The study of influencing factors to straw mixed with livestock manure anaerobic fermentation

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Abstract. Anaerobic fermentation of mixed raw materials is an effective method to solve the single raw material fermentation instability and adjust the carbon to nitrogen ratio during the fermentation process. In this study, manure and straw were used as mixed fermentation raw materials. The effects of mixed raw material types, total solid concentration, inoculum types and inoculation ratio on gas production efficiency were studied. The experimental results show that the total TS has the greatest effect on the biogas gas production effect. The pig manure and rice stalks are used as the mixed raw materials. The total TS is set to 10%. The inoculum is selected from the biogas slurry. The inoculation ratio is 30%. The gas rate is 0.61 L/(L·d). The influence of four factors on gas production performance is: total solid concentration > fermentation raw material > types of inoculum > inoculation ratio. The test results have certain reference significance for anaerobic digestion of mixed raw materials.

1. Introduction
With the development of society, China's agriculture and animal husbandry have been developing towards intensification and industrialization, and the ensuing agricultural and animal husbandry wastes have also caused certain environmental pollution problems [1]. According to the survey and statistics, in 2015, the theoretical amount of straw in China was 1.04 billion tons, and about 900 million tons of straw could be collected. 180 million tons of straw were not effectively utilized, and most of them were burned in the field. At the same time, China's animal husbandry is developing rapidly, and the scale of livestock and poultry breeding industry is constantly expanding. Large-scale livestock and poultry farms produce 2.05 billion tons of livestock manure every year, and 56% of them are still not effectively utilized [2]. Biogas digestion technology is a clean and renewable energy technology, which can make full use of waste biomass resources such as crop straw and livestock manure, realize harmless treatment and resource utilization of these wastes, and alleviate environmental pollution and energy supply pressure [3,4]. So far, a lot of research has been done on the application of single fermentation material in anaerobic fermentation, and the technology has been relatively mature. However, crop straw has high cellulose and lignin content, and there is a layer of wax on the surface that is not conducive to the adhesion of microorganisms, resulting in low degradation rate, long anaerobic digestion time, prone to floating delamination, crusts in the tank, and difficulty in discharging[5, 6]. As a fermentation substrate, mixed raw materials have the advantages of stable and efficient gas production, which has been widely studied by scholars. It is found that the mixture ratio, carbon and nitrogen ratio, total solid concentration,
inoculant, temperature and pretreatment have great influence on the gas production effect of anaerobic fermentation. Yao X [7], Li Y [8], Li Y [9] et al. studied the mixed anaerobic fermentation of cow manure and corn straw, and the research results showed that the mixed raw material could improve the yield of the mixed raw material, and the mixed anaerobic fermentation of cow manure and corn straw produced gas better than the fermentation of single raw material. Ning J [1], Wang H [10] et al. studied the gas production effect of mixed anaerobic fermentation of pig manure and corn straw, and found that the stable operation and gas production performance of the mixed raw material anaerobic co-digestion reaction system was more stable. The degradation of organic matter and the change of pH in the total solid concentration anaerobic fermentation system have a certain influence, and the biogas yield will increase with the increase of concentration within a certain concentration range [11]. Song S et al. [12] studied the influence of temperature and total solid concentration on the gas production characteristics of mixed fermentation of faecal stalk, and the results showed that the material liquid concentration had a great influence on the biogas fermentation, and the optimal concentration of fermentation material liquid was different at different temperatures. Meng X found in his study that gas production increased linearly with substrate concentration (8% ~15%) [13]. The inoculum can provide the bacteria and microorganisms in the start-up phase for anaerobic fermentation, which can shorten the start-up time, and the size of the inoculation ratio will also affect the gas production and methane production. Within a certain range, the methane production increases with the increase of the inoculation ratio [14,15].

The purpose of this study is to study the influence of different experimental factors on the anaerobic fermentation of mixed raw materials. By observing and studying the change rules of pH, gas production and methane content, better mixed fermentation technological parameters are obtained, which provides certain theoretical basis for the practical application of mixed anaerobic fermentation to produce methane.

2. Methods

2.1. Raw material and inoculum
In the straw experiment, three types of wheat straw, corn straw and rice straw were selected, which were taken from the experimental field of the production and breeding base of Henan Agricultural University. After natural air drying, they were mechanically crushed with a crusher to a particle size of 0.5-1 cm, and pre-treated before the experiment. The experimental manure was selected from cow manure, chicken manure and pig manure. Taken from the production and breeding base of Mao Zhuang, Zhengzhou, Henan and the pig farm of Xin Zheng. Three types of inoculum were selected, which were cow dung, sludge and biogas slurry after pile-up treatment. Table 1 shows the characteristics of fermented food.

| Material         | TS (%) | VS (%) | C/N Ratio |
|------------------|--------|--------|-----------|
| Cow manure       | 79.33  | 39.57  | 13.07     |
| Chicken manure   | 82.16  | 68.01  | 3.43      |
| Pig manure       | 82.33  | 66.98  | 7.31      |
| Wheat straw      | 61.99  | 83.03  | 55.25     |
| Corn straw       | 61.26  | 71.55  | 27.82     |
| Rice straw       | 65.18  | 83.14  | 35.26     |
| Inoculum         | 4.79   | 64.38  | —         |

Table 1. Characteristics of substrates and inoculums.
2.2. Experimental device
This experimental device is a self-made small anaerobic fermentation device. The experimental device includes a 500ml jar with a rubber plug as a fermenter, a gas collecting port and a sampling port arranged on the rubber plug, a gas collecting bag with a 1L gas collecting device, the rubber plug and the gas collecting bag are connected with a latex tube and a glass tube, and the sampling port is sealed with an airtight clamp.

2.3. Analytical methods
TS and VS in the samples were determined according to the standard method; C, N and C were analysed and determined by elemental analyser; samples were taken from the fermenter for pH determination every two days, and the biogas yield was collected and measured by PHS-3E pH; through the gas collection bag, and the methane content was determined by Agilent 6820 GC gas chromatograph every two days.

2.4. Analytical methods
The medium temperature fermentation was used in this experiment, and the fermentation temperature was controlled at 35 ±2 degrees Celsius. According to the type of fermentation raw materials, total TS, the type of inoculum and the proportion of inoculation as the influencing factors of the experiment, (A1: Chicken manure and wheat straw, A2: Pig manure and rice straw, A3: Cow dung and corn straw), the experiment was designed according to the orthogonal test table, 9 experimental groups were set up to carry out the experiment, and 3 parallel experiments were set up in each experimental group. The test period was 34 days, the orthogonal experiment table is shown in Table 2.

| reactor No. | Types of raw materials, A | Total TS (%) , B | Inoculated species, C | Inoculant ratio (%) , D |
|-------------|---------------------------|------------------|-----------------------|------------------------|
| R1          | A1                        | 6                | Biogas slurry         | 20                     |
| R2          | A1                        | 8                | Sludge                | 30                     |
| R3          | A1                        | 10               | Compost cow manure    | 40                     |
| R4          | A2                        | 6                | Sludge                | 40                     |
| R5          | A2                        | 8                | Compost cow manure    | 20                     |
| R6          | A2                        | 10               | Biogas slurry         | 30                     |
| R7          | A3                        | 6                | Compost cow manure    | 30                     |
| R8          | A3                        | 8                | Biogas slurry         | 40                     |
| R9          | A3                        | 10               | Sludge                | 20                     |

3. Results and discussion
3.1. Gas production changes in different experimental groups
It can be seen from the graph of daily gas production in Figure 1a that the experimental fermentation period is 34 days. There is a certain difference between the rise rate of the gas production rate and the time to reach the peak of gas production in different experimental groups in the initial fermentation period, of which experimental groups 1, 3, 4 and 6 both increased rapidly in the early stage of the experiment, and reached the peak of gas production on the 8th day, of which the peak value of the experimental group 6 reached a maximum of 996.33ml/d, and the period from the 8th day to the 16th day During the time, the gas production state was maintained at a high level. The experimental group 6 reached the gas production peak 6 days earlier than other experimental gas production peaks, indicating that the experimental group is rich in nutrients, the carbon to nitrogen ratio is appropriate, and appropriate the inoculum provides a wealth of microorganisms that can be quickly started.
It can be seen from the graph of cumulative gas production in Figure 1b that the cumulative gas production of the different experimental groups differs significantly, among which experimental group 6 has the most cumulative gas production, reaching 6799.8 ml after 34 days of fermentation, which is significantly higher than other experimental groups. The cumulative gas production of the experimental groups 7 and 8 is very low. It may be that the cow dung experimental group in this experiment can play a good role in the deployment of nutrients and rapid start under the combination of this ratio and other factors. The gas production effect of the different experimental groups is obviously different. The biogas production of the experimental group with cow dung and straw as mixed raw materials is low. The reason for the analysis may be that cow dung contains more cellulose. Cellulose is a more difficult to degrade material, so fermentation The start of gas production is slow, and the effect of gas production is poor, and the microbial content in pig manure and chicken manure is high, and it can be rich in nutrients after being mixed with straw to achieve rapid start.

3.2. Variation curve of pH value and methane content

PH value is an important index to judge the stability of anaerobic fermentation. Figure 2a shows that in the start-up stage of fermentation, with the acidification of organic matter, the pH value decreases at first, then propagates rapidly with the growth of methanogens, the accumulated organic acid is rapidly consumed, and the pH value gradually increases and finally stabilizes in weak alkalinity, and the anaerobic reaction reaches a stable state. The pH fluctuation range of R6 experimental group is the smallest, and the fermentation process can start quickly and produce gas stably, while R2 experimental group has the lowest pH value in the start-up stage, which leads to the phenomenon of acidosis in anaerobic fermentation, indicating that attention should be paid to the change of pH value in the process of fermentation to ensure that the fermentation can be carried out stably.

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Methane content in biogas is an important index to measure the gas production efficiency of biogas anaerobic fermentation. As shown in the figure 2b, the methane content in the fermentation start-up stage increases rapidly, and the methane content in the experimental group which can produce gas stably can be stabilized at more than 55%. The change trend of methane content is positively correlated with the change of gas production rate.

3.3. Range analysis of volumetric biogas production rate

The volumetric biogas production rate (VBPR) is an important index to measure the gas production effect of biogas fermentation. In a certain range of volumetric load, the volumetric gas production rate will increase with the increase of substrate concentration. In this experiment, the results of orthogonal test on volumetric gas production rate are shown in the table 3:

| Reactor No. | Types of raw materials | Total TS (%) | Inoculated species | Inoculant ratio (%) | VBPR mL/(L·d) |
|-------------|------------------------|--------------|--------------------|---------------------|----------------|
| R1          | A1                     | 6            | Biogas slurry      | 20                  | 410.62         |
| R2          | A1                     | 8            | Sludge             | 30                  | 473.60         |
| R3          | A1                     | 10           | Compost cow manure | 40                  | 496.55         |
| R4          | A2                     | 6            | Sludge             | 40                  | 395.84         |
| R5          | A2                     | 8            | Compost cow manure | 40                  | 410.96         |
| R6          | A2                     | 10           | Biogas slurry      | 30                  | 605.29         |
| R7          | A3                     | 6            | Compost cow manure | 30                  | 168.53         |
| R8          | A3                     | 8            | Biogas slurry      | 40                  | 280.21         |
| R9          | A3                     | 10           | Sludge             | 20                  | 439.02         |

| Mean K1     | 460.49                 | 325.00       | 432.04             | 420.20              |
| Mean K2     | 470.70                 | 388.25       | 436.15             | 415.81              |
| Mean K3     | 295.92                 | 513.62       | 358.68             | 390.87              |
| Range       | 174.77                 | 188.63       | 77.47              | 29.33               |

It can be seen from table that there are significant differences in gas production among different experimental groups under different factor combinations. The volumetric gas production rate of R6 group is the highest, reaching 605.29 mL/(L·d). Orthogonal analysis of the experimental data: the data in the table reflect the influence of various factors on the unit gas production, and the influence value is expressed by the range. The greater the range is, the greater the influence of the factor on the objective function is, and vice versa. It can be concluded from the table that the influence of various factors on volumetric gas production rate is as follows: total solid concentration > fermentation raw material > inoculum type > inoculation ratio.

4. Conclusions

In this experiment, a variety of manure and straw were used as fermentation raw materials, and the effects of fermentation raw materials, total TS, types of inoculum and inoculation ratio on biogas production by mixed fermentation were studied by orthogonal experiment.

1) The pig manure rice is used as the mixed raw material, the total TS is set to 10%, the inoculum is selected as the normal fermentation biogas slurry, the inoculation ratio is set to 30%, the fermentation start time is short, the gas production rate and cumulative gas production are the highest, and the methane content It was 59.69%, and the volumetric gas production rate was 0.61 L/(L·d).

2) Fermentation raw materials, total TS, inoculum type and inoculation ratio will all have a certain effect on the anaerobic fermentation of mixed raw materials. The orthogonal analysis shows that the influence of four factors on gas production performance is: total solid concentration > fermentation raw materials > Type of inoculum > Inoculation ratio. Within a certain concentration range, the volumetric gas production rate will increase with the increase of the substrate concentration; both biogas slurry and sludge have a better promotion effect on fermentation.
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