INTRODUCTION

At present, the resource utilization of solid waste is the focus of research, especially livestock and poultry manure. With the rapid development of China's agriculture and the animal husbandry industry, there will be an increasing amount of organic waste, such as livestock manure, per year. The livestock and poultry industry have gradually changed from the original scattered operation to a large-scale, intensive aquaculture production. However, the utilization rate of livestock manure has not increased with the increase in total stocking. These feces are directly discharged without treatment, and harmful substances such as salts, bacteria, viruses, and so on in the feces enter the soil and water through various channels, thus posing a great threat to human health. In addition, China is the world's largest producer and exporter of edible fungus. The national edible fungus production exceeds 30 million tons, which accounts for more than 75% of the world's total output and ranks first in the world in the industrialization of edible fungus. Increasing waste fungus has been produced...
after the production of edible fungus flourished, and the annual production of waste fungus in China is more than 7 million tons. In the disposal method of waste fungus, except for a small amount was returned to the field to be cultivated as substrate, the majority is directly discarded or burned in situ, thus discarding and breeding a variety of harmful organisms, such as mold, mosquitoes, and mites. It has become a source of pollution and a breeding ground for insects in the production of edible fungus, although burned on the spot may cause air pollution, which wastes resources and pollutes the environment. Studies have shown that the waste fungus is often rich in organic matter, nitrogen, phosphorus, and other nutrients, although rich nutrients can provide a basis for the long-term reproduction of methanogens, not only full utilization can reduce environmental destruction and pollution, but also resource recycling can alleviate the existing energy crisis. In particular, the preparation of organic fertilizers as raw materials can improve soil properties.

Livestock and poultry manure have a low organic carbon to nitrogen (C/N) ratio, contain many easily decomposable compounds, has a short fermentation cycle, and are prone to NH3-N inhibition during their single anaerobic digestion (AD). These are the reasons why they are not used in biogas production. Therefore, an optimum growth environment is required. The C/N ratio of the bacillus is relatively high, and at the same time, waste mushroom fungus contains a large number of nutrients and microorganisms needed for AD. Therefore, using livestock manure and mushroom fungus as a substrate for AD to produce biogas can not only adjust the C/N ratio of the substrate to the optimal state but also make up for the AD undernutrition of a single raw material, promote the conversion of refractory components, such as the lignocellulose that is rich in straw, improve the stability of AD process, and enhance the synergistic effect of microorganisms, thereby making the fermentation substrate more balanced and improving the buffering capacity and stability of the fermentation system. In addition to providing a new way for mushroom fungus recycling, stability is increased and the toxicity inhibition is reduced. At the same time, mushroom fungus plays the role of adding biological bacteria in the AD process. AD of deer manure is influenced by many factors, such as temperature, pH, and biogas slurry addition. In addition, the addition of organic wastewater or limestone can increase AD CH4 production of deer manure. Therefore, the AD of livestock and poultry manure mixed with mushroom residue to produce biogas is relatively complex. In this experiment, cow dung, deer manure, and mushroom fungus were studied. The research on AD of cow dung to produce biogas has been basically mature, and the research on deer manure is less. Therefore, this paper takes deer manure as the research focus to explore the resource utilization of livestock and poultry manure, reduce the cost of treatment of livestock and poultry manure, and save resources at the same time. The purpose of this study was to explore the effects of mixed raw materials on biogas production under different mass ratios and different temperatures. The controllable research provides a theoretical reference for scientific guidance to farmers in the production of livestock manure and mushroom fungus biogas production.

2 | MATERIALS AND METHODS

2.1 | Test materials

Cow dung and deer manure were taken from a cattle farm in the Jingyue District of Changchun and a deer farm in Luxiang Town of Changchun City in November 2018. The cow dung is dried naturally to reduce its moisture content to less than 20%. Considering that deer manure has no odor, it does not need to be air-dried. These were thoroughly mixed together, crushed with a pulverizer (XA-1 solid pulverizer, produced by Changzhou Dingxin Experimental Instrument Co., Ltd.), packed in a zip lock bag, sealed, and stored at 4°C. The discarded mushroom fungus was taken from the fungus base of Jilin Agricultural University, and the mushroom fungus was watered and cultured every day to ensure the normal growth of the strain, the culture time was 1 month. The inoculum is a biogas fermentation bacterium (It is a compound microbial agent containing cellulytic bacteria, methanogens, etc. Produced by Guangxi Desheng New Energy Technology Co., Ltd.), and the physical and chemical properties of the fermentation raw materials are shown in Table 1. According to the moisture content of raw materials and considering the loss of raw materials size reduction process, the consumption of cow dung, deer manure, and mushroom fungus are estimated to be 60 g, 1300 g, and 75 g, respectively.

| TABLE 1 Main properties of cow dung, deer manure, and mushroom fungus |
|-------------------|-----------------|---------------|--------------|----------|-----------|-----------|
| Material          | TS (%)          | VS (%)        | TOC (%)      | TN (%)   | TP (%)    | C/N ratio |
| Deer manure       | 33.71           | 78.86         | 36.88        | 1.64     | 0.62      | 22.48:1   |
| Cow dung          | 85.14           | 70.06         | 49.36        | 2.13     | 1.33      | 23.17:1   |
| Mushroom fungus   | 58.28           | 80.92         | 56.00        | 1.52     | 1.27      | 36.84:1   |

Note: Calculate C/N ratio by determining organic carbon (C) and nitrogen (N).
Abbreviations: TN, total nitrogen; TOC, total organic carbon; TP, total phosphorus; TS, total solid; VS, volatile solids.
2.2 | Experimental device

The experimental device used in this experiment is mainly divided into three parts: the fermentation device, the gas collecting device, and the water collecting device. These devices are connected by a glass tube and a rubber tube. The fermentation bottle is a conical flask with a volume of 500 mL. After filling, it is enclosed in a water bath (HH-4 digital thermostat water bath). The gas collecting bottle is a wide-mouth glass bottle with a volume of 1000 mL, and it contains a saturated NaCl solution. The bottle mouth of fermentation bottle and gas collecting bottle is sealed with a rubber stopper and glass glue. The sampling needle is embedded in the bottle from the rubber stopper for sampling. The water collecting bottle adopts a plastic measuring cylinder with a volume of 1000 mL, and the volume of the generated gas can be accurately determined by the cylinder reading. The specific device is illustrated in Figure 1.

2.3 | Experimental design

Codigestion of two or more substrates can promote biogas production. Deer manure was taken as the main research object in this experiment. The fermented raw materials were cow dung, deer manure, and mushroom fungus in this experiment. Mushroom fungus acts as fermentation raw material and also plays the role of inoculation. Five matching groups were set up and recorded as U1, U2, U3, U4, and U5. The dry matter ratio of raw materials is shown in Table 2. Five hundred grams of fermentation broth was placed in a conical flask at a total solids concentration of 6%, and then, 0.08 g of the microbial agent was added. The experiment was carried out at three constant temperatures of 20°C, 35°C, and 50°C (±1°C). Set up three repeats for each group. The gas collection method was adopted, and the gas sample was collected at the same time every day to record the gas production amount.

Because a lot of research works have been done in the early stage, including AD of deer manure and AD of mixed deer manure and mushroom fungus, the characteristics of AD of deer manure mixed with mushroom fungus have been well understood. The focus of this study is to understand the AD of deer manure after adding cow dung in detail and to obtain the best proportion of cow dung. Therefore, the proportion of cow dung in mixed digestive materials ranges from 0% to 50% and is set by 10% gradient. Considering the small difference between the setting ratio of 10% cow dung and the background value (U1), the proportion experiment was canceled.

The C/N ratios ranged from 22.83 to 27.06 by calculating. Except for group U5, there was little difference in C/N ratio among other groups. The results showed that the AD of cow dung had better gas production effect when the C/N ratio was between 20 and 25.23,24 The C/N ratio of deer manure ranged from 25.72 to 30.06, and the gas production effect is the best at about 25.20,21 Therefore, the C/N ratio index will not have a significant impact on AD gas production.

2.4 | Determination method

1. The TS was measured after drying to a constant weight in an oven (DHG-9076A electrothermal constant temperature blast drying oven) at 105°C ± 2°C.
2. The VS were measured by burning in a box-type resistance furnace (2.5-10 box-type resistance furnace) at 550°C ± 5°C to a constant weight.
3. The TOC test method used was the potassium dichromate oxidation method; the TN was determined by the Kjeldahl method.

| Item             | Experimental group |
|------------------|--------------------|
|                  | U1     | U2    | U3    | U4    | U5    |
| Proportion       |        |       |       |       |       |
| Cow dung         | 0      | 0.2   | 0.3   | 0.4   | 0.5   |
| Deer manure      | 0.7    | 0.5   | 0.4   | 0.3   | 0.5   |
| Mushroom fungus  | 0.3    | 0.3   | 0.3   | 0.3   | 0     |
| C/N ratio        | 26.79  | 26.93 | 27.00 | 27.06 | 22.83 |
method (Ben Ang KA-1 automatic Kjeldahl nitrogen analyzer).25

4. The pH was measured by a lightning magnetic pH meter (Ray-Magnetic PHS-25 pH meter); the volatile fatty acids (VFAs) were determined by colorimetric method.

5. The CH\textsubscript{4} content in the biogas was measured by a gas chromatograph (Agilent 7890A gas chromatograph). The unit gas production (L/g) was calculated by dividing the total gas production (L) by the total dry matter mass (g).26

3 | RESULTS AND DISCUSSION

3.1 | Changes in the daily biogas production at different temperatures

Temperature is the main factor affecting AD of livestock manure.27 Normally, the AD can be divided into low-temperature AD (<25°C), medium-temperature AD (25-45°C), and high-temperature AD (>45°C).28 The 20, 35, and 50°C, respectively, represent three reaction types in different temperature ranges.29-31 Therefore, 25, 35, and 50°C conditions were selected to carry out the experimental study. The effect of different temperatures and different mass fractions on the daily biogas production is shown in Figure 2, when AD of the mushroom fungus and the livestock manure was a combined substrate. Except for at 20°C, the mixed raw materials of various ratios can start to ferment and produce biogas in a short time. Moreover, the overall gas production trend of the experimental group with mushroom fungus was significantly different from that of the experimental group without mushroom fungus. At 20°C, the biogas production was extremely low compared with that of the other two groups. Low temperature not only increases the viscosity of the reaction solution, inhibits the activity of microorganisms including methanogens, and reduces the rate of biochemical reaction, but also increases the solubility of CH\textsubscript{4} in the reaction solution and significantly reduces CH\textsubscript{4} production.32
It is observed from Figure 2B that at a constant temperature of 35°C, the peak of U3 biogas production appeared the earliest (the 8th day after the start of the experiment), the peak value was 283.2 mL·d⁻¹, and a small peak of biogas production appeared on the 41st day. The U1, U2, and U4 reached their respective biogas production peaks on days 10, 12, and 10, with peaks of 502.6, 429.3, and 342.6 mL·d⁻¹, respectively. The peak of U2 came at the latest, reaching the peak of biogas production on the 17th day, with a peak of 217.8 mL·d⁻¹. Except for U2, the biogas production rate of each group changed significantly during the 50-day fermentation process, and the biogas production peak was obvious. The biogas production period was mainly concentrated during the 8th to 20th days and the 37th to 44th days. The peak of U5 biogas production was mainly concentrated during the 17th to 33rd days. After reaching the peak biogas production, the biogas production of each group showed a significant downward trend, and the decline rate of U5 was the fastest.

It is observed from Figure 2C that at a constant temperature of 50°C, the peak of U3 biogas production appeared the earliest (the fourth day after the start of the experiment) and the peak value was 475.3 mL·d⁻¹. U1, U2, and U4 reached their respective biogas production peaks of 234.2, 463.6, and 426.6 mL·d⁻¹, respectively, on the 12th day. The peak of U5 appeared at the latest, reaching the peak of biogas production of 234.7 mL·d⁻¹ on the 23rd day. Except for U5, the biogas production rate of each experimental group changed significantly during the 50-day fermentation process, and the biogas production peak was obvious. The biogas production period was concentrated mainly during the 8th to 25th days. The peak of U5 biogas production was concentrated mainly during the 17th to 29th days. After peak biogas production, the biogas production of each group showed a significant downward trend, and the decline rate of U1 was the fastest.

Mushroom fungus is conducive to rapid biogas production during AD of deer manure, but the effect of composting of other manure is less studied.20 As shown in Figure 2B,C, the peak of biogas production showed that the trend of U5 experimental group was different from that of the other experimental groups at the same temperature, and the peak of biogas production appeared at the latest. This indicated that the addition of mushroom fungus was beneficial to rapid biogas production. The peak of the biogas production period was the earliest in the U3 experimental group, and the peak time of the other three groups appeared to be relatively close, but the high peak had no obvious law. Under the condition of 35°C, U1, U2, U4, and U3 showed a downward trend after the first high peak of biogas production. In the later stage of AD, during the 37th to 44th days, a small peak of biogas production appeared, which indicated that the stability of the system was strong, and the AD system could be brought to the appropriate conditions again by its own regulation. Under the condition of 50°C, the high peak of biogas production during the early stage was concentrated, the biogas production was larger, and the rate of decline was slightly faster than 35°C. There was no small peak during the later AD process. The increase in the production period was shorter, which indicated that the elevated temperature can significantly increase the biogas production rate, but the occurrence time of the biogas production peak did not show a consistent relationship with the temperature increase. Relevant studies show that high-temperature AD is superior to medium- and low-temperature AD.33 The biogas production cycle is shortened at 50°C compared with 35°C, which is obviously advantageous. The U3 experimental group first reached the peak of biogas production, which was conducive to rapid biogas production, and the biogas production effect was better than other experimental groups.

3.2 | Changes in the cumulative biogas production at different temperatures and in different proportions

Accumulated biogas production is an important index for evaluating the effect of biogas production. The variation in cumulative biogas production under different temperature conditions is shown in Figure 3. It is observed from the figure that at the end of AD, the cumulative biogas production under both temperature conditions was U3 > U2 > U4 > U1 > U5. Except for U5, within the first 0 to 25 days of the experiment, the biogas production rate of each group at 50°C was significantly higher than at 35°C, which was due to the intense microbial activity under high-temperature environmental conditions during AD. Conducive to the efficient operation of the experiment and the decomposition of organic matter, the biogas production rate in the early stage was faster at 50°C, the decomposition of organic matter was mainly concentrated in the early stage, and the accumulation at 50°C after the 25th day was due to insufficient fermentation substrate in the system. The biogas production tended to be flat. At 35°C, the microbial activity was relatively slow, the biogas production rate was relatively uniform, the cumulative biogas production had been on the rise and the biogas production rate had increased in the later stage of AD (45-50 days), and overall, the system was more stable relative to 50°C. In the U5 experimental group, due to the lack of mushroom fungus, not provided a suitable microbial flora and a suitable C/N ratio for the AD system. Therefore, during the initial microbial growth period, the cumulative biogas production increased slowly, and the total biogas production was lower than that of the other groups.

It was found that the biogas production regularity of U3 experimental group was basically the same as that of AD of cow dung alone at 55°C.34 As shown in Figure 3, the total biogas production of the U3 experimental groups was about 6000 mL at 35°C, while that of the total biogas production
was close to 7000 mL at 50°C, and the biogas production increased by about 10%. After calculation, the highest daily biogas production of U3 experimental group at 55°C is 521.4 mL·d⁻¹, and the unit TS biogas production rate can reach 0.23 L·g⁻¹. By analyzing the cumulative biogas production data, it is observed that the production cycle is effectively shortened and biogas production is increased in high-temperature conditions.

3.3 | Changes in the VFAs and pH of each proportion at different temperatures

The changes of VFAs in each experimental group were shown in Figure 4 at 35 and 50°C. At 35 and 50°C, the changes of VFAs in all experimental groups except U5 experimental group increased first and then decreased. It shows that the acidification of U5 experimental group is serious, which has seriously affected the biogas production. The content of VFAs in U4 experimental group was significantly higher than that in U1, U2, and U3, which indicated that the acidification also existed in U4 group.

The pH is an important condition for AD of livestock manure. The pH changes under different temperature conditions are shown in Figure 5. Methanogens are anaerobic bacteria, and the requirements for the acid-alkaline environment are also high. Under acidic conditions, the AD process cannot be carried out normally, so the pH of the fermentation system is critical. Under the condition of 20°C, the pH in the experimental period was always between 4 and 6. Under the acidic environment, suitable environmental conditions for the methanogens could not be reached, so the AD experiment failed.

The results showed that the pH of AD was affected by temperature. As shown in Figure 5, the U5 experimental group is low in pH, which indicates that mushroom fungus can effectively alleviate the unstable state of the AD
system to a certain extent, thereby improving the buffer capacity of the system, effectively inhibiting the acidification process, and increasing the biogas production. Under the two temperature conditions, the pH of U1, U2, U3, and U4 showed a decreasing trend first and then increased after 0-10 days. This pattern occurs because the acid production stage predominates during the initial stage of the AD reaction, thus producing a large amount of VFAs, which results in a low pH. As the reaction progresses, the acid-producing stage and the methanogenic stage gradually reach a state of equilibrium, the pH is maintained in a relatively stable range, so the pH is maintained at approximately 7 in the middle and late stages of the reaction. Under the high-temperature condition of 50°C, the AD system returned to a weakly alkaline state during the 5th to 10th days, which was earlier than under the temperature condition of 35°C (10-15 days), indicating that the system has a self-regulation ability under high-temperature conditions, the pH can reach the appropriate state earlier. Therefore, the starting speed is faster under high-temperature conditions, the time required for AD is short, and the total biogas production is relatively large.

3.4 Changes in the CH4 content of different proportions at different temperatures

The CH4 content in biogas directly affects the quality of biogas. Generally, the CH4 content is more than 50%, which is enough to ensure the combustion quality of biogas. In the initial stage of the AD experiment, the CH4 content in the produced biogas is relatively low, this is because acid-producing bacteria play an important role in the early stage of fermentation, which store acetic acid and other substances for methanogens. Severe acid-producing processes can lead to a decrease in the number or activity of methanogens. With the increased rate of CH4, methanogens are enriched and the corresponding CH4 content shows a rapid increase. The CH4 content in biogas is basically maintained at a certain level after reaching a certain peak.

As observed in Figure 6, after the start of fermentation, the experimental group at 50°C increased the CH4 content faster than the experimental group at 35°C. At 50°C, the CH4 content of U3 experimental group reached 52.21% at the 4th day after the start of fermentation and the CH4 content remained above 50% after the 6th day; the U1 and U2 experimental group CH4 contents reached more than 50% on the 8th day, and the U4 and U5 experimental group CH4 contents reached more than 50% on the 9th day. The change at 35°C was basically the same as that at 50°C. In the later stage, the CH4 content of U5 decreased significantly compared with that of the other three groups. The CH4 content of the other four groups showed a different degree decline after the high peak, but toward the end of AD, the CH4 content also maintained a high level above 35%, which shows that the amount of CH4 is related to the pH and the amount of organic acid. Since U5 experimental group is not added with mushroom fungus, the system is gradually acidified in the later stage, and the system cannot self-regulate, so the CH4 content is low. These results fully indicate that the mixed fermentation of mushroom fungus and livestock manure can buffer the acid-producing behavior during the fermentation process, which improve the quality of biogas. The CH4 content of U3 experimental group is the highest and the total biogas production is the largest, so the total CH4 production is the largest (Figures 3 and 6).
3.5  Relationship between cumulative biogas production, the fermentation temperature, and the raw material ratio

It is observed from Figures 7 and 8 that at different temperatures, the cumulative biogas production is $U_3 > U_2 > U_4 > U_1 > U_5$, only the cumulative biogas production of $U_1$ experimental group at 35°C was higher than at 50°C. The cumulative biogas production except $U_1$ experimental group increased with increasing temperature. There was a significant difference in the cumulative biogas production at all temperatures, which indicated that the AD at 50°C temperatures was significantly better than at 50°C. The cumulative biogas production except $U_1$ experimental group increased with increasing temperature. Under the same temperature conditions, the effect of $U_3$ experimental group is the best (Figure 8). The result shows that the heat generated by AD can basically maintain 35°C temperature condition in actual biogas engineering, while the experimental condition at 50°C needs to provide external heating. Compared with the condition of 35°C, the total output of biogas at 50°C is only increased by about 10%. Therefore, from the point of view of engineering application, the temperature condition of 35°C may be more advantageous.

Because the C/N ratio of mushroom fungus is significantly higher than the optimal C/N ratio of biogas AD, it is not suitable for biogas production by AD alone. It was found that under the condition of sludge inoculation, the biogas production of mushroom fungus was 0.119-0.143 m$^3$·kg$^{-1}$, and the average CH$_4$ content was...
The results showed that AD of single cow dung resulted in a biogas yield of 0.032 m$^3$·kg$^{-1}$ and CH$_4$ content of 65.6%. The maximum potential of biogas production by AD of cow dung alone is significantly lower than that of mixing deer manure, cow dung, and mushroom fungus. The results of AD of deer manure showed that the cumulative biogas production was less than 0.02 m$^3$·kg$^{-1}$ and the CH$_4$ content was less than 50%. As shown in Figures 2 and 3, the biogas yield by AD of mixing deer manure and mushroom fungus was significantly lower than that of mixing deer manure, cow dung, and mushroom fungus, because the mixing of various materials can overcome the deficiency of unbalanced nutrient content in single material AD and provide a better environment for the growth of anaerobic microorganisms, thereby significantly improving biogas production. By comparing the U2, U3, and U4 experimental groups, it can be seen that the cumulative biogas production of U3 experimental group is the highest, and the CH$_4$ content is 60%-70% at 50°C.

4 | CONCLUSIONS

In this paper, mushroom fungus, cow dung, and deer manure were used as raw materials for AD. AD experiments were carried out at 20, 35, and 50°C with different dry matter mass ratios. The primary conclusions are as follows:

1. Adding a certain amount of mushroom fungus to the AD system of deer manure and cow dung can effectively inhibit the acidification process, improving the buffer capacity of the system and increasing biogas production.
2. There is a positive correlation between the temperature and biogas production. With the increase in temperature, the cumulative biogas production of each proportion increases accordingly.
3. During whole AD when the proportion is 0.3:0.4:0.3, biogas production is optimal, the maximum biogas production is close to 7000 mL, the highest daily biogas production is 521.4 mL·d$^{-1}$, and the unit TS biogas production rate can reach 0.23 L·g$^{-1}$, which is significantly higher than that of other experimental groups.
4. Under the same amount of mushroom fungus, the total biogas produced by AD of mixing deer manure and cow dung is obviously better than that of single deer manure, and this provides important enlightenment for improving the utilization level of livestock and poultry manure by AD of mixed manure.

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