1^\circ\text{C}$ constant-temperature water bath to enable mesophilic AD. The AD time was set to 40 days, and the quantity of biogas produced, and its composition was measured every day. In addition, the VFA concentration of the sludge in the reaction system was measured daily. Each group of experiments was designed with three parallel experiments, and the data are the average of these three parallel experiments.

Analysis of biogas production performance

The water displacement method was used to record daily biogas production^[17]. A SP-2100 gas chromatograph (BeiFenRuiLi, Beijing, China), which was furnished with a TDX-01 molecular sieve and a thermal conductivity detector (TCD), was used to determine the CH_4 , CO_2 , H_2 , and N_2 composition of the

biogas, and argon was used as the carrier gas. The temperatures of the oven, injector port, and TCD were 140°C, 150°C, and 150°C, respectively.

Chemical composition analysis

TS and volatile solids (VSs) were measured using APHA standard methods^[18]. The total carbon (TC) and total nitrogen (TN) were determined using a Vario EL/micro cube elemental analyzer (Elementar, Germany). The composition of cellulose, hemicelluloses, and lignin in the RS and digestate were measured using an A2000I fiber analyzer (ANKOM, USA). Samples were taken from the sampling port of the anaerobic digester every day to measure VFAs. A GC-2014 gas chromatograph (Shimadzu, Japan) was used to analyze the VFAs, and a flame ionization detector (FID) was equipped in the gas chromatograph. The gas chromatograph column was a capillary column of 30 m × 0.25 mm × 0.25 μm (Agilent, DB-WAX), and the carrier gas that was used was nitrogen. The operational temperatures of the injector, detector, and column were 250°C, 250°C, and 180°C, respectively.

Kinetic analysis

The modified Gompertz model was used in this study to evaluate AD performance. It is the most widely used model for the analysis of AD processes of complex organic matter^[19].

The modified Gompertz model formula is shown in equation (1):

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

Where $P_{(t)}$ is the degree of hydrolysis/gasification of the material on day t (%), P_m is the maximum degree of hydrolysis/gasification (%), R_m represents the maximum hydrolysis/gasification rate (%), λ is the delay time (d), t is the digestion time (d), and e is the natural constant, 2.71828.

Microbial community analysis

Total DNA extraction was performed using an EZNA Soil DNA Kit (Omega Bio-tek, Norcross, GA, US), DNA concentration and purity were detected using NanoDrop2000, and the quality of DNA extraction was detected using 1% agarose gel electrophoresis, using two primers for polymerase chain reaction (PCR) amplification of the V3-V4 variable region: 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3').

Purified amplified fragments were constructed into a PE2 × 300 library using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) and following standard operating procedures. Sequencing was then performed using the Illumina MiSeq PE300 platform (Shanghai Meiji Biomedical Technology Co., Ltd.).

The original sequences were determined using Trimmomatic software for quality control and FLASH software for splicing. UPARSE software (version 7.1 <http://drive5.com/uparse/>) was then used, and the

sequences were clustered based on 97% similarity of operational taxonomic units (OTUs). Single sequences and chimeras were removed. The ribosomal database processor classifier (<http://rdp.cme.msu.edu/>) was used to annotate the species classification of each sequence, and these were compared with data in the Silva database (SSU132), using an alignment threshold of 70%.

MetaGene analysis

Sample DNA was extracted using the EZNA Soil DNA Kit (Omega Bio-tek, USA). After genomic DNA extraction was completed, DNA concentration was measured using TBS-380, DNA purity was measured using NanoDrop2000, and DNA integrity was detected using 1% agarose gel electrophoresis. The DNA was fragmented using Covaris M220 (Gene Corporation, China), and a fragment of about 400 bp was interrupted by screening. A PE library was constructed using a NEXTFLEX Rapid DNA-Seq (BioScientific, USA) library construction kit. Metabonomic sequencing was then performed after bridge PCR amplification using the Illumina NovaSeq/Hiseq Xten (Illumina, USA) sequencing platform.

The Fastp software was used to perform quality control on the original data, and BWA software was used to align the reads to the host's DNA sequence and remove contaminated reads with high similarity. The stitching software MEGAHIT, which is based on the principle of succinct de Bruijn graphs, was used to stitch and assemble optimized sequences. Contigs ≥ 300 bp were selected as the final assembly result in the splicing results. MetaGene was used to perform open reading frame (ORF) prediction on the assembled contig, and then CD-HIT software was used to cluster the predicted gene sequences of all samples to construct a non-redundant gene set. Finally, using SOAPaligner software, the high-quality reads of each sample were compared with non-redundant gene sets (95% identity), and the gene abundance information in the corresponding samples was counted.

BLASTP was used to align non-redundant gene set sequences with the Kyoto encyclopedia of genes and genomes (KEGG) gene database (GENES). KEGG orthology-based annotation system (KOBAS) 2.0 was used for function annotation based on the results of the comparison. The sum of gene abundances corresponding to KO, Pathway, EC, and Module was used to calculate the abundance of corresponding functional categories.

Data analysis

Statistics and data processing were performed using Microsoft Excel software. Origin 9.0 software was used for dot-line, histogram, and nonlinear fitting. The composition of microbial communities and their genetic functions were analyzed using the ggplot2 package of R language for Windows 3.5. 1. Fisher's exact test was performed using the Statistical Analysis of Metagenomic Profiles (STAMP).

Abbreviations

AD

Anaerobic digestion

RS

Rice straw

TS

Total solids

VS

Volatile solid

TC

Total carbon

TN

Total nitrogen

VFAs

Volatile fatty acids

TCD

Thermal conductivity detector

FID

Flame ionization detector

STAMP

Statistical Analysis of Metagenomic Profiles

KEGG

Kyoto Encyclopedia of Genes and Genomes

OTUs

Operational taxonomic units

Declarations

Author's contributions

Xiaoyu Zuo initiated the project and responsible for the coordination of the whole study and the funding. Jie Liu completed analyzing of the experiment data and the writing of the manuscript, and assisted in completing the experiment. Ke Peng and Rui He completed most of the experiments.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data of biogas and VFAs generated or analysed during this study are included in this manuscript. The raw reads of 16s were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRR12131890). The raw reads of metagenome were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRPSRR12132972).

Consent for publication

Not applicable

Ethics approval and consent to participate

Not applicable

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Tables

Table 1 The physicochemical characteristics of raw material and inoculum.

Indexes	Rice straw	Cellulose	Hemicellulose	Inoculum
Total Solid (TS) ^a (%)	93.70	98.86	96.02	11.59
Volatile Solid (VS) ^a (%)	80.82	96.46	96.02	7.61
Total Carbon (TC) ^b (%)	38.14	44.44	42.75	35.15
Total Nitrogen (TN) ^b (%)	0.51	0.00	0.00	2.5
C/N	74.78	-	-	14.06
Cellulose ^b %	40.43	99.00	0.00	ND
Hemicellulose ^b %	29.27	0.00	97.00	ND
Lignin ^b %	5.17	0.00	0.00	ND

^a Content of fresh matter. ^b Content of dry matter.

Table 2. The parameters estimated by the modified Gompertz model.

Substrate	parameter			
	P_m/g	R_m/g	λ/d	R^2
Rice straw	282.03	37.67	0.15	0.98711
Celloluse	621.42	138.17	-0.12	0.99311
Hemicellulose	414.60	91.95	2.86	0.99384

Table 3 Module abundance related to methane generation

Module	RS	CL	HC	Total
M00357	172436	161772	180200	514408
M00567	103960	97314	110754	312028
M00356	84442	76450	86572	247464
M00563	5238	4148	4628	14014

Figures

Figure 1

The biogas-producing performance of the anaerobic digestion.

Figure 2

The changes of daily VFAs concentration during the anaerobic digestion.

Figure 3

Venn analyze and Community structures of bacterial and archaea at genus level.

Figure 4

Composition of functions at enzyme level and the abundance pathway.

Figure 5

Fisher's exact test on Module level

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