COMPARISON OF METHANOGENESIS BETWEEN SUNFLOWER AND CORN STALKS MIXED WITH PIG MANURE AT DIFFERENT TEMPERATURES

FENG, L.[1] – YU, Q.[1] – ZHEN, X. F.[2*] – DONG, H. Y.[2] – ZHENG, J.[3] – WANG, Y.[3]

[1]*Liaoning Province Clean Energy Key Laboratory, Shenyang Aerospace University*
Shenyang Daoyi Street 37, Shenyang 110136, China
(phone: +86-1804-0038-889)

[2]*School of New Energy and Power Engineering, Lanzhou Jiaotong University*
No. 88, Anning West Road, Anning District, Lanzhou 730070, China
(phone: +86-1391-9302-012)

[3]*School of Energy and Power Engineering, Lanzhou University of Technology*
No. 287, Langongping Road, Qilihe District, Lanzhou 730050, China
(phone: +86-1391-9257-393)

[*]*Corresponding author*
e-mail: farming478@outlook.com; phone: +86-1391-9302-012

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Abstract. The gas production effect of two kinds of straws mixed with pig manure via anaerobic fermentation under two sets of temperatures 32℃ and 52℃ has been studied in this experiment. In the process, sunflower and corn straw were used as raw materials with activated sludge as inoculum in a batch anaerobic fermentation reactor. The results showed that the average methane content of sunflower straw reached 62.55% at 37℃, which was 20.41% higher than that of sunflower straw at 52℃.The cumulative methanogenic yield of corn stalks was higher than that of sunflower stalks at different temperatures. The cumulative methane production of corn stalks was the highest at 52℃, and 67.99 L of methane was produced in 54 days. The cumulative methane production of sunflower stalks was the lowest at 52℃, and the methane production was 34.09 L in 53 days. The results showed that corn stalks produce more methane than sunflower stalks and are more suitable for high temperature anaerobic fermentation. On the other hand, sunflower stalks were found suitable for medium temperature fermentation.
Keywords: *anaerobic fermentation, biomass, resource utilization, livestock manure, biogas*

Introduction

The People’s Republic of China as a large agricultural country has abundant straw resources. In 2017, the country's collectible straw production from the main crops reached 900 million tons (Liu et al., 2018). This production included the straw from corn (*Zea mays* L., Fam.: Poaceae) and sunflower (*Helianthes annuus* L., Fam.: Asteraceae). The amount was nearly more than 1/3 of the total straw production of China. In China, reasonable conversion of straw to available energy is crucial field of research (Du et al., 2015). At present, a large amount of straw in China is not being effectively utilized, resulting in wastage of resources and pollution to the environment due to indiscriminate burning (Fan et al., 2018). The anaerobic fermentation technology is an effective means to ensure the utilization of straw resources and the technology can sufficiently solve the problem of straw waste management and generate renewable energy such as methane gas to alleviate the energy shortage in China (Duan et al., 2016). The effort will promote

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the sustainable development of the society and improve the economy and ecological condition of the environment (Wan et al., 2010; Jha et al., 2011; Sun, 2016).

Studies related to the methane production by straw is affected by a number of factors. Among which temperature is an important factor affecting anaerobic fermentation (Brown et al., 2012; Huang et al., 2018). It plays an important role in microbial growth rate, enzyme activity, biochemical reaction rate, etc. Generally, the anaerobic fermentation temperature is divided into three gradients: low temperature, medium temperature and high temperature (Liu et al., 2008; Yin et al., 2014). Juan et al. (2018) found that the methane production efficiency of corn straw increased with increasing temperature. During anaerobic fermentation at high temperature like 55℃, the total organic load (TS) was 100 g/L. The yield of TS in the corn straw unit was 44.68% higher than that under the anaerobic digestion at 45℃, which was 64.08% higher than 35℃. Studies by Pang et al. (2018) and others showed that the cumulative methane production of anaerobic fermentation of cow manure and straw at 35℃ was 91.84 L, which was 20% higher than that at 25℃ and 30℃. Contreras et al. (2012) explored the potential for methane production in rice straw and rice at 37℃ and 55℃. It was found that the biogas production at high temperature was 0.43 m^3·kg/vs, which was 0.09 m^3·kg/vs higher than that under medium temperature. It was confirmed that the high-temperature fermentation of rice straw was better.

In the present research, studies on the effects of temperature on anaerobic fermentation of different types of straw have been reported (Gu et al., 2016). Some previous studies have shown different types of gas production by using different compositions of straw. However, there exists very few studies on the effect of temperature and on the potentiality of anaerobic fermentation of sunflower straw and its anaerobicity. In order to fill up the gap between theoretical research and the application of the technology in practice, the concept of present research has been developed. In this research, using batch anaerobic fermentation reactor, the effect of gas production changes by sunflower and corn straw at 37℃ and 52℃ has been compared during the research, the changes of various parameters while the anaerobic fermentation of two straws were in operation have also been studied. So, the aim of the present research is to maximize the utilization of straw anaerobic fermentation, improve the resource utilization of sunflower straw and corn straw and to provide data support for straw anaerobic fermentation technology.

Materials and Methods

Experiments

The corn straw for the present study was collected from the Shenbei New District of Shenyang City, Liaoning Province, China. The whole corn was harvested and after separating the grains of the cobs, the straws were air dried and crushed. The crushed biomass of the straw was passed through a 20-mesh sieve. The source and treatment of sunflower straw were the same as those applied for the corn straw. Fresh pig manure was collected from Shenbei New District, Shenyang City, Liaoning Province. The inoculum used for the experiment was the biogas slurry collected after carrying out in vitro anaerobic fermentation. Fresh pig manure (mass ratio 1:10) was added before the experiment which was carried out at medium (37℃) and high temperature (52℃) for 15

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days. The characteristic parameters of the raw materials used in the anaerobic digestion processes have been presented in *Table 1*.

Table 1. *Presenting the characteristic parameters of anaerobic digestion feedstock*

Raw material	TS/% Total solids	VS/% volatile solids	C/N
Corn straw	89.78±0.01	71.68±0.01	ND
Sunflower straw	94.44±0.01	88.64±0.01	ND
Pig manure	29.28±0.02	19.8±0.01	24±0.03
Biogas slurry inoculum	2.09±0.01	0.885±0.001	ND

Data shown were the average and standard deviation based on triplicate runs; ND meant not determined

Main devices

A controllable constant temperature fermentation unit was used for carrying out the tests involved in the present investigation. The experimental protocol has been presented in *Fig. 1*.

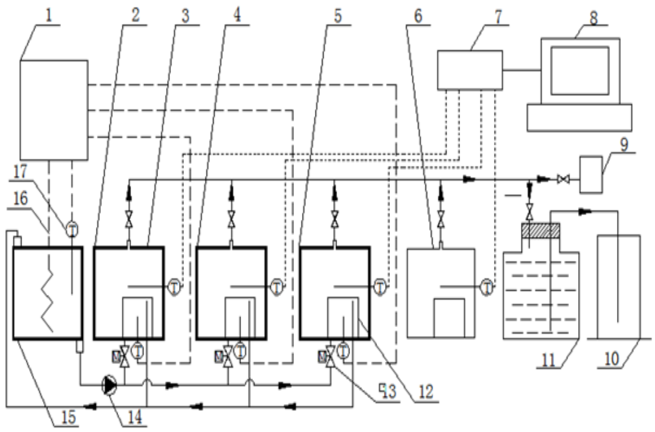

Figure 1. *Schematic diagram of controllable constant- temperature fermenting equipment 1. Temperature controller 2. Insulation layer 3. Fermentation cylinder 4. Fermentation cylinder 5. Fermentation cylinder 6. Fermentation cylinder 7. Data collecting instrument 8. Computer 9. Biogas analyzer 10. Water storage tank 11. Gas storage tank 12. Internal water tank 13. Solenoid valve 14. Hot water pump 15. Constant temperature water tank 16. Heating wire 17. Temperature sensor*

The device is mainly composed of a heating water tank, a temperature controller, a cylindrical 304 stainless steel fermenter with an effective volume of 10 L (with a height to diameter ratio of 1:1) and a gas collecting device. The fermenter of the device is insulated from the environment and the temperature of the fermenter is adjusted. The flow rate of the circulating heating water tank is precisely controlled, and the temperature is measured with a Pt100 platinum resistance temperature detecting device with an accuracy of ±0.1℃.

Other instruments used for determining various other experimental parameters were: TDL-5-A centrifuge (Shanghai Anting Scientific Instrument Factory); GZX-9240MBE digital display blast drying oven (Shanghai Boxun Industrial Co., Ltd.); UV-9200 UV-visible spectrophotometer (Beijing Ruili Analytical Instruments Co., Ltd.); Biogas check

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biogas analyzer (Geotech, UK); JSM5600LV scanning electron microscope (JEOL, Japan) and D/MAX-2004 powder X-ray diffractometer (Nippon Science).

Anaerobic digestion test design

The mass ratio of corn and sunflower stalk and pig manure was 1:2 and the amount of biogas slury inoculation was 30% of the mass. The corn straw group contained: straw, pig manure, acclimated biogas slurry at the rate of 0.30 kg, 0.59 kg and 1.70 kg, respectively. On the other hand, sunflower straw group contained: 0.30 kg, 0.58 kg and 1.69 kg as straw, pig manure and acclimated biogas slurry, respectively after feeding, 6.0 kg distilled water was added.

The fermenter has a volume of about 6 L, and the temperature is controlled at 52 ± 1℃ and 37±1℃, respectively. The reaction tank is shaken 3 times per day, and the digestion process is continued until no gas is produced. The test run cycle was fixed for 54 d. The pH value, gas composition and gas production of the fermentation broth were measured every 5 days, and indicators like the chemical oxygen demand (COD), volatile fatty acids (VFAs) and ammonia nitrogen (NH_4^+-N) of the fermentation broth were measured at intervals of 4 days. After the fermentation, the corn straw residue is separated, and the weight after drying was taken and recorded.

Analysis methods

The total solids (TS) and volatile solids (VS) of corn straw, sunflower straw, pig manure and biogas slurry inoculum samples were measured according to the standard methods (APHA, 1998). The biogas components were measured using a Biogas Check. The pH value was measured using an Oriol PHS3C portable pH meter. VFAs and COD determination is based on Liu et al. (2013), using spectrophotometry and potassium dichromate method respectively. While phenol sodium hypochlorite colorimetry method was used to determine NH_4^+-N concentration. The measurement of alkalinity was carried out using an acid-base indicator titration method.

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) measurements were done via Paradigm analysis. Cellulose (CL) and hemicellulose (HC) quality scores were done after ADF-ADL and NDF-ADF. The structural analysis were carried out using scanning electron microscope (SEM) as well as X-ray diffraction (XRD).

Results and Discussion

Gas production changes of corn straw and sunflower straw under anaerobic fermentation at different temperatures

It can be seen from *Figs. 2-3* that the gas production amount is significantly different depending upon the temperature and straw varieties. During the 54 d fermentation period, the gas yield of corn straw reached the peak at 11.72 L/d on the 42nd day at 52℃. At this temperature, the sunflower straw reached the highest daily gas production at 4.94 L/d on the 44th day. At 37℃, the gas yield of corn straw reached the peak at 4.83 L/d on the 51st day, while the peak gas yield of sunflower straw appeared at 47 d, which was 5.92 L/d. In contrast, the peak gas production time during high temperature fermentation at 52℃ was earlier than that at 37℃. In addition, the cumulative gas production of corn straw at 52℃ was 140.31 L while the cumulative gas production of sunflower straw was 80.68 L.

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On the other hand, the cumulative gas production of corn straw at 37℃ was 92.46 L and the peak gas yield of sunflower straw was 67.55 L. Cumulative total biogas production in the present investigation followed an order: 52℃ corn straw>37℃ corn straw>52℃ sunflower straw>37℃ sunflower straw. The cumulative gas production of corn straw and sunflower straw at 52℃ is higher than the cumulative gas production at 37℃, indicating that the bioactivity of the bacteria is stronger under high temperature. During which, the decomposition efficiency of cellulose, hemicellulose and other substances is higher and the metabolic rate of microbial cells is accelerated. The anaerobic fermentation efficiency is improved. In addition, the cumulative gas production of corn straw was higher than that of sunflower straw. The daily gas production of sunflower straw during the late stage decreased to the initial stage of the reaction, indicating that the content of cellulose and other substances in sunflower straw was lower than that of corn straw. Insufficient accumulation leads to a decrease in gas production efficiency and a decrease in gas production during the later stages of methanogen production.

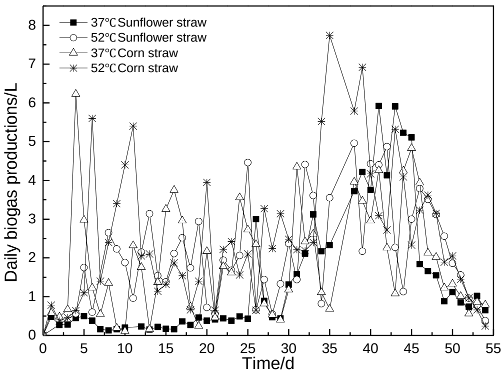

Figure 2. *Daily biogas production trends of corn straw and sunflower straw at different temperatures*

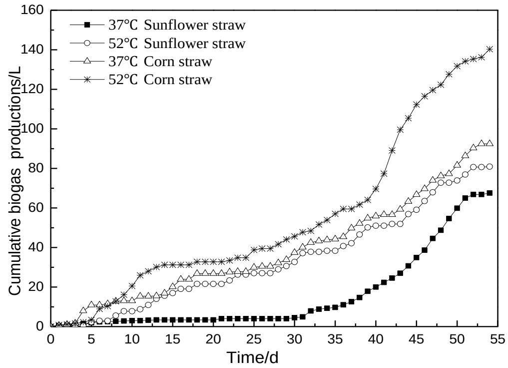

Figure 3. *Cumulative biogas production trends of corn straw and sunflower straw at different temperatures*

Anaerobic fermentation pH and fatty acid changes of corn straw and sunflower straw at different temperatures

Because, acid-producing bacteria have a wide range of pH adaptation and methane producing bacteria are sensitive to pH changes, pH plays a great role in the anaerobic digestion processes.

During the 54-day fermentation period, the pH of each group can be roughly divided into three stages: “stable-decline-fast rise”. The overall pH of the fermentation was between 6.5 and 7.9, indicating that the self-regulating pH inside the fermentation can maintain a stable fluctuation range (Zhou et al., 2014). Since the added activated sludge itself is weakly alkaline, the pH gradually rises during the initial stage of the reaction. During the period of 18 d-30 d, the pH value may decrease. It may be that the hydrolysis and acidification of the acetogenic bacteria decomposes the available materials in the straw and converts them into volatile fatty acids. At this time, the ammonia nitrogen content of the alkalinity in the fermentation system is insufficient, and the methanogens are still insufficiently adapted to the environment. A large amount of fatty acids accumulate, leading to a drop in pH (Fang et al., 2017). From 35 d to 47 d in the later stage of the experiment, as the reaction continued, the methanogens gradually adapted to

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the environment. As a result the activity of the strains increased and began to reproduce, consuming large amounts of fatty acids and producing ammonia nitrogen. The pH level rose again. As seen from figures (*Fig. 4 and Fig.5*) there is an opposite trend of change of pH and VFAs in anaerobic fermentation. During the mid-stage pH drop, the fatty acid concentration was at a higher value; while the pH value increased at the late stage of fermentation, the concentration of volatile fatty acids began to decrease rapidly.

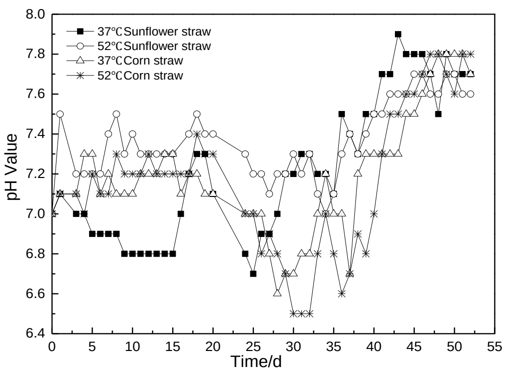

***Figure 4.** Change trend of pH value of corn straw and sunflower straw under different temperatures*

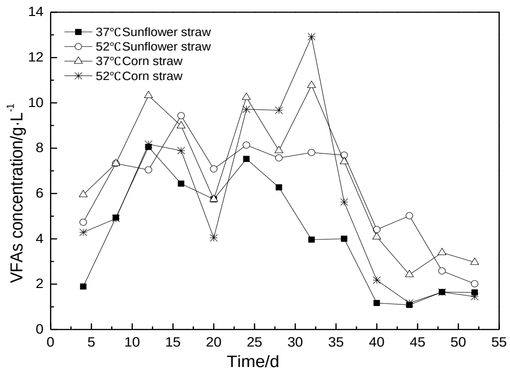

***Figure 5.** Change trend of volatile fatty acids concentration of corn straw and sunflower straw under different temperatures*

Volatile fatty acids are important intermediary compounds for reflecting microbial metabolism during anaerobic fermentation of straw (Xu et al., 2016).

At 37°C, sunflower straw reaches almost in the acidification and gas-free stage within 30 days before the middle temperature anaerobic digestion. However, the average concentration of VFAs was the lowest at this stage, and the theoretical inhibition intensity was the lowest, but the cumulative gas production was the lowest. The analysis showed that the sunflower stalks were under medium temperature conditions. The volatile acid produced had the highest propionic acid content, while propionic acid had the most obvious inhibitory effect on methanogens, which led to the inhibition of gas production in the first and middle stages. The average concentration of VFAs in corn straw at 37℃ was higher than 52℃. Although, the average content of methane in corn straw at 52℃ and 37°C was not much different, only 2.8% higher, but the cumulative yield of corn straw methane at 52℃ was much higher than that of sunflower straw. The results showed that different temperatures had different effects on the methanogenesis of sunflower straw and corn straw. At 37℃, the methane production increased rapidly and the average methane content was the highest. The analysis showed that the methanogens used acetic acid, the acetic acid content decreased, and the acetic acid was resistant to propionic acid. Oxygen digestion has the strongest inhibitory effect. The decrease in acetic acid content leads to an increase in the degradation ability of propionic acid, and the gas production efficiency of methanogens increases. The content of VFAs decreased at the beginning of 52℃ corn straw for 30 days. At the same time, the methane production grew rapidly and the average methane content was higher than 37℃, indicating that corn straw was more suitable for 52℃ high temperature anaerobic fermentation.

Ammonia nitrogen concentration changes in corn straw and sunflower straw under anaerobic fermentation at different temperatures

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The change of ammonia nitrogen concentration is an important parameter for the stability of the reaction fermentation system. It can be seen from *Fig. 6* that the average ammonia nitrogen value of sunflower straw is 1.19 g/L at 37℃ and 1.87 g/L at 52℃ and the average ammonia nitrogen value of corn straw at 37℃ is 1.85 g/L and at 52℃, it is 1.98. g/L. At 37℃, the ammonia nitrogen concentration and pH of the sunflower straw fermentation were lower than other groups. It may be that at 37℃ sunflower straw has higher activity of acid-producing bacteria at this stage, while the methanogen has not adapted to the environment, resulting in low ammonia nitrogen concentration. On the 40th day of fermentation, the daily methane production of sunflower straw increased significantly at 37°C. It is known from *Figure 4* that the pH increased rapidly. At this stage, the ammonia nitrogen concentration decreased rapidly and the volatile fatty acid reached the lowest value (Lin et al., 2011). It indicated that low concentration of ammonia nitrogen can promote the proliferation and division of methanogens and increase the yield of methane, which is consistent with the experimental study of Shun et al. (2018).

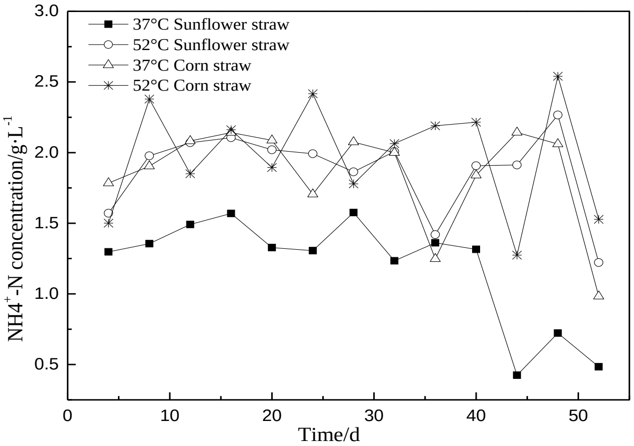

Figure 6. *Change trend of ammonia nitrogen concentration of corn straw and sunflower straw under different temperatures*

The average concentration of ammonia nitrogen in high temperature anaerobic fermentation of corn straw and sunflower straw was higher than that of medium temperature anaerobic fermentation. In the appropriate temperature range, as the temperature increases, the activity of the strain gradually increases resulting an increasing rate of ammonia nitrogen.

Anaerobic methanogenesis of corn straw and sunflower straw at different temperatures

It can be seen from *Figs. 7-8* that the methane production in the middle and early days before the reaction is low, and in the late, rate rises rapidly, The peak value appears between 42 d and 51 d, and the gas production fluctuates greatly, indicating that the experimental fermentation factors are excessive. At the temperature of 52℃, the methanogenic rate of corn stalks increased rapidly from 40 d to44 d. It was found that a large amount of methanogenic precursors such as acetic acid, formic acid, ethanol and CO_2 were formed in the middle and early stages of corn stalks at the same temperature. Methanogens can be utilized, and gas production increases, leading to peak values. The peak daily methane production of sunflower straw at 37℃ and sunflower straw at 52℃

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was 47 d and 44 d, respectively. The peaks of daily methane production at 37℃ and 52℃ of corn straw were 51 d and 42 d, respectively. It can be seen that as the temperature increases, the peak value of the daily methane gas conversion is advanced. However, there is no such rule in methane production. From *Table 2*, the highest content of methane in corn stalks at 52℃ is 73.7%, while the highest methane content in corn stalks at 37℃ is 70.9%. However, sunflower straw and corn stalks showed an opposite trend. The maximum content of methane from sunflower straw at 37℃ was 78.37%, while the same at 52℃ was only 58.70%. It can be speculated that the cumulative methane production of corn stalks increases with increasing temperature within a certain temperature range. The accumulated methane production of sunflower straw at 37℃ was higher than 52℃. The result showed that 37℃ was closer to the optimum temperature of sunflower straw anaerobic fermentation. While the activity of methanogen was inhibited at 52℃, and the methane production decreased.

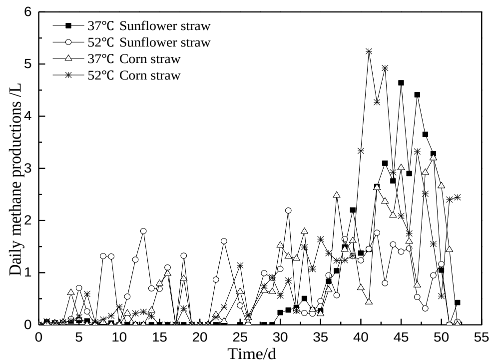

Figure 7. *Daily methane production of corn straw and sunflower straw under different temperatures*

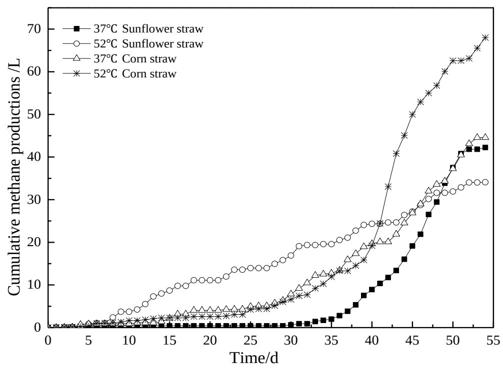

Figure 8. *Cumulative methane production of corn straw and sunflower straw under different temperatures*

Table 2. *Biogas production and methane production changes*

Grouping	Sunflower straw at 37℃	Sunflower straw at 52℃	Corn straw at 37℃	Corn straw at 52℃
Daily biogas productions/L	1.25	1.49	1.71	2.59
TS biogas productions/L·kg^{-1}TS	141.35	169.27	193.53	293.69
VS biogas productions/L·kg^{-1}VS	175.06	209.64	266.59	404.55
TS methane productions/L·kg^{-1}TS	88.41	71.33	93.28	142.32
VS methane productions/L·kg^{-1}VS	109.45	88.34	128.49	196.05
Average methane content%	62.55	42.14	48.20	48.46
Maximum methane content%	78.37	58.70	70.90	73.70

Conclusions

From the present investigation, experiments show that temperature has a significant effect on the gas production characteristics of combined sunflower straw and corn straw anaerobic fermentation.

In the 100-day fermentation test, the cumulative methane production of corn stalk at 52°C was 77.99 L, which was 128.77% higher than that of anaerobic fermentation of sunflower straw at the same temperature. When the fermentation temperature was

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maintained at 37°C, the cumulative production of methane from sunflower straw The lowest amount, only 34.09 L. However, the average content of methane in sunflower straw at the fermentation temperature of 37°C was 62.55%, which was higher than the average content of corn stalks at 37 degrees Celsius and sunflower stalks at 52 degrees Celsius by 14.35% and 20.41%. In general, in order to improve the methane yield of anaerobic fermentation of straw, corn straw is more suitable for anaerobic fermentation at high temperature (52℃). Unlike corn straw, sunflower straw is more suitable for anaerobic fermentation at medium temperature (37℃).

The methane production characteristics of straw and pig manure under medium and high temperature anaerobic fermentation conditions were studied. It was proved that corn stalk and pig manure could effectively improve the C/N ratio of materials and improve anaerobic digestion efficiency, and synergistically in anaerobic fermentation.

There is no research on low temperature (<15°C). The winter temperature in northern China is low, and the anaerobic mixing of straw and pig manure at low temperature is studied. Fermentation has high value for the utilization of biomass resources.

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