Scholar Publishing Group
Academic Journal of Agricultural Sciences
https://doi.org/10.38007/AJAS.2022.030401
ISSN 2789-8415 Vol. 3, Issue 4: 1-18

Two-Phase High-Temperature Anaerobic Digestion Experiment of Crop Straw

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Keywords: Crop Straw, Anaerobic Digestion, Methane Production, Ammonia Nitrogen Content, Lignocellulose Degradation Rate

Abstract: With the rapid development of agriculture, the output of crop straw has also increased year by year. The treatment and development and utilization of crop straw have become the focus of attention from all walks of life. In order to improve the utilization rate of crop stalks, save energy and protect the environment, this article conducted an in-depth study on the two-phase high-temperature anaerobic digestion test of crop stalks. First, the response surface method is used to control the organic loading ratio and the inoculation ratio respectively, and analyze the daily methane production and daily cumulative production. The test results showed that the A5 group on the 5th day (the organic loading ratio was 20gVS/L) had the highest single-day and cumulative methane production, which were 6.15mL/gVS and 19.57mL/gVS, respectively. When the inoculation ratio was 0.4 on the 25th day, the B1 group had the highest methane production, which was 2.88 mL/gVS. Then, the rice straw was treated by ammoniating treatment and multi-strain cooperative treatment to analyze the differences in pH value, ammonia nitrogen content and lignocellulose degradation rate. The test data showed that the pH values of the two groups were between 7 and 8. The ammonia nitrogen content of the ammoniated treatment reached 4485.1mg/L, and the degradation rates of lignocellulose on the 35th day of the ammoniated treatment and the multi-strain co-treatment were 24.14% and 35.33%, respectively. This shows that the effect of multi-strains synergistic treatment is better than ammoniated treatment, with lower ammonia nitrogen content and higher lignocellulose degradation rate. Finally, under the same experimental conditions, two-phase high-temperature anaerobic digestion experiments were carried out using rice straw and corn straw, respectively, to analyze the differences in pH, ammonia nitrogen content and lignocellulose degradation rate. The pH values of the rice straw and corn straw reactors were 7.56 and 8.21, the ammonia nitrogen content was 984.2 and 1128.6 mg/L, respectively, and the lignocellulose degradation rate was 34.54% and 25.38%, respectively. This shows that under the same reaction conditions, rice straw is better than corn straw, has lower ammonia nitrogen content and higher lignocellulose degradation rate, and its gas production and utilization are higher.

1. Introduction

1.1. Background Significance

Most of the crop straws are handled too simply and crudely, which not only seriously pollutes the environment, but also causes a waste of resources. Crop straw contains a lot of carbohydrates, which can be converted into biogas and bioethanol [1]. Anaerobic digestion technology is an environmentally friendly and efficient way of resource utilization, which has been used in many fields [2]. Lignocellulose in crop straw will hinder the contact and reaction of microbial extracellular enzymes and cellulose, reducing its utilization rate. Therefore, studying the influencing factors and optimal parameter settings of the two-phase high temperature anaerobic digestion of crop straw can improve the utilization rate of crop straw.

1.2. Related Work

The treatment, development and utilization of crop stalks have always been the focus of everyone's attention, because improper treatment methods will lead to waste of resources and environmental pollution. ZHANG used straw return to the field for a long-term location test plot to study the effects of combined application without straw (CK), combined application of 100% or 50% straw (SI1, SI2) and combined application of 50% straw burning (SI2B) on soil ammonia volatilization [3]. Huo J used rice straw and corn stalks as raw materials to produce fresh straw burning particles in the laboratory. The chemical composition and mixing state of fresh straw burning particles were studied with an aerosol time-of-flight mass spectrometer (ATOFMS) [4]. Their research has analyzed the effects of straw burning, but during the experiment, they did not notice that the natural conditions of burning in the laboratory and burning in the field are different. Anaerobic digestion is a worldwide organic waste treatment technology with obvious environmental benefits. The addition of inorganic and biological additives has shown good results in improving the performance of the digestion tank. Romero-Gueiza MS reviewed the application progress of inorganic and biological additives in recent years [5]. Pellera F M studied the effect of alkaline (NaOH) pretreatment on anaerobic digestion of olive pomace [6]. He conducted batch hydrolysis experiments on olive pomace under different amounts of NaOH, reaction time and temperature, investigated the solubility changes of olive pomace in the liquid phase, and studied the effect of pretreatment on anaerobic digestion by measuring the biochemical methane potential. Their research provides a data reference for anaerobic digestion technology, but the processing of variables during the experiment is not reasonable.

1.3. Innovative Points in this Paper

In order to improve the utilization rate of crop straw in the anaerobic digestion process, save natural energy, and protect the natural environment, this article conducted an in-depth study on the parameter settings and influencing factors of the two-phase high temperature anaerobic digestion test of crop straw. The innovations of this study are as follows: (1) The response surface method was used to detect the daily methane production and daily cumulative production of crop straw under different organic loading ratios and inoculation ratios. When the organic loading ratio is 20gVS/L and the inoculation ratio is 0.4, the methane production is the highest. However, if the inoculation ratio is too low, it will cause a waste of resources, so controlling around 1 is the best setting. (2) Two different pretreatment methods, ammoniating treatment and multi-strain co-treatment, were used for rice straw, and the effects of pretreatment methods on pH, ammonia nitrogen content and lignocellulose degradation rate were analyzed. The pretreatment method has

little effect on the pH value, but the multi-strain co-processing has lower ammonia nitrogen content and higher lignocellulose degradation rate. (3) Under the same test conditions, the rice straw and corn straw were used for the experiment. The pH value of the rice straw is more suitable for methanogenesis, the ammonia nitrogen content is lower, and the lignocellulose degradation rate is higher.

2. Straw Treatment and Anaerobic Digestion Technology

2.1. Types and Development and Utilization of Crop Straw

(1) Composition and types of crop straw

Crop straw contains a lot of cellulose, hemicellulose, lignocellulose and a small amount of protein and fat. The cellulose in the straw is mainly a crystalline state composed of microfibers, which is the main component of the cell wall, and the content is as high as 40% to 50% [7]. Cellulose is chemically stable and can be hydrolyzed into glucose under high temperature, high pressure, and acidic conditions. Cellulose hydrolyzed by microorganisms can be broken down into volatile fatty acids.

The function of lignocellulose is mainly to protect the straw from the invasion of microorganisms and act as a scaffold between cells [8]. Lignocellulose is entangled with cellulose and hemicellulose, which will affect the microbial degradation efficiency of cellulose and hemicellulose. And the degradation of any one of these costs will be affected by other components. Therefore, a better way to use straw is to use it as biomass energy, which can effectively utilize various components.

The crop straws are mainly rice straw, wheat straw and corn straw [9]. In addition, there are sorghum straw, sugar cane straw, peanut soybean and sesame straw. The grain and cash crops grown in the north are mainly wheat, corn and sorghum, while in the south are mainly rice, peanuts and sugarcane. Therefore, the types of crop stalks produced in the north and south are different.

(2) Development and utilization of crop straw

The use of straw as feed can supplement the shortage of feed, and can also save energy and reduce resource waste. It is an effective method for the reuse of crop straw. Crop straw feed is rich in fiber and is the main feed for ruminants. The cellulose in the feed becomes monosaccharides for absorption [10].

The cellulose in crop stalks can be used as a new raw material for industrial polymers, partially replacing bricks and wood and other materials, becoming environmentally friendly and beautiful building materials. As a new energy source, crop straw can also be used for power generation, ethanol production and molding fuel. Effectively protect arable land and forest resources, and solve the shortcomings of traditional building materials and energy.

Crop straws can also be directly returned to the field, which can ensure a certain amount of nutrients in the field, regulate the temperature and humidity of the field and suppress weeds. Therefore, insisting on returning straw to the field all the year round can effectively improve the ecological environment of the farmland, and has an obvious and continuous effect of increasing production.

2.2. Pretreatment Methods of Crop Straw

(1) Physical pretreatment

The physical pretreatment of straw generally refers to treatment methods such as mechanical processing, thermal processing and salinization, and the physical pretreatment process is relatively mature. Mechanical processing generally involves cutting, crushing and kneading the straw with

mechanical equipment. Thermal processing uses thermal spray and puffing technology to improve the digestibility of straw by reducing lignin barriers [11]. Compared with mechanical processing, the digestion and utilization rate of straw after thermal processing is higher, so the most commonly used methods are thermal spraying and puffing.

Thermal spray technology treats straw roughage by reducing pressure and spraying hot air. Hot steam can achieve disinfection and improve the palatability of animals. In the process of thermal spraying, non-protein nitrogen compounds can be added to improve the nutritional value of feed [12].

Puffing treatment can obviously increase the soluble and digestible components of straw feed, and improve its feed value. However, this process requires specialized equipment, and the cost is relatively high, and it cannot be applied in a wide range.

(2) Chemical pretreatment

The chemical pretreatment of straw generally refers to the treatment of crop straw with chemical agents. The chemical pretreatment methods currently in use include ammoniating, alkalizing, dilute acid and oxidation [13-14].

Ammonia treatment will break the lipid bond between lignin and polysaccharide chains to form ammonium salt, and produce ammonia, urea, liquid ammonia and ammonium bicarbonate. These substances are happy to provide a source of nitrogen, thereby increasing the activity of microorganisms in the rumen. Specific methods with better effects in ammoniating treatment include liquid ammonia ammoniation and urea ammoniation.

Alkalization treatment can increase the permeability and microbial activity of rumen juice, and improve the palatability, feed intake and digestibility of straw feed. Commonly used alkalis in alkalization are sodium hydroxide, lime water and calcium hydroxide [15]. Compound alkalization treatment can also be used to shorten the treatment time.

(3) Biological pretreatment

The biological pretreatment of straw generally refers to the use of microorganisms to selectively degrade the lignin in the straw, decomposing the cellulose and other substances in the straw into small molecular substances, which is conducive to the further degradation and utilization of the subsequent microorganisms [16]. This can reduce the time for subsequent anaerobic digestion, improve the enzymatic hydrolysis performance of cellulose, and increase the degradation rate and methane yield. The most important thing in biological pretreatment is the microorganisms used, the commonly used white rot fungi, lactic acid bacteria, yeasts and so on.

White rot fungi secrete lignin peroxidase and manganese peroxidase in an environment with insufficient carbon and nitrogen nutrients. After oxidation of the ferric iron of these two enzymes, the catalytic cycle will start to release the polymerization state of lignin [17]. Eventually, the chemical bonds of lignin are broken into small molecules, and finally oxidized and degraded. Lactic acid bacteria are the dominant strains of straw silage. After fermentation, they can improve the nutrient composition of the straw and improve the quality of the straw.

The synergistic effect of multiple strains on straw pretreatment is more effective than a single strain. The use of microbial strains with lignin degradation ability can significantly increase the lignocellulose content of ginger straw, and the volatile matter content in the fermentation broth will also increase. The content of propionic acid, which has an inhibitory effect on methanogens, will decrease [18]. However, the price of co-pretreatment of multiple strains is higher than that of a single strain, and economic factors should be considered when selecting.

2.3. Anaerobic Digestion Technology

(1) Aerobic anaerobic two-phase fermentation process

The aerobic anaerobic two-phase process needs to achieve high utilization of equipment in actual production applications, so the load matching between the two phases is very important [19]. The calculation formula for the load ratio of the oxygen-consuming hydrolysis phase and the anaerobic methane production phase is shown in Formula 1:

$$r_{OLR} = \frac{OLR_1}{OLR_2} = \frac{(F_1 M_1 - F_2 M_2) V_2 f_2}{F_2 M_2 V_1 f_1} \quad (1)$$

Among them, OLR_1, OLR_2 are the organic load of the aerobic hydrolysis phase and anaerobic fermentation phase, and the unit is kg/(m3·d). M_1, M_2 are the feed volume and the output volume respectively, the unit is kg; V_1, V_2 is the effective volume, the unit is L. f_1, f_2 is the hydraulic retention time in d[20].

The formula for calculating the reflux ratio of biogas slurry is shown in Formula 2:

$$L = \frac{N_1}{N_2} = \frac{(F_3 - F_1) N_3}{(F_1 - F_4) N_2} \quad (2)$$

Among them, N_1, N_2, N_3 are the amount of biogas slurry, the amount of methane-producing phase discharged biogas slurry, and the amount of straw. F_3, F_4 are the total solids mass fraction of the original straw and the total solids mass fraction of the methane-producing phase.

The dissolved oxygen concentration of the fermentation broth depends on oxygen supply and oxygen consumption, both of which need to be balanced [21], as shown in formulas 3 to 7:

$$D = K_L a(n_1 - n_2) = H_{O_2} J \quad (3)$$

$$K_L a = \frac{H_{O_2} J}{n_1 - n_2} \quad (4)$$

$$\frac{dn_t}{d_t} = K_L a(n_1 - n_2) - H_{O_2} J \quad (5)$$

$$n_2 = n_1 - \frac{H_{O_2} J}{K_L a} \quad (6)$$

$$K_L a = k\left[\left(\frac{p}{v}\right)^{\varepsilon} (v_a)^{\gamma} (\eta_{app})^{-\mu}\right] \quad (7)$$

Among them, D, J are the rate of oxygen transfer and the concentration of bacteria in the fermentation broth; n_1, n_2 are the saturated dissolved oxygen concentration in the solution and the mainstream dissolved oxygen concentration; $K_L a, H_{O_2} J$ are the dissolved oxygen coefficient and the respiratory intensity, respectively; $p/v, v_a$ are the actual power consumed per unit fermentation broth and the linear air flow rate; η_{app}, k are the apparent viscosity and empirical constant of the fermentation broth; ε, γ, μ are all exponents.

The calculation of the air flow per unit volume of the fan is shown in Formula 8:

$$Q = \frac{W}{bkv_1} \quad (8)$$

Among them, W is the amount of dissolved oxygen required by the fermentation liquid, and b,k are the volume fraction of oxygen in the air and the dissolved oxygen coefficient in the liquid, respectively.

(2) Influencing factors and classification of anaerobic digestion technology

The influencing factors of anaerobic digestion technology include pretreatment method, carbon to nitrogen ratio, pH value, temperature and organic load [22]. Different pretreatment methods will change the characteristics of the reaction object to varying degrees. The proper carbon-nitrogen ratio determines the balance of nutrients, too high or too low will cause system disorders and reduce the efficiency of anaerobic digestion. In the anaerobic digestion system, the pH value will directly affect the osmotic pressure of the internal microbial cells and affect the microbial activity. Therefore, the adjustment and stability of the pH value is very important for the stable operation of the anaerobic digestion system. Under different temperature conditions, the activity of enzymes and coenzymes in microorganisms will also change, which affects the effect of hydrolysis and acidification and the efficiency of methane production. Organic load is an important indicator of biomass conversion capacity of anaerobic digestion system.

Anaerobic digestion technology can be divided into single-phase anaerobic digestion and two-phase anaerobic digestion according to the difference of digested substances, control conditions and feeding methods [23]. Anaerobic digestion technology can be divided into high temperature, medium temperature and low temperature anaerobic digestion technology in terms of temperature. Generally, anaerobic digestion experiments are carried out under medium and high temperature conditions. According to the solid content of the anaerobic digestion system, it can be divided into wet and dry anaerobic digestion technologies. According to the time and number of feeding materials, anaerobic digestion technology can be divided into sequential batch, semi-sequential batch and continuous [24].

(3) Response surface method

The mathematical model of the response surface method is shown in Formula 9:

$$f = \alpha_0 + \sum_{i=1}^{k} \alpha_i G_i + \sum_{i=1}^{j-1} \sum_{j=1}^{k} \alpha_{ij} G_i G_j + \sum_{i=1}^{k} \alpha_{ij} G_i^2 \quad (9)$$

Among them, f, G are the predicted response value and independent variable code respectively. The conversion formula between the independent variable code and the actual value g of the independent variable is shown in Formula 10:

$$G_i = \frac{2\left(g_i - g_j\right)_0}{g_i(high) - g_i(low)} \quad (10)$$

Rewrite Formula 9 into a matrix form and solve it to get the equation system shown in Formula 11:

$$\frac{\partial f}{\partial G} = \left[\frac{\partial f}{\partial G_1}, \frac{\partial f}{\partial G_2}, ..., \frac{\partial f}{\partial G_k} \right] = 0 \quad (11)$$

The eigenvalues of the quadratic coefficients of the response surface function can determine its shape at the stagnation point, thereby determining the optimal test value [25]. Usually after establishing an optimization model for the gas production efficiency of anaerobic digestion, the

response surface function and experimental data can be used to determine the best points of the gas production efficiency of anaerobic digestion, and to determine the most advantageous values of each optimization parameter.

3. Experiments on Two-Phase High Temperature Anaerobic Digestion

3.1. Experimental Materials and Equipment

The straw used in this experiment includes two kinds of rice straw and corn straw, which are crushed to about 20 meshes by a pulverizer. The inoculum is the biogas slurry after anaerobic digestion, with TS of 13.5% and VS of 10.64%. Pretreatment methods include ammoniating treatment and multi-strain co-treatment.

The hydrolysis acidification system uses a 5L organic glass reactor, and the hydrolysis acidification process is carried out in a secondary vessel. The methane production system uses a stainless steel anaerobic fermentation reactor with a capacity of 8L and the upper part is made of transparent glass for easy observation. The reactor is equipped with automatic stirring, and the speed is 50r/min.

3.2. Two-Phase High Temperature Anaerobic Test Plan

The central combination design in the response surface method was used to set the parameters, and the experiment was conducted with the organic load 20gVS/L and the inoculation ratio 1 as the center. This experiment step is to add rice straw and inoculum to the reactor in turn, and add deionized water to adjust the initial pH to about 7. There are 5 experimental groups for testing the organic load ratio, and the organic load ratio is set to 4, 8, 12, 16, 20 gVS/L, and the daily production and cumulative production of methane are compared. There are also 5 experimental groups for testing the inoculation ratio. The inoculation ratios are set to 0.4, 0.8, 1.2, 1.6, and 2, respectively, to compare the daily and cumulative methane production.

The basic parameters are the same as the previous experiment. Before the straw is tested, different pretreatment methods are used to pretreat the straw to detect the changes in methane daily output, lignocellulose content and pH value.

The technical roadmap of this article is shown in Figure 1:

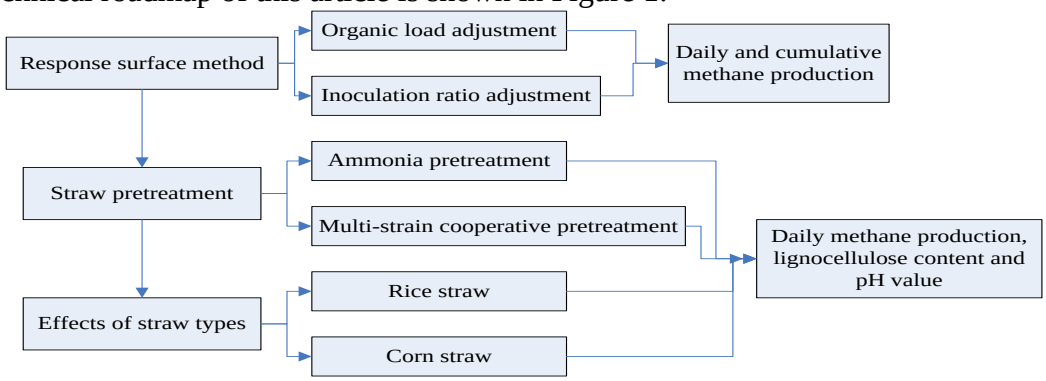

Figure 1. Technology roadmap

3.3. Test Measurement Indicators and Methods

(1) Detection of methane production

Use a handheld pressure gauge to detect the headspace gas pressure in the serum bottle and calculate the pressure difference. The calculation formula for daily methane production is shown in

Formula 12:

$$V = \frac{\Delta Y \times V_0 \times M}{R \times D} \quad (12)$$

Among them, ΔY is the pressure difference; V_0, M are the reactor headspace volume and the gas molar volume under standard conditions, respectively; R, D are the ideal gas constant and absolute temperature, respectively.

The anaerobic digestion performance of straw is analyzed by calculating the biodegradation rate of straw, and the calculation method is shown in Formula 13:

$$V_B = \frac{EMY}{MMY} \times 100\% \quad (13)$$

Among them, EMY, MMY are the experimental cumulative methane production and the maximum theoretical methane production respectively.

In order to study the methane production potential of straw, the kinetic fitting of the experimental cumulative methane production was performed, and the cumulative methane production after t days of reaction was calculated:

$$C_t = C_0 \exp\left\{-\exp\left[\frac{\gamma e}{C_0}(\sigma - t) + 1\right]\right\} \quad (14)$$

Among them, C_0, γ are the maximum methane production and the maximum methane production rate of the reaction, respectively; e, σ respectively represent the constant (generally 2.72) and the lag period of the reaction.

(2) Detection of lignocellulose

The method for determining the neutral detergent fiber is as follows: place the pulverized sample in an oven to dry to a constant weight, sieving it, and put it into a filter bag, mark the weight and seal it. Put the filter bag on the filter bag holder and put it in the digester, put a metal hammer and add the prepared neutral detergent for washing. After the digestion, rinse with distilled water, lightly press the filter bag to squeeze out part of the water, soak in acetone, dry it, dry it in an oven, and finally take it out to cool. The mass fraction of neutral detergent fiber is calculated as shown in Formula 15:

$$NDF(\%) = (M - M_k \times r) \times 100 / M_y \quad (15)$$

Among them, M, M_k, M_y are the mass sum of the sample residue and filter bag after washing, the empty bag mass, and the sample mass respectively; r is the calibration index of the blank bag.

The method for measuring acid detergent fibers is as follows: Put the filter bag with neutral detergent fibers in the digester, and introduce the prepared acid detergent solution for digestion. After the digestion, rinse with distilled water, lightly press the filter bag to squeeze out part of the water, soak in acetone, dry it, dry it in an oven, and finally take it out to cool. The mass fraction of acid detergent fiber is calculated as shown in Formula 16:

$$ADF(\%) = (N - N_k \times r) \times 100 / N_y \quad (16)$$

Among them, N, N_k, N_y are respectively the mass of the sample residue and filter bag after washing, the mass of the empty bag, and the mass of the sample.

The determination method of acid washing lignin is as follows: Put the filter bag tested by acid washing fiber into a beaker, and add concentrated sulfuric acid that has been prepared. Put it in a small beaker and lift it up and down 30 times, remove the filter bag and rinse it with water until the pH is 7, then wash it with acetone, air dry it and put it in an oven to dry. Put the filter bag into the crucible and put it into the muffle furnace, burn it for 100 minutes and take it out to cool. The acid washing lignin content is calculated as shown in Formula 17:

$$ADL(\%)=[(G-G_k\times r)-(G_z-G_i)]\times 100/G_y \quad (17)$$

Among them, G, G_k, G_y are the mass of the filter bag and the sample after washing, the mass of the empty bag, and the mass of the sample respectively; G_z, G_i are the mass of the crucible and the ash content, and the mass of the crucible after burning.

(3) Testing of other indicators

The total solid content (TS) of the fermentation raw materials is measured by the oven constant temperature drying method, and the total volatile solid (VS) is measured by the muffle furnace burning method. The pH value was measured with a Shanghai Ray Magnetic Acidity Meter.

4. Discussion on Anaerobic Digestion Experiment Results

4.1. Methane Production of Two-Phase High Temperature Anaerobic Digestion Response Surface Method Test

(1) Impact of organic load

There are 5 experimental groups for testing the organic load ratio. The organic load ratios are set to 4, 8, 12, 16, 20 gVS/L, and the group numbers are A1-A5. The comparison is in the 5th, 10th, 15th, 20th, and 20th. Single-day methane production and cumulative production on 25, 30, 35, 40, 45 and 50 days:

Table 1. Impact of organic loading ratio on methane production per day (mL/gVS)

Time (d)	A1	A2	A3	A4	A5
5	0.51	1.89	2.85	4.2	6.15
10	0.81	1.5	1.64	2.57	2.75
15	0.46	0.62	1.44	2.01	2.02
20	0.18	0.84	1.5	2.04	2.21
25	0.41	1	1.1	1.52	1.94
30	0.19	0.44	0.92	0.98	1.14
35	0.15	0.23	0.51	0.55	0.87
40	0	0.21	0.35	0.41	0.85
45	0	0.18	0.31	0.37	0.83
50	0	0.11	0.24	0.34	0.81

As shown in Table 1, when the organic loading ratio is the same, generally speaking, as time

increases, the single-day methane production will decrease. The highest single-day methane production was in the A5 group on the 5th day, which was 6.15 mL/gVS, followed by the A4 group on the 5th day, which was 4.2 mL/gVS. After the 30th day, the daily output of all groups was lower than 1mL/gVS, so we will focus on analyzing the daily output of the first 30 days.

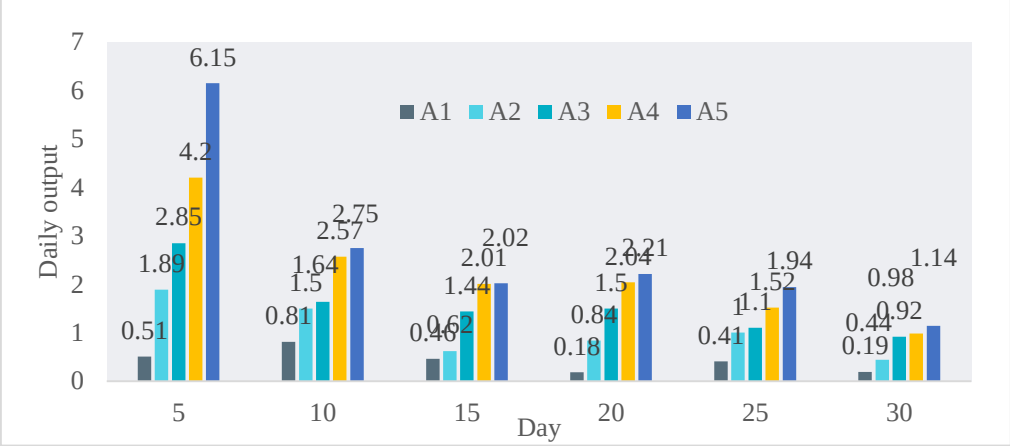

Figure 2. Comparison of daily methane production in the first 30 days

As shown in Figure 2, the daily output on that day is the highest in Group A5 and the lowest in Group A1. Moreover, the daily methane production of groups A1 to A5 increases in sequence. This indicates that the higher the organic loading ratio, the higher the daily methane production. Then calculate the daily cumulative methane production. The details are as follows:

Table 2. Daily cumulative production of methane under different load ratios (mL/gVS)

Time (d)	A1	A2	A3	A4	A5
5	0.51	1.89	2.85	4.2	6.15
10	1.32	3.39	4.49	6.77	8.9
15	1.78	4.01	5.93	8.78	10.92
20	1.96	4.85	7.43	10.82	13.13
25	2.37	5.85	8.43	12.34	15.07
30	2.56	6.29	9.35	13.32	16.21
35	2.71	6.25	9.86	13.87	17.08
40	2.71	6.73	10.21	14.28	17.93
45	2.71	6.91	10.52	14.65	18.76
50	2.71	7.02	10.76	14.99	19.57

As shown in Table 2, the final cumulative yields of the A1-A5 groups were 2.71, 7.02, 10.76,

14.99, and 19.57 mL/gVS, respectively. The cumulative output of group A1 did not increase from day 35 to day 50, while the increase rate of other groups gradually slowed down. The trends are as follows:

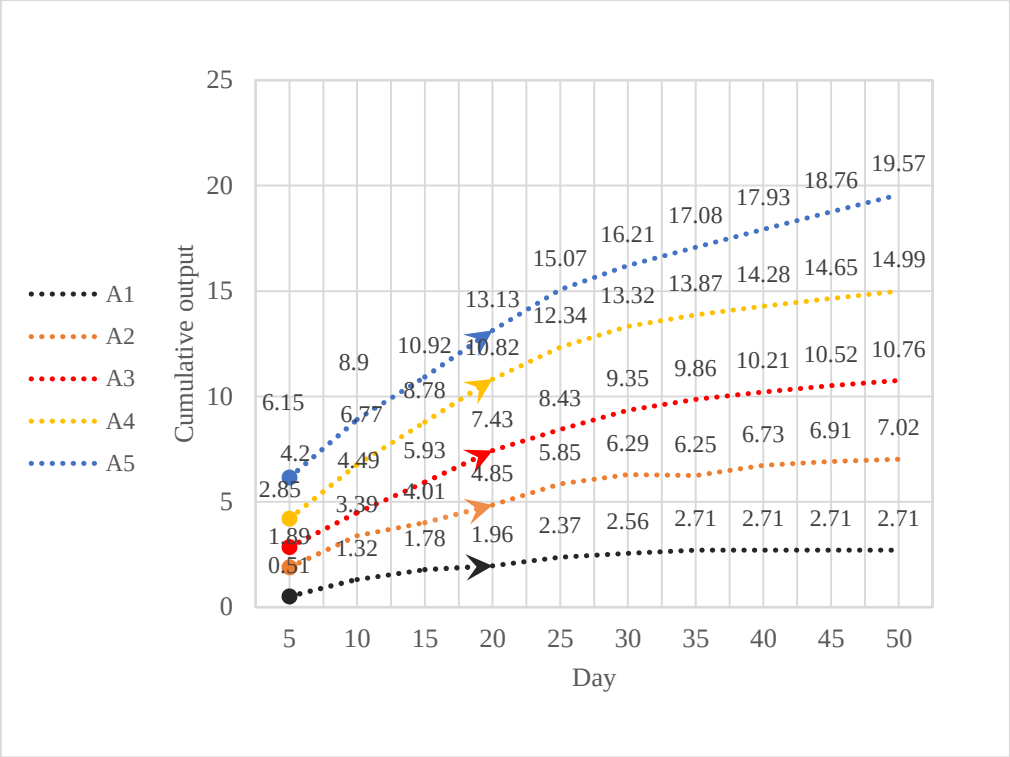

Figure 3. The change trend of daily cumulative output under different load ratios

As shown in Figure 3, all the change curves were steeper from the 5th to the 30th day, and gradually smoothed after the 30th day. This also shows that the cumulative output gradually decreases with the passage of time.

(2) Influence of inoculation ratio

There are also 5 groups in the experimental group for testing the inoculation ratio. The inoculation ratios are set to 0.4, 0.8, 1.2, 1.6, and 2, respectively, and the group numbers are B1-B5 to compare the daily methane production.

Table 3. Daily methane output under different inoculation ratios (mL/gVS)

Time (d)	B1	B2	B3	B4	B5
5	2.73	2.34	1.84	1.51	1.11
10	1.81	1.58	1.54	1.42	1.29
15	1.79	1.05	0.89	0.64	0.5
20	2.48	1.51	1.35	0.84	0.31
25	2.88	2.01	1.24	1.02	0.74
30	0.99	0.84	0.52	0.48	0.24
35	0.25	0.53	0.51	0.35	0.17
40	1.21	0.61	0.35	0.31	0.15
45	0.52	0.28	0.21	0.17	0.13
50	0.74	0.41	0.34	0.24	0.11

As shown in Table 3, too high an inoculation ratio will result in a decrease in the daily methane production. When the inoculation ratio is 0.4 on the 25th day, the daily methane production is the highest at 2.88 mL/gVS. The trends are as follows:

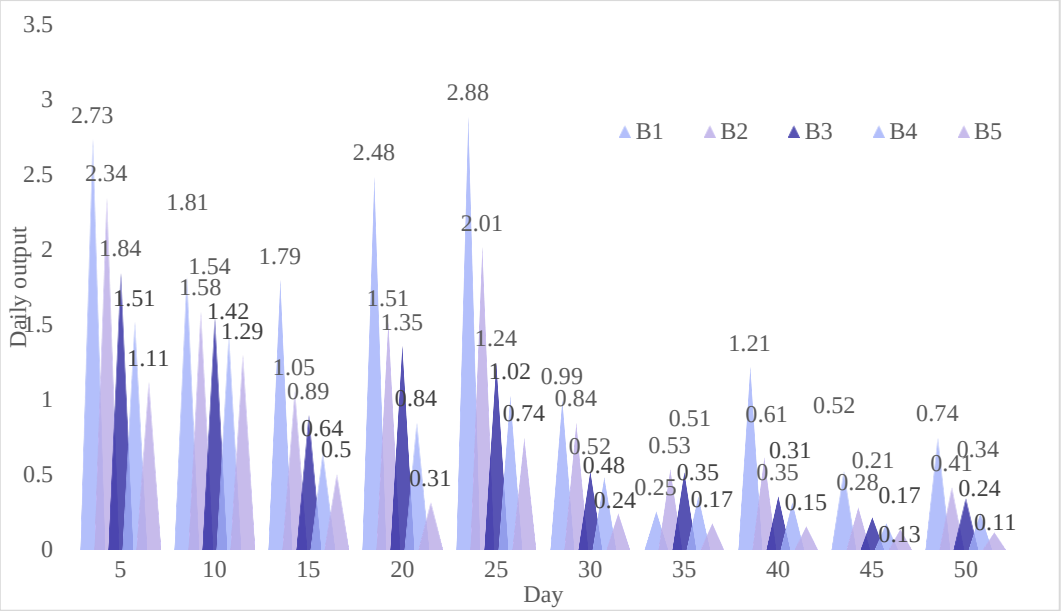

Figure 4. Daily methane production under different inoculation ratios

As shown in Figure 4, there was a peak of daily output on the 5th and 25th days. After 30 days, there was a cliff-like decline and remained stable.

4.2. Influence of Pretreatment Method on Anaerobic Digestion Test

The same straw was subjected to different pretreatments, and then the high-temperature two-phase anaerobic digestion test was carried out under the condition that the organic loading ratio was 20gVS/L and the inoculation ratio was 1. Group C1 and C2 were treated with ammoniating treatment and multi-strain co-treatment, respectively. The changes in pH, ammonia nitrogen content and lignocellulose degradation rate on the 5th, 10th, 15, 20th, 25th, 30th, and 35th days are as follows:

Table 4. Test results under different pretreatment methods

Time (d)	pH		Ammonia nitrogen content (mg/L)		Lignocellulose degradation rate (%)	
	C1	C2	C1	C2	C1	C2
5	7.5	7.51	954.7	874.3	9.4%	8.74%
10	7.24	7.22	1088.4	911.6	13.66%	12.97%
15	7.55	7.26	998.8	984.5	15.53%	16.49%
20	7.6	7.13	1120.3	1018.1	19.07%	20.52%
25	7.49	7.47	2136.1	1314.9	21.88%	25.11%
30	7.32	7.38	4485.1	1498.2	23.24%	29.63%
35	7.56	7.71	3984.2	1138.7	24.14%	35.33%

As shown in Table 4, different pretreatment methods will bring different test results, and the pH value, ammonia nitrogen content and lignocellulose degradation rate in the reactor after anaerobic digestion are different.

(1) Changes in pH value under different pretreatment methods

Under ammoniating treatment and multi-strain co-treatment, the pH value in the reactor changes as follows:

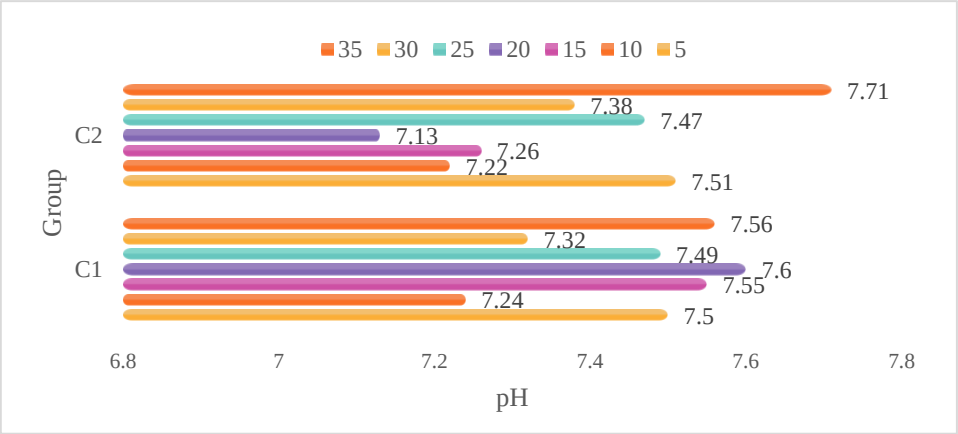

Figure 5. pH value under different pretreatment methods

As shown in Figure 5, the pH value dropped a few days before the fermentation because the reactor was in the acid production stage. With the increase of time, the pH value of the two groups is between 7 and 8, which is very suitable for methanogenesis and survival. This shows that ammoniating treatment and multi-strain co-treatment have little effect on pH.

(2) Changes of ammonia nitrogen under different pretreatment methods

The content of ammonia nitrogen will directly affect the rate of anaerobic digestion, and too high a concentration will inhibit the production of methanogens. Under the ammoniation treatment and multi-strain co-treatment mode, the ammonia nitrogen content in the reactor changes as follows:

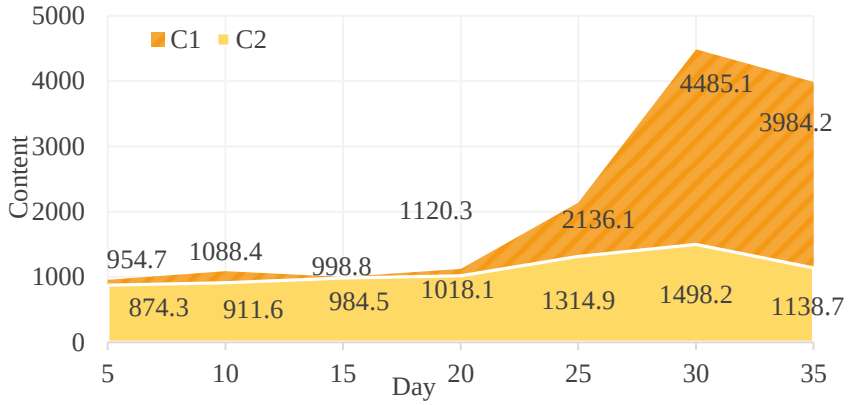

Figure 6. Ammonia nitrogen content under different pretreatment methods

As shown in Figure 6, the ammonia nitrogen content of the two groups was similar in the first 20 days. After the 25th day, the ammonia nitrogen content of the ammoniated C1 group increased significantly, reaching a maximum of 4485.1 mg/L. This indicates that the ammoniating treatment will produce high concentrations of ammonia nitrogen and inhibit the production of methane bacteria.

(3) Changes in lignocellulose degradation under different pretreatment methods

Lignocellulose and hemicellulose will hinder the contact between straw cellulose and enzyme molecules and reduce its utilization rate. Therefore, the degradation rate of lignocellulose is an important indicator to measure the effect of anaerobic digestion. Under the ammoniation treatment

and multi-strain co-processing, the degradation rate of lignocellulose in the reactor changes as follows:

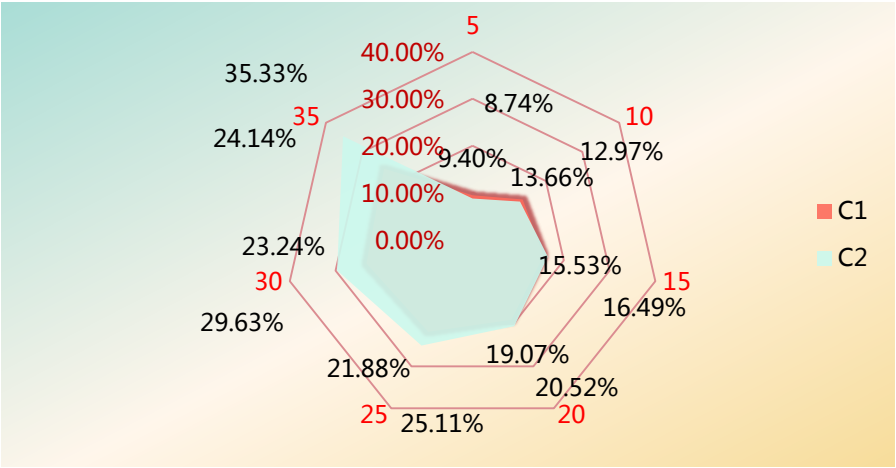

Figure 7. Lignocellulose degradation rate under different pretreatment methods

As shown in Figure 7, with the increase of time, the degradation rate of lignocellulose continues to increase, and the degradation rate of lignocellulose in the ammoniated treatment C1 group is ultimately lower than that in the multi-strain co-treatment group C2. On the 35th day, the two were 24.14% and 35.33%, respectively.

The above results show that the effect of multi-strain synergistic treatment is better than that of ammoniating treatment, with lower ammonia nitrogen content and higher lignocellulose degradation rate, which can increase gas production.

4.3. Effect of Straw Types on Anaerobic Digestion Test

The rice straw and corn straw were treated with multi-strains, and then the high temperature two-phase anaerobic digestion test was carried out under the condition that the organic loading ratio was 20gVS/L and the inoculation ratio was 1. Group D1 and D2 represent rice straw and corn stalk, respectively. The changes in pH, ammonia nitrogen content and lignocellulose degradation rate on the 5th, 10th, 15, 20th, 25th, 30th, and 35th days are as follows:

Table 5. Test results of different straw types

Time (d)	pH		Ammonia nitrogen content (mg/L)		Lignocellulose degradation rate (%)	
	D1	D2	D1	D2	D1	D2
5	6.23	6.71	634.7	714.3	9.41%	9.94%
10	6.47	6.92	687.3	811.1	19.62%	12.88%
15	7.15	7.26	738.8	883.5	21.35%	17.79%
20	7.26	7.43	820.4	918.4	25.27%	19.58%
25	7.39	7.57	836.2	994.3	29.18%	20.17%
30	7.42	7.88	885.7	1098.1	33.04%	22.68%
35	7.56	8.21	984.2	1128.6	34.54%	25.38%

As shown in Table 5, different straws have different test results even under the same reaction conditions because of their different compositions. After anaerobic digestion, the pH value, ammonia nitrogen content and lignocellulose degradation rate in the reactor are different.

Effect of straw types on pH

The pH value changes in the rice straw and corn straw reactor are as follows:

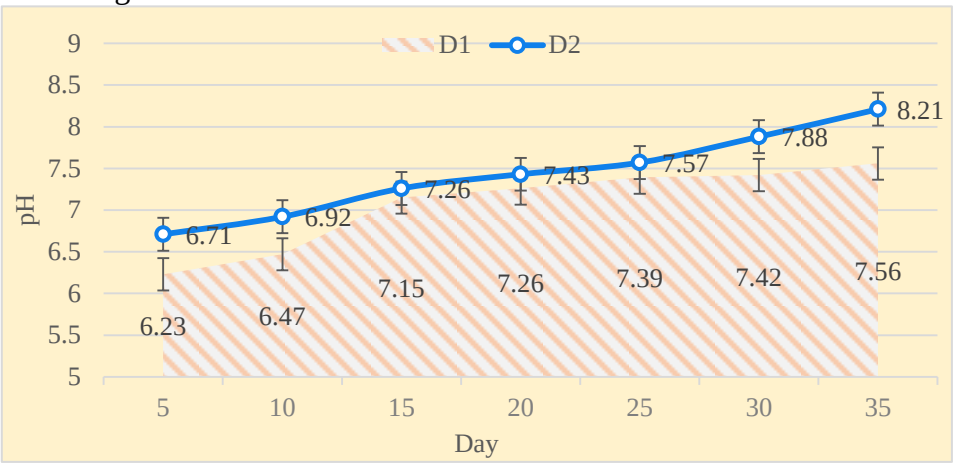

Figure 8. The effect of straw types on pH

As shown in Figure 8, the pH values in the acid production stage were all low, and with the increase of time, the pH values of the two groups continued to increase. On the 35th day, the pH values of the rice straw and corn straw reactors were 7.56 and 8.21. The pH value of the rice straw reactor is acidic, and the environment is more suitable for methane production.

(2) Effect of straw types on ammonia nitrogen content

The changes of ammonia nitrogen content in rice straw and corn straw reactors are as follows:

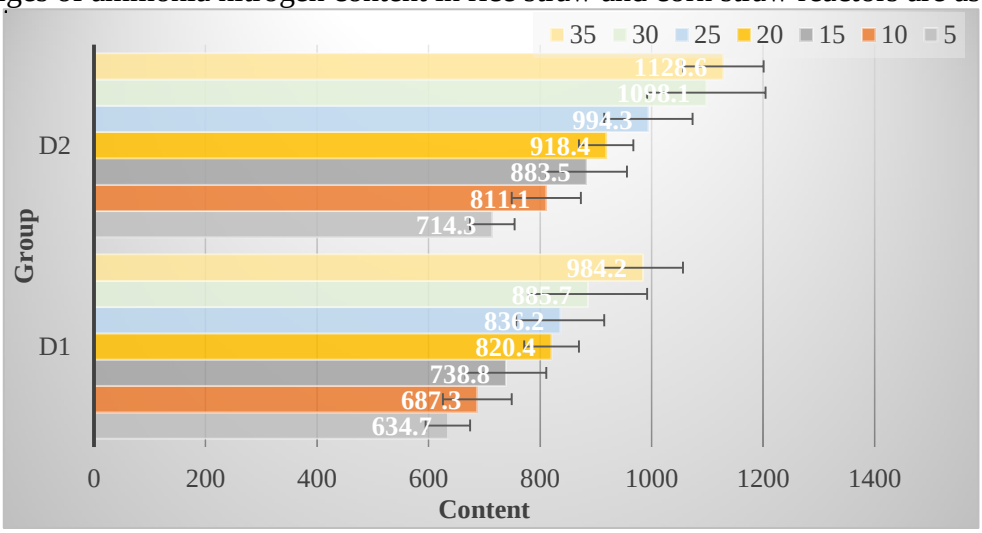

Figure 9. Effect of straw types on ammonia nitrogen content

As shown in Figure 9, the ammonia nitrogen content of the two groups was significantly different. On the 35th day, the ammonia nitrogen content of rice straw and corn straw were 984.2 and 1128.6 mg/L, respectively. This indicates that corn stover will produce high concentrations of ammonia nitrogen, inhibiting the production of methanogens.

(3) Effect of straw types on the degradation rate of lignocellulose

Although the cellulose content of rice straw and corn stalk is different, the comparison of degradation rate is of reference significance. Changes in the degradation rate of lignocellulose in the reactor are as follows:

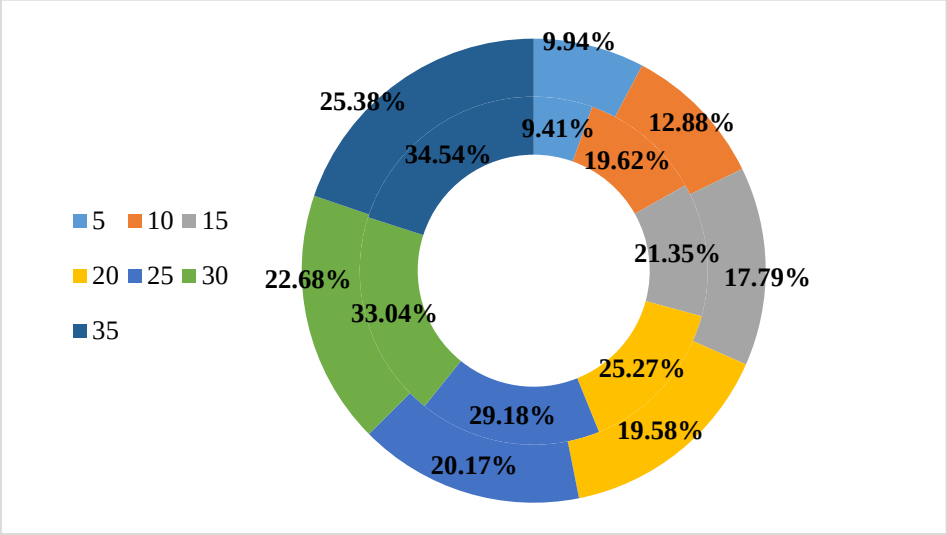

Figure 10. Effect of straw types on the degradation rate of lignocellulose

As shown in Figure 10, the degradation rate of lignocellulose of rice straw was higher than that of corn straw, and the degradation rates of the two were 34.54% and 25.38% on the 35th day.

The above results show that under the same reaction conditions, rice straw is better than corn straw, has a lower ammonia nitrogen content and a higher degradation rate of lignocellulose, and its gas production and utilization are higher.

5. Conclusion

The use of crop straw as feed can supplement the shortage of feed, and can also save energy and reduce resource waste. It is an effective method for the reuse of crop straw. The cellulose in crop straw can be used as a new raw material for industrial polymers. Crop stalks can also be directly returned to the field, which helps to improve the ecological environment of the farmland and has an obvious and continuous effect of increasing production.

The influencing factors of anaerobic digestion technology include pretreatment method, carbon to nitrogen ratio, pH value, temperature and organic load. Different pretreatment methods will change the characteristics of the reaction object to varying degrees. Anaerobic digestion technology can be divided into single-phase anaerobic digestion and two-phase anaerobic digestion. High temperature, medium temperature and low temperature anaerobic digestion technology, wet and dry anaerobic digestion technology, sequential batch, semi-sequential batch and continuous anaerobic digestion technology.

The higher the organic loading ratio, the higher the single-day methane production and the more accumulated daily methane production. When the inoculation ratio is about 1, its utilization is the highest. The effect of multi-strain synergistic treatment is better than that of ammoniating treatment, with lower ammonia nitrogen content and higher lignocellulose degradation rate, which can increase gas production. Under the same reaction conditions, rice straw has better effects than corn straw, has lower ammonia nitrogen content and higher lignocellulose degradation rate, and its gas production and utilization are higher.

Funding

This article is not supported by any foundation.

Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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