Optimization of the methane production in batch anaerobic digestion of maize straw by adjustment of total solid and substrate-to-inoculum ratio based on kinetics

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Abstract: Anaerobic digestion (AD) operating under conditions of organic overload stress typically exacerbates the potential for process instability, thereby resulting in significant economic and ecological ramifications. In this investigation, an augmented substrate-to-inoculum ratio (S/I) along with varying total solid content (TS) levels was employed to replicate diverse organic loadings, utilizing maize straw and cattle manure. The findings reveal that a moderate augmentation in S/I and TS proves advantageous in augmenting methane yield, while an excessive substrate loading diminishes methane yield, hampers the kinetics of methane production, and even induces severe process instability. Kinetic study also displayed the variation of the model parameters for the first-order model, the modified Gompertze model, and the transfer function model. Both the modified Gompertze model and transfer function model exhibited the same environmental stress trend. Thus, both the increase in particulate content and the increase in S/I had a substantial effect on the substrate conversion rate to methane. Microbial analysis demonstrates the dominant influence of Firmicutes and Methanosarcina under different organic loading stresses. From both a kinetic and a microbiological point of view, this work provides novel insights into the fundamental processes that regulate anaerobic digestion (AD) under varying loading stress. Furthermore, it has significant implications for improving the operating efficiency of AD, which is a significant benefit.

Keywords: maize straw, dry anaerobic digestion, methane production, microbial characteristics, kinetic model
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1 Introduction

China is constructing a "1+N" policy system consisting of "carbon peaking and carbon neutrality"[1]. Not only can the development of renewable energy reduce environmental pollution, but it can also satisfy the most important strategic requirements of national energy security. The development of innovative renewable energy technologies is essential for China to achieve its carbon-neutral objectives. The portion of material and energy losses produced during the utilization of resources in agricultural production is referred to as agricultural waste. Without adequate treatment, it could have detrimental impacts on the ecosystem[2]. It is estimated that 495 million t of agricultural straw produced annually in China can be used to produce methane, with a methane production potential of approximately 8225 million m^3/a, which is equivalent to 29.20% of China's natural gas consumption in 2018 and 2.25% of its total energy consumption in 2019[3]. Due to its widespread source and high utilization rate, maize straw is one of the most extensively used agricultural wastes. As a result, finding high-value uses for maize straw has recently gained attention[4]. Anaerobic digestion is regarded as a key method for the high-value usage of maize straw because it has the qualities of resource recovery, reduction, and safety[5] which is of great significance to China's "3060 double-carbon" goal[6]. Compared to wet anaerobic digestion (fixation rate 10%), dry anaerobic digestion (fixation rate>15%) has the characteristics of high organic load capacity, low biogas slurry yield, high biogas yield, less energy consumption, simple operation, etc.[7], meanwhile, by-products from anaerobic digestion such as biogas slurry can also be recycled in other fields[8-10]. Therefore, dry anaerobic digestion of maize straw has attracted the attention of numerous researchers.

During anaerobic digestion, the inoculum supplies the system with the initial microbiome, which will later be involved in the constituent organic matter degradation process. In addition, the inoculum contains a number of macronutrients[11]. The efficacy of dry anaerobic digestion is heavily influenced by total solid content (TS) and substrate-to-inoculum ratio (S/I). The TS will directly influence the effect of gas-liquid mass transfer and the accumulation of organic acids in the system, which will in turn influence the effect of gas production[12]. In the context of batch anaerobic digestion, the term "S/I" denotes the ratio between the digestion

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matrix and the inoculum. A lower inoculation ratio can lead to complete contact between the digestion matrix and microorganisms, resulting in local acidification. On the other hand, a higher inoculation ratio is beneficial for reducing the starting cycle and enhancing the matrix conversion rate. However, an excessive inoculation ratio reduces the rate of gas production[13].

At present, due to the differences in reactor types, operating conditions, and matrix characteristics, there is no generally agreed optimal condition for the fixation content and inoculation ratio of dry anaerobic digestion. Abbassi-Guendouz et al.[14] studied the effect of TS (10%-35%) on anaerobic digestion, and showed that when the TS is 10% to 20%, the maximum methane yield gradually increases with the TS; when the TS is 20% to 35%, the maximum methane yield gradually decreases with the increase of TS. Gonzalez-Fernandez et al.[15] investigated the effect of different inoculation conditions on anaerobic digestion. The result of research proved that when the ratio of pig manure and inoculation sludge was 1:1, the experimental group of volatile fatty acids (VFA) could be degraded more rapidly. Using a higher proportion may be due to the accumulation of VFA reactor imbalance, resulting in the "acid inhibition" phenomenon. Therefore, determining the effect of TS and inoculation ratio on the anaerobic digestion system during the dry anaerobic digestion of maize straw necessitates the development of a practical analysis method.

In light of this, this study utilized maize straw as the substrate for anaerobic digestion and cattle manure as the inoculum, investigated the effect of TS and S/I on the gas production performance of maize straw dry anaerobic digestion under thermophilic conditions, and elucidated the pertinent kinetic mechanism. This study aims to: 1) investigate the methane production performance of dry anaerobic digestion under varying TS and inoculation ratio conditions; 2) analyze the effect of TS and inoculation ratio on maize straw dry anaerobic digestion using kinetic models; 3) reveal the mechanism of TS and inoculation ratio using microbial community and diversity analysis.

2 Materials and methods

2.1 Substrate and inoculum

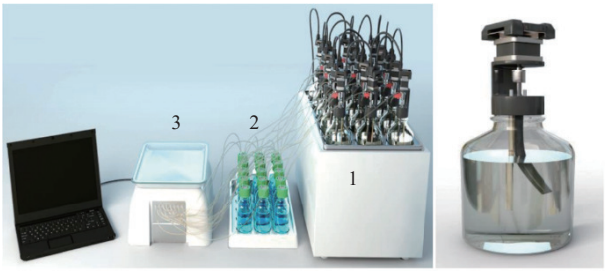
The maize straw was taken from Fanshan Town, Zhangjiakou City, Hebei Province. The resulting raw material was reduced to 1-2 cm, crushed, and frozen at −20°C for subsequent experiments. Cattle manure was collected from Kaiping Cattle Farm in Guangzhou. The original cattle manure was cleaned by hand to remove debris such as stone sand and straw, combined evenly, and separated into solid and liquid through a sieve with a 22 mm grid before being frozen at −20°C for future experiments. Inoculum was obtained from the laboratory's continuously stirred tank reactor (CSTR). The operating temperature of the CSTR was set to (50±1)°C, the current organic loading rate (OLR) was 3 g VS (volatile solid)/(m^3·d), and the hydraulic retention time (HRT) was 20 d During the operation of three consecutive HRTs, fluctuations in daily methane production, methane content, and organic matter removal rate did not exceed 10%. Before inoculation, a certain amount of biogas slurry was removed and placed in the water bath under the same temperature operation conditions for pre-incubation, so as to remove the residual organic matter in the inoculum, until the residual organic matter could be completely consumed when the gas production in the inoculum was almost 0. The inoculum with a high concentration of solids was centrifuged and used as the inoculum for subsequent experiments. Table 1 displays the physical and chemical characteristics of maize straw, manure, and inoculum.

Table 1 Physical and chemical characteristics of maize straw, cow manure and inoculum

Physical and chemical characteristics	Maize straw	Cattle manure	Inoculum
TS/%	17.40±0.25	31.00±0.30	11.03±0.34
VS/%	14.40±0.25	28.21±0.31	10.68±0.20
VS/TS/%	82.76±0.37	90.32±0.35	96.88±0.32

2.2 Experiment design

The device used for batch-type dry anaerobic digestion experiments utilized Bioprocess Automated Methane Potential Measuring System (MultiTalent 203, Nova Skantek Instruments, China), as depicted in Figure 1.



1. Anaerobic digestion unit 2. CO_2 adsorption unit 3. Test unit.

Figure 1 Batch type dry anaerobic digestion experimental device

In serum flasks with a total volume of 1.0 L and an effective volume of 0.8 L, batch experiments were conducted. The maize straw to cattle manure ratio was maintained at 1:1 (in VS). After adding all of the substrates, including maize straw, manure, and inoculum, deionized water was added to reach the effective volume of the above reactors, and the initial pH of the AD reactors was adjusted to 7.2 with 1 mol/L HCl and NaOH. The reactors were then sealed with rubber plugs and charged with nitrogen for 5 min to produce a strictly anaerobic environment. Each assembled reactor was then deposited in a water bath with a predetermined temperature of (50±1)°C. The experiment was timed after the internal temperature of the reactor attained the corresponding water bath temperature, and the gas produced by thermal expansion was expelled using a syringe. When the daily gas production was less than 1% of the accumulated gas production, the experiment was terminated. Using mixed substrate and inoculated sludge as the base variables, two levels of S/I=25 and 30 were introduced into the anaerobic reactor, and three sets of TS levels of 10, 15, and 20 were set at the same level for each. Based on the aforementioned recommendations labeled R1, R2, R3, R4, R5, and R6. For each experimental condition, three parallels were established (Table 2).

Table 2 Specific experimental design of batch tests

Reactor	S/I	TS/%	Maize straw/g	Cattle manure/g	Inoculum/g
R1	25	10	225.65	115.89	38.25
R2	25	15	338.48	173.83	57.37
R3	25	20	451.31	231.77	76.49
R4	30	10	227.88	117.49	32.28
R5	30	15	341.82	176.24	48.42
R6	30	20	455.76	234.98	64.56

Note: S/I is substrate-to-inoculum ratio; TS is total solid content.

2.3 Characterization methods

The biogas produced by the AD system was predominantly composed of CH_4 and CO_2, along with trace quantities of N_2, H_2, NH_3 and H_2S. During this experiment, biogas was collected using gas canisters and then measured by volume using a syringe.

According to Equation (1), the volumes were converted to standard conditions based on the current operating conditions.

$$V_{STP} = \frac{V_T \times 273.15 \times (760 - P_w)}{(273.15 + T) \times 760} \quad (1)$$

where, V_{STP} is the volume of gas at standard temperature (273.15 K) and pressure (760 mm Hg), mL; V_T is the volume of biogas derived from the test at the current operating temperature, mL; P_w is the actual gas pressure, mm Hg; T is the temperature of the laboratory ambient space, °C.

The biogas components (CH_4, CO_2, H_2) were determined by gas chromatography (7890A, Agilent, USA) equipped with a thermal conductivity detector. The detailed setup information of the equipment was as follows. The GC column was a GS-Gas Pro capillary column (Agilent, 113-4362, −80°C-260/300°C, 60 m ×0.320 mm, USA). The initial temperature was 60°C and was maintained at this temperature for 2.5 min, followed by an increase in temperature at a rate of 15°C/min up to 180°C and then maintained for 5 min.

The VS removal rate (VS reduction, VS_r) for the experiments of the batch formula is shown in Equation (2).

$$VS_r = \frac{(F + I)a - Ib}{F} \times 100\% \quad (2)$$

where, F is the VS content of the substrate, g; I is the VS content of the inoculated sludge, g; a is the VS removal rate before and after digestion with, %; b is the VS removal rate before and after digestion for the blank control, %.

Bacteria and archaea were identified by high-throughput sequencing on an Illumina MiSeq platform (MiSeq 3000, Illumina, USA). DNA extraction and PCR amplification (16S rDNA, V3-V4 region) were performed as previously described. Sample data were homogenized and analyzed using the Majorbio I-Sanger Cloud Platform (www.i-sanger.com).

2.4 Kinetics Analysis

Analysis of the kinetics of the AD system provides a comprehensive and in-depth understanding of the effects of various factors on substrate degradation. In view of this, the first-order model, modified Gompertze model and transfer model are further used to describe the gas production dynamics of batch experiment to describe the influence of differences in experimental conditions on gas production performance.

The first-order model can be used to describe the relationship between methane generation rate and substrate concentration. The advantage of this model lies in its simplicity and intuitiveness, as it can quickly provide basic trends in methane production. The equation is as follows:

$$M_{(t)} = M_{Max}(1 - e^{-kt}) \quad (3)$$

where, $M_{(t)}$ is the methane yield at time t, mL/g VS; M_{max} is the final methane yield, mL/g VS; e is the natural logarithm, take 2.718 28; k is the hydrolysis rate constant, d^{-1}; t is the digestion time, d.

The modified geometric model is based on the first-order model and takes into account the feedback mechanism of substrate consumption and product generation during anaerobic fermentation. The advantage of this model lies in its consideration of more biological processes, such as substrate inhibitory effects and product feedback stimuli. This enables the modified geometric model to more accurately describe the dynamic changes of substrates and products during anaerobic fermentation processes. The equation is as follows:

$$M_{(t)} = M_{max} \exp\left\{-\exp\left[\frac{R_{max} e}{M_{max}}(\lambda - t) + 1\right]\right\} \quad (4)$$

where, $M_{(t)}$ is the methane yield at time t, mL/g VS; M_{max} is the final methane yield, mL/g VS; R_{max} is the maximum rate of methane production, mL/(g VS·d); e is the natural logarithm, namely 2.718 28; t is the digestion time, d; λ is the stagnation period, d.

The transfer function model is a model based on differential equations that can describe time-dependent processes. In anaerobic fermentation of methane production, transfer function models can be used to describe the dynamic relationships between variables such as substrate concentration, product concentration, and microbial activity. The advantage of this model is that it can consider more biological processes and interactions, such as substrate consumption, product generation, microbial growth and death, etc. The transfer function model is as follows:

$$M_{(t)} = M_{max}\left\{1 - \exp\left[\frac{R_{max}}{M_{max}}(t - \lambda)\right]\right\} \quad (5)$$

where, $M_{(t)}$ is the methane yield at time t, mL/g VS; M_{max} is the final methane yield, mL/g VS; R_{max} is the maximum rate of methane production, mL/(g VS·d); t is the digestion time, d; λ is the stagnation period, d.

2.5 Analysis and statistics

The mean and standard deviation of all tested indexes were calculated using Excel; One-way ANOVA was used to assess the variability between the physical and chemical data of different batch reactors, and the p-value was considered statistically different when it was less than 0.05.

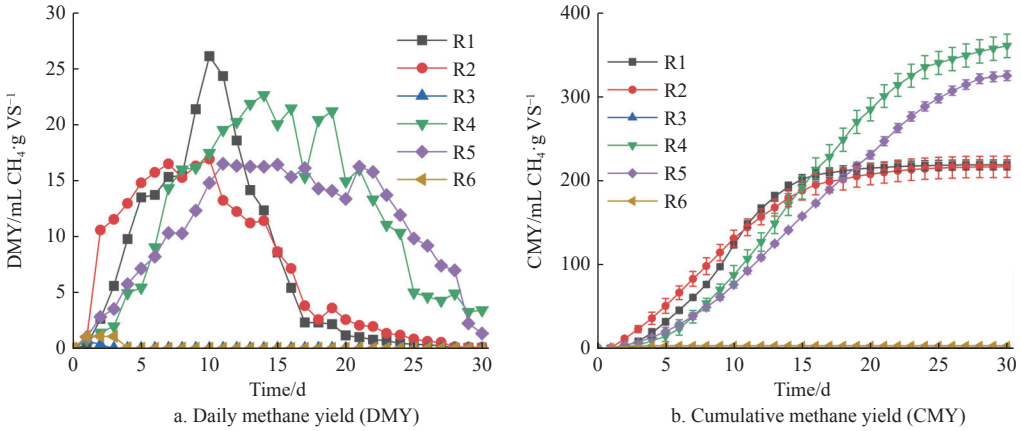
3 Results and discussion

3.1 Methane production performance of AD

Figure 2 depicts the variation of daily methane yield (DMY) with digestion duration for the batch system. It was apparent that the overall trajectory of DMY for the mixture of R1, R2, R4, and R5 feedstocks was roughly the same. On the second to third day of digestion, anaerobic microorganisms degraded readily available components of the feedstock, such as soluble monosaccharides, oligosaccharides, and proteins, resulting in the production of the peak gas[16]. Further analysis of the biogas composition at this time revealed that the overall percentage of CH_4 was between 10%-15%, whereas the overall percentage of CO_2 was greater than 50%. This phenomenon could be attributed to the fact that anaerobic microorganisms had not yet adapted to the current AD system environment and was related to the fact that acid-producing bacteria held a certain advantage during the early stage of digestion. Subsequently, the gas production of the mixed feedstock entered a stable trend and then reached a peak, which was maintained for a relatively long period of time, during which the percentage of CH_4 was always greater than 55%, indicating that this was the period of maximum CH_4 production and that microorganisms gradually adapted to the current changing environment. Notable, however, it was the fact that, with the exception of R3 and R6, which collapsed due to system overload, the time to reach the peak for each of the above normal gas production groups was delayed with the elevation of S/I and the increase of TS content, indicating that the AD system overload enhanced the anaerobic microbial inhibition.

After gas production reached its peak, the DMY of R1, R2, R4, and R5 progressively decreased until it stabilized, at which point gas production ceased. The Cumulative Methane Yield (CMY) of R1, R2, R4, and R5 reached (152.92±5.91), (151.28±12.72), (251.19±13.94), and (227.25±5.83) mL CH_4/g VS, respectively, as

shown in Figure 2. When S/I was set to 25, CMY did not decrease significantly with increasing TS content (p>0.05), whereas when S/I was set to 30, CMY decreased substantially with increasing TS content (p<0.05), with a 10% decrease. Moreover, when the value of TS was held constant, CMY increased substantially (p<0.05) with increasing S/I, and then both variables collapsed at TS=20. It can be seen that when feeding with a mixture of straw and cattle manure, an increase in its solids content will have a significant effect on the CH_4 yield of the AD system when the TS reaches 20%; thus, an appropriate amount of mixed substrate can promote the production of CMY to a certain extent, whereas an excessive amount can cause the system to fail.



a. Daily methane yield (DMY) b. Cumulative methane yield (CMY)

Figure 2 Methane generation performance of batch tests

3.2 Organic matter removal rate

Based on Figure 3, the performance of the substrate removal rate and the variation of the gas production efficiency were in good agreement. Specifically, the VS Removal Rate (VS_r) of R1, R2, R4, and R5 was (48.12±3.45)%, (51.62±2.56)%, (60.55±2.34)%, and (51.45±7.24)%, respectively, whereas R3 and R6 were destabilized due to overloading and the VS_r was only (12.34±5.34)%. The overall results were slightly lower than previous experimental results, but it was understandable. Initially, the high TS range (10-20) and the S/I settings chosen for this study were biased toward high stress, which in turn limited the activity of the anaerobic microorganisms and decreased the operational efficiency of the AD reactor. The principal components of both the straw and the cattle manure co-digested with it were lignocellulose, i.e., cellulose, hemicellulose, and lignin. According to earlier studies, the outer epidermal and cortical regions of plant cell walls contained a large number of lignin-rich vascular bundles, which were not available for conversion in the AD process and also limited the bioconversion efficiency of cellulose and hemicellulose.

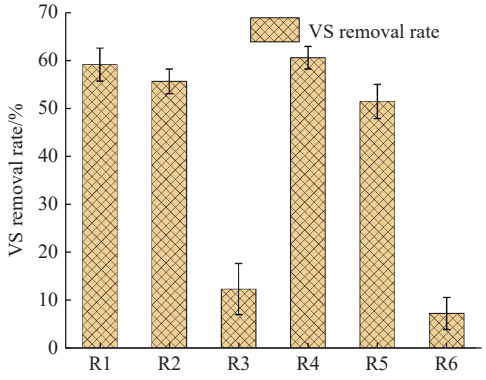


Figure 3 List of VS removal rates in different reactors

Furthermore, Monlau et al.[17] compared the effects of the structural and component properties of twenty lignocellulosic feedstocks on their anaerobic digestion performance and found that soluble sugar concentration, protein content, and hemicellulose content were positively and linearly correlated with the methane yield of the feedstocks, whereas lignin content and cellulose crystallinity were negatively and linearly correlated with the methane yield of the feedstocks. Consequently, an increase in substrate and, consequently, an increase in lignin content had a significant impact on the degradation efficacy of a mixed substrate.

3.3 Kinetic models analysis

This study used the first-order model, the modified Gompertze model, and the transfer function model to simulate the aforementioned various groups of AD reactors in order to clarify the effects of TS and inoculation ratio on the kinetics of mixed substrate degradation. R^2 is one of the key indicators used to evaluate the accuracy of models; the closer R^2 is to 1, the more accurate the model simulation is assumed to be[18]. As can be seen from the table, the R^2 of the three models are located in the range of 0.98-0.99, 0.72-0.86, and 0.93-0.98, respectively. the R^2 of the first-order model was significantly lower than that of the modified Gompertze model and the transfer function model. it can be seen that the fit of the modified Gompertze model and the transfer function model were better than that of the first-order model. The similar results of Li et al.[19] were obtained when the above models were used for gas production simulation of the AD reactor.

Tables 3-5 also displayed the variation of the model parameters k for the first-order model, R_{max} and λ for the modified Gompertze model and the transfer function model. k denoted the hydrolysis rate constant, and it was commonly believed that the higher the value of k, the quicker the substrate's hydrolysis rate[20]. Correspondingly, R_{max} in both the modified Gompertze model and the transfer function model was the methanogenic rate constant, which was commonly used to evaluate the methanogenic activity of AD reactors; the larger the value of R_{max}, the higher the methanogenic activity of its AD system. As shown in Table 3, among the groups of reactors with stable gas production, the k values for R1, R2, R4, and R5 fell within the range (0.01-0.06) d^{-1}, which was significantly lower than the values reported in previous studies[21], which might be a result of the lower simulation accuracy of the first-order model.

As shown in Tables 4 and 5, the R_{max} of the modified Gompertze and transfer function models was (11.88±0.11)-(16.42±0.51) mL CH_4/(g VS·d) and (9.07±0.25)-(15.84±0.38) mL

CH_4/(g VS·d), respectively. Both models observed a gradual decrease with increasing TS content and S/I, indicating that an increase in TS and S/I would have a significant impact on the methanogenic activity of the AD system. These R_{max} values were comparable to those of other lignocellulosic feedstocks [(16.3-32.1) mL CH_4/(g VS·d)][22], but considerably lower than those of the AD experiments with kitchen waste as substrate [(28.03-174.63) mL CH_4/(g VS·d)][19], which can be attributed to the physical and chemical differences of the feed substrate itself. Compared to lignocellulose-rich biomass such as straw and manure, the above-mentioned food waste had more readily degradable components and, as a result, a faster conversion rate in a stable reactor. In addition, the complex crystalline structure of lignocellulose in mixed substrates frequently caused a period of stagnation (λ) in the AD system.

Table 3 Kinetic parameters obtained by fitting methane yield via first order model of different groups

Group	First order model		
	M_{max}	k	R^2
R1	203.34±36.00	0.06±0.01	0.72
R2	172.30±16.51	0.06±0.01	0.86
R4	235.36±21.12	0.02±0.01	0.82
R5	245.12±5.63	0.01	0.86

Table 4 Kinetic parameters obtained by fitting methane yield via modified Gompertze model of different groups

Group	Modified Gompertze model			
	M_{max}	R_{max}	λ	R^2
R1	154.88±1.03	16.42±0.51	4.38±0.15	0.99
R2	153.25±0.69	12.42±0.21	2.34±0.07	0.99
R4	274.26±1.94	15.57±0.17	6.22±0.08	0.99
R5	270.23±2.81	11.88±0.11	5.74±0.08	0.99

Table 5 Kinetic parameters obtained by fitting methane yield via transfer functional model of different groups.

Group	Transfer functional model			
	M_{max}	R_{max}	λ	R^2
R1	165.02±8.80	14.48±2.56	2.85±0.33	0.94
R2	151.19±2.84	11.37±1.89	2.30±0.21	0.97
R4	231.51±4.78	15.84±0.38	2.87±0.24	0.98
R5	252.14±5.61	9.07±0.25	2.25±0.12	0.93

λ refers to the amount of time needed for anaerobic microorganisms to reorganize in response to a shifting environment[23]. The values for the modified Gompertze model and transfer function model were (2.34±0.07)-(6.22±0.08) d, and (2.25±0.12)-(2.87±0.24) d, respectively, and increased progressively with increasing TS content and S/I. Overall, although the values simulated by the two types of models differed more significantly, the trends of both with environmental stress were comparable. Both models exhibited the same environmental stress trend. Thus, both the increase in particulate content and the increase in S/I had a substantial effect on the substrate conversion rate to methane.

3.4 Microbial community structure analysis

Microorganisms are essential to the proper functioning of the AD process. Variations in environmental factors, such as temperature, solids content, and S/I, will substantially impact the structure of the microbial community and, consequently, the performance of the AD system.

Figure 4 demonstrated that the dominant bacterial community composition at the backdoor level at the end of AD was similar across the various categories mentioned. These categories mainly consisted of *Firmicutes*, *Proteobacteria*, *Chloroflexi*, *Euryarchaeota*, *Candidatus_Cloacimonetes* *Acidobacteria*, *Actinobacteria*, *Fibrobacteres*, and *Bacteroidetes*. Upon further examination of the prevalence of dominant clades, it was observed that *Firmicutes* were the primary dominant clade in R1-R6, comprising 30%-50% of the total. However, in the destabilized R3, the percentage of *Firmicutes* exceeded 70%. Conversely, in R6, *Firmicutes* accounted for only 23.82%, while *Proteobacteria* dominated with 31.53%. This demonstrated that the internal microbial population of the AD system exhibited distinct alterations when destabilized by high total solids (TS) and overloading. The bacteria *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, and *Proteobacteria* were identified as the prevailing microorganisms in the mesophilic anaerobic digestion reactor across various circumstances. The genera *Firmicutes* and *Clostridium* encompassed a variety of metabolic capabilities, such as protein hydrolysis and glycosylation, as well as microorganisms involved in VFA degradation. On the other hand, *Proteobacteria* were primarily associated with acidifying bacteria[24].

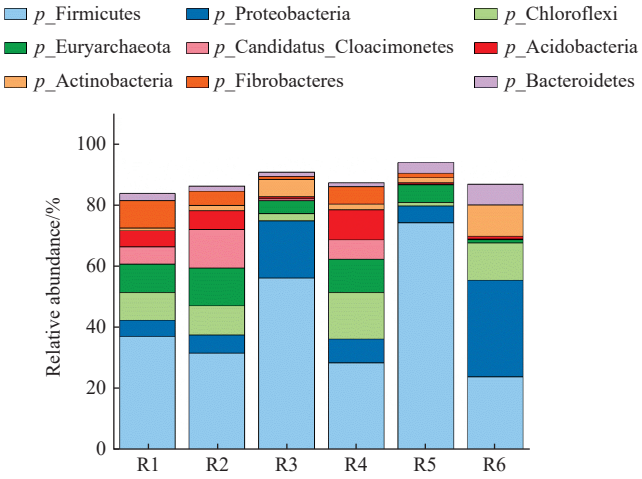


Figure 4 List of microbial community structures identified in different groups at the phylum level

The dominant bacteria at the genus level cover unclassified_*p*__Firmicutes, unclassified_*f*__Ruminococcaceae, unclassified_*p*__Chloroflexi, Methanosarcina, Fibrobacter unclassified_*f*__Clostridiaceae, unclassified_*p*__Proteobacteria, Candidatus_Syntrophosphaera, Candidatus_Solibacter, Sporolactobacillus, Vulgatibacter, Methanoculleus, and Clostridium (see Figure 5). The relative abundance of the dominant microorganisms at the genus level varied among the different groups. Methanogenic archaea were generally considered to be more sensitive to changing environments compared to hydrolytic acidifying bacteria[25,26]. From the genus level, the mixed-nutrient Methanosarcina dominated, with the ability to use acetic acid, methylamine, methanol, H_2 and CO_2 for growth, recognized as having relatively fast growth rates, and adaptable to variable environments. Methanoculleus was a hydrogen-type methanogenic bacterium that can use CO_2 and H_2 to produce methane, i.e., acetic acid, which was directly used by methanogenic bacteria, was first oxidized to CO_2 and H_2 by mutualistic acetic acid oxidizing bacteria under stress conditions before being used by Methanoculleus. Methanoculleus, which were used to generate methane, consume acetic acid in the reactor. Differently, as the TS content increased, Methanosarcina in R1, R2,

and R3 increased from 6.82% to 9.32% and then decreased to 2.67% in R3, where the system collapsed, while Methanoculleus increased slightly from 1.56% to 1.64% and then decreased to only 1.04%, again due to system collapse, with the trend of methane production in the various reactors. The findings from the examination of the microbial community were consistent with previous research[27], indicating a high level of trustworthiness.

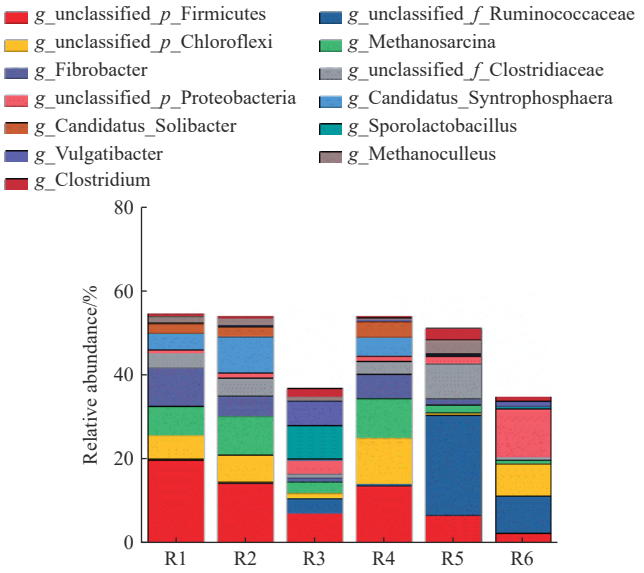


Figure 5 List of microbial community structures identified in different groups at the genus level

4 Conclusions

An investigation of the impacts of increased solids content and S/I on the operational performance of batch AD systems was carried out in this study. The variables that were used for this investigation were TS and S/I. An increase in TS within a specified range in a dry anaerobic system led to an increase in the rate of methane generation; however, an overly high TS led to a reduction in the performance of the anaerobic digestion reactor. According to the findings of the kinetic analysis, an increase in both the total stoichiometry (TS) and the load would have a detrimental effect on the performance of the AD system. This would be accomplished by reducing the rate of hydrolysis and the rate of methane production, as well as by extending the stagnation period. Furthermore, the distribution of the microbial community structure would be affected to varying degrees. As a result of mass transfer constraints and an excessive concentration of local intermediates, the batch anaerobic process without agitation and methane reflux was shown to be particularly vulnerable to block destabilization. This was discovered through the use of substrate sampling and microbiological analysis. This discovery was restricted to the anaerobic digestion of maize straw as the primary substance, and more investigation on various substances and inoculum was required to be conducted extensively. The use of this information will be of great assistance in identifying the ideal ratio of substrate to inoculum in AD, which will in turn ease the speedy start-up and directional control of maize straw batch AD.

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