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Anaerobic Digestion Technology for Methane Production Using Deer Manure Under Different Experimental Conditions

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Abstract: Anaerobic digestion (AD) is an important technology for the treatment of livestock and poultry manure. The optimal experimental conditions were studied, with deer manure as a fermentation material and mushroom residue as an inoculum. At the same time, methane production was increased by adding zeolite and changing the magnetic field conditions. The results showed that a 6% solid content was the best condition for producing methane. The optimal conditions for methane production were obtained by adding 35 g of mushroom residue to 80 g of deer manure at 35 °C. The addition of organic wastewater (OW) improved methane production. The result of improving the methane production factor showed that adding zeolite during the reaction process could increase the methane production rate. When the amount of zeolite was over 8% total solids (TSes), methane production could improve, but the rate decreased. Setting a different magnetic field strength in the AD environment showed that when the distance between the magnetic field and the reactor was 50 mm and the magnetic field strength was 10–50 mT, the methane production increment and the content of methane in the mixed gases increased.

Keywords: deer manure; mushroom residue; anaerobic digestion (AD); methane

1. Introduction

China's livestock and poultry industry is developing rapidly. According to statistics, livestock manure waste production in China was approximately 3.8 billion tons in 2015 [1]. The pathogenic bacteria and malodorous gas that manure produces have puzzled poultry feeders and surrounding residents. Nitrogen and phosphorus in the breeding industry's wastewater can easily cause surface water body eutrophication and result in the death of zooplankters because of asphyxia. The stacking of manure can damage the soil's physicochemical properties because of the accumulation of dissolved salts, such as nitrite [2,3]. Many countries have realized that a preponderance of environmental problems is caused by livestock manure and have performed treatment and resource utilization on livestock manure [4]. At present, a shortage of energy and the utilization of renewable energy are of great significance [5–8]. Resource utilization of livestock manure is an effective method to reduce pollution, specifically the production of methane, and anaerobic digestion (AD) has great potential in this connection [9,10]. At present, AD biogas production technology is relatively more mature than hydrogen production technology [11]. The effect factors of AD include the raw material ratio, temperature, inoculum, feed liquid content, and pH [12–17]. The inoculum plays an important role in accelerating the start of fermentation, improving the gas production rate and maintaining a constant reaction [18,19].

The use of AD started earlier in Europe than it did in China, and earlier research emphasized how to effectively improve methane production [20]. The experiment indicated that adding an effective microorganism to a reactant could enhance the enzymatic activity of AD and increase the gas production quantity [21]. The study found that a method of heat treatment or alkali treatment could improve the AD rate [22–25]. Research has shown that mixed AD could reduce the adverse effects of heavy metals and toxic materials on the whole system better than single-material AD could [26,27]. The AD of pig manure and silage grass or crude syrup could produce more than 300 L/kg of biogas [28,29]. It was found that a mixture of livestock manure and other wastes was better than a mixture of livestock manure and fermentation alone [30].

In the 1880s, southern China had already started using AD to produce biogas, but the application of biogas developed slowly because relevant technology was not available [31,32]. In recent years, the application range of AD has become wider. The results showed that a mixture ratio of rice straw, kitchen waste, and pig manure was 0.4:1.6:1. As for the materials for AD, the C/N ratio was 21.7, and the methane content could reach 72% [33]. Higher proportions of vegetable wastes and higher organic loading rates improved volumetric methane production for the co-digestion of vegetable wastes and swine wastewater, as well as organic matter removal rates, by up to 98% [34]. Most antibiotics could be effectively eliminated by AD, and removal rates ranged from 11% to 86% for livestock manure. AD could completely remove ampicillin, tetracycline, and sulfamethoxydiazine [35,36]. It has been observed that research on fermentation technology is of great significance. Because of the different components in raw materials, the best experimental conditions for biogas production by AD are different. Results have shown that the disintegration performance of waste-activated sludge was effectively improved in the presence of a magnetic field [37]. The magnetic field could improve the AD of municipal sludge to produce biogas [38]. A correlation between the time of exposure to the constant magnetic field and the values of parameters characterizing the methane production was found in the AD of algal biomass [39]. The results showed that biochar, as an additive, could simultaneously promote AD process stability in organic wastes [40,41]. Natural Australian zeolite was shown to be a potential sorbent for the removal of NH^{4+} during the AD of swine manure [42]. The results indicated that zeolite had a positive effect on the AD process of the wastewater [43]. Clay residue had a positive effect as an inorganic additive for stimulating the anaerobic process [44]. In conclusion, additives are of great significance in the improvement of the AD of organic wastes.

Statistics show that there are more than 3 million deer in stock in China. The price of venison is five times that of beef. The price of deer antler is as high as 5000 yuan per kilogram. The cost of raising an adult deer is about 0.15 times that of a cow. The breeding scale of deer is growing, because they have a superior economic value. The AD of deer manure to produce biogas has the appropriate ratio of carbon to nitrogen, and the biogas residue produced by AD is used to prepare organic fertilizer for indoor flower plants, with a high economic value [45]. The use ratio of a large amount of deer manure is low, but there has not been enough research aimed at the resource utilization of deer manure. In addition, cow manure, chicken manure, sheep manure, and horse manure have been studied more than deer manure. Therefore, it is important to research the preparation of biogas using deer manure. AD experiments are also affected by inoculants. Sludge is the main inoculant currently used [46,47]. Because sludge contains heavy metals, it is difficult to treat the fermented biogas residue. At present, sludge is the main inoculant used, but the sludge contains heavy metals, which makes it difficult to treat biogas sludge. Mushroom residue has been used as an inoculant to effectively avoid this problem. In addition, the treatment of mushroom residue as solid waste is also a technical problem, and it is more meaningful to use mushroom residue as an inoculant. Adding zeolite to the AD system can improve gas production, but the gas production of different dosages is unknown. A magnetic field can promote the AD of manure and increase methane production [48]. Methane production can be improved under appropriate magnetic field conditions, but the gas production of different magnetic field intensities on AD is unknown. This paper studies the effects of total solids (TSes), an additive amount of mushroom residue, organic wastewater (OW), and temperature condition on producing methane by determining the total amount of biogas, pH, volatile fatty acids (VFAs), and ammonia nitrogen content. Under the most suitable conditions, the efficiency of deer manure AD was improved by adding OW and zeolite. Additionally, the yield and methane production rate of deer manure was improved under the optimal magnetic field. Finally, biogas production of the AD of deer manure could be increased.

2. Materials and Methods

2.1. Experimental Materials

Deer manure: Fresh deer manure was collected from the Shuangyang District Changchun City deer farm. It was fully mixed after collection, installed in a valve bag, and hermetically preserved at 4 °C. Mushroom residue: Mushroom residue was collected from the polysaccharide waste of a *Pleurotus ostreatus* production plant and was sprayed with water every day after being retrieved to ensure the activity of the strain. OW:OW was collected from a tofu workshop, and the pH was adjusted to approximately 7 by adding lye. Zeolite: Natural zeolite was produced in Liaoning, China, with a particle size of 8 to 10 mm. Magnetic powder: Iron powder was magnetized with a magnet, and the magnetic field intensity was measured by an HT 20 portable digital Tesla meter (Shanghai Hengtong Magnetoelectric Technology Limited Company, Shanghai, China).

2.2. Experimental Method

The physicochemical properties of the fermentation materials were determined before the AD experiment. The composition content of the experimental materials and the carbon/nitrogen ratio are shown in Table 1. The experimental study was carried out to explore the appropriate experimental conditions for the AD of deer manure by setting the conditions concerning the amount of mushroom residue, temperature, and TSes. Basic physical and chemical properties were characterized by testing the TS, volatile solid (VS), total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) indicators in the experimental process. The samples were dried to a constant weight in an oven at 105 °C. Then, TSes were measured, and after this, the sample was ignited to a constant weight in a box-type resistance furnace at 550 °C. Next, VSes were measured, and the selected testing method for TOC was the potassium dichromate method. TN was determined using the alkaline potassium persulfate digestion UV spectrophotometric method, and TP was determined using the ammonium molybdate spectrophotometric method. VFAs were determined by colorimetry. The change in ammonia nitrogen content was determined by Nessler's reagent spectrophotometry [10,46,49,50].

Table 1. Fermentative substrate characteristics. TS: total solid; VS: volatile solid.

Material	TS (%)	VS (%)	C (g/g·TS) (%)	N (g/g·TS) (%)	P (g/g·TS) (%)	C/N
Deer manure	67.82–73.69	77.83–81.59	49.89–54.43	1.68–2.02	0.49–0.65	25.72–30.06
Mushroom residue	50.63–53.96	87.65–90.38	33.69–35.85	1.51–1.63	1.39–1.57	21.96–23.11

Note: Calculate C/N by determining carbon (C) and nitrogen (N).

The experiments were conducted using batch fermentation. Solid materials were no longer added during the fermentation process. The fermentation mixture was stirred manually every two days. Sampling was conducted every 5 days to determine the TSes, while the water was supplemented and the TSes were prevented from changing. The water content was supplemented and adjusted during the experiment. This prevented the total solid content from changing. The experimental device was divided into an AD flask, a biogas collecting bottle, and a graduated cylinder (Figure 1). The filter flask, which had a volume of 1500 mL, was selected for the AD flask. During the AD experiment, the biogas produced by the AD flask entered the biogas collection bottle through a biogas conduit. The biogas pushed the saturated salt water, which was stored in the biogas collection bottle, into the graduated cylinder. Biogas samples were taken from the biogas outlet. Biogas residue and slurry were taken from the liquid outlet. The experiment was carried out with a water bath heater, and the temperature was maintained under the set conditions.

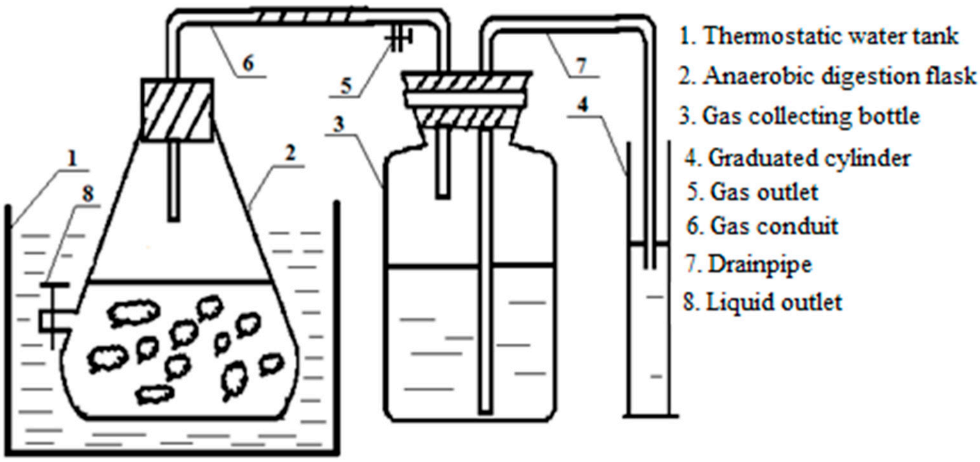

Figure 1. Sketch map of the anaerobic digestion (AD) equipment.

The pH was measured by a pH meter. The daily biogas production was determined by the saturated brine discharge volume. The methane content was determined by gas chromatography. The VFAs were determined by the colorimetric method. The change in the ammonia nitrogen content was determined by Nessler's reagent spectrophotometric method.

Deer manure was used as the fermentation medium in this experiment. Mushroom residue acted as both an inoculant and a fermentation substrate while participating in the reaction. A flow chart of the experiment is shown in Figure 2. The formulas for calculating the production of methane and methane were as follows:

$$V_{biogas} = P_1 V_1 / P_0, \tag{1}$$

where V_{biogas} represents the daily biogas volume (mL), P_1 is the pressure of the gas in the container (kPa), V_1 refers to the liquid volume in the graduated cylinder (mL), and P_0 refers to the atmospheric pressure (101.325 kPa). Equation (2) is

$$V_{d.CH_4} = V_{biogas} \times m_d, \tag{2}$$

where $V_{d.\mathrm{CH}_4}$ represents the daily methane volume (mL), and m_d refers to the daily average methane content (%). Equation (3) is

$$V_{T.CH_4} = V_{biogas} \times m_d \times t, \tag{3}$$

where $V_{T.\mathrm{CH}_4}$ represents the total methane volume (mL), and t is the day (d).

The main parameters of the experiment are shown in Table 2. In order to study the effect of OW on the AD system, two groups of experiments were set up. One group used distilled water (DW) to regulate the water content, and the other group used OW to regulate the water content. The other conditions were the same in both experiments. The effect of zeolite addition on the AD system was studied, and the zeolite addition was 0%, 2%, 4%, 6.25%, 8%, 10%, and 12% (TSes), all of which were evenly mixed with fermentation materials. The magnetic field intensity was set at 0, 5, 10, 20, 50, 70, 90, 120, 150, and 190 mT in the sixth group. The magnetic field source used in our experiment was provided by a magnetic powder. The weight of the magnetic powder was 6 g. The magnetic field strength was measured at 50 mm from the magnetic powder. The measured magnetic powder was evenly mixed with the fermentation material. The data were sorted out using IBM' (International Business Machines Corporation, Armonk, NY, USA) SPSS 21.0 version software.

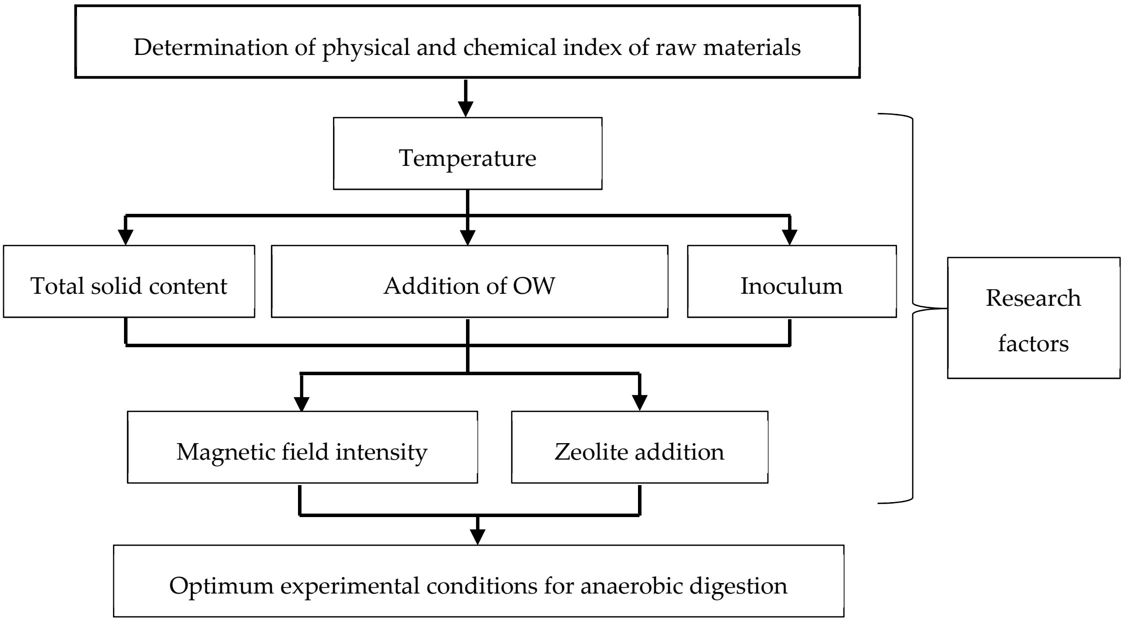

Figure 2. Flow chart of the experiment.

Table 2. Experimental conditions under different research factors. DW: distilled water; OW: organic wastewater; VFAs: volatile fatty acids.

Research Factors	Experimental Condition					Total Volume (mL)	Liquid Addition
	Temperature (°C)	Deer Manure (g)	Mushroom Residue (g)	TS (%)	Inoculum (%)		
Temperature	20						
	35	80	50	8	38.5	965.8	DW
	50						
Addition of mushroom residue	35	80	20	8	20.0	808.6	DW
			35		30.4	887.2	
			50		38.5	965.8	
			80		50.0	1123.1	
TS	35	80	50	6	38.5	1287.1	DW
				8		965.8	
				10		772.1	
OW	35	80	——	8	——	965.8	DW
						703.8	OW
Zeolite	35	80	35	6	30.4	1296.3	DW
Magnetic field	35	80	35	6	30.4	1296.3	DW

Note: (1) Use a water bath pot to control the experimental temperature; (2) the TSes are controlled by adding water; (3) the biogas production was measured every day, and the pH, VFAs, and ammonia nitrogen contents were measured every 5 days.

3. Results

3.1. *Effect of Temperature on the AD of Deer Manure*

3.1.1. The Change in Methane Content and pH in the Fermentation Period

The experimental results showed that total methane production and daily methane production were the lowest at 20 °C. At 50 °C, the fluctuation in daily methane production was larger: The maximum methane production was 840 mL on the 28th day, and unit solid methane production was 11 mL/g·TS. At 35 °C, the fluctuation in daily methane production was the largest: The maximum methane production was 840 mL on the 56th day, and the unit solid methane production was 11 mL/g·TS. Because the AD system had no acid–base adjustment, and the experimental container capacity was small, the system was easily affected by the outside world. Thus, methane production fluctuated greatly, and heat preservation needed to be further strengthened. Based on the experimental data and analysis results, at 35 °C and 50 °C, cumulative methane production was about 200 mL/g·TS, and daily average methane production was about 3.0 mL/g·TS (Figure 3).

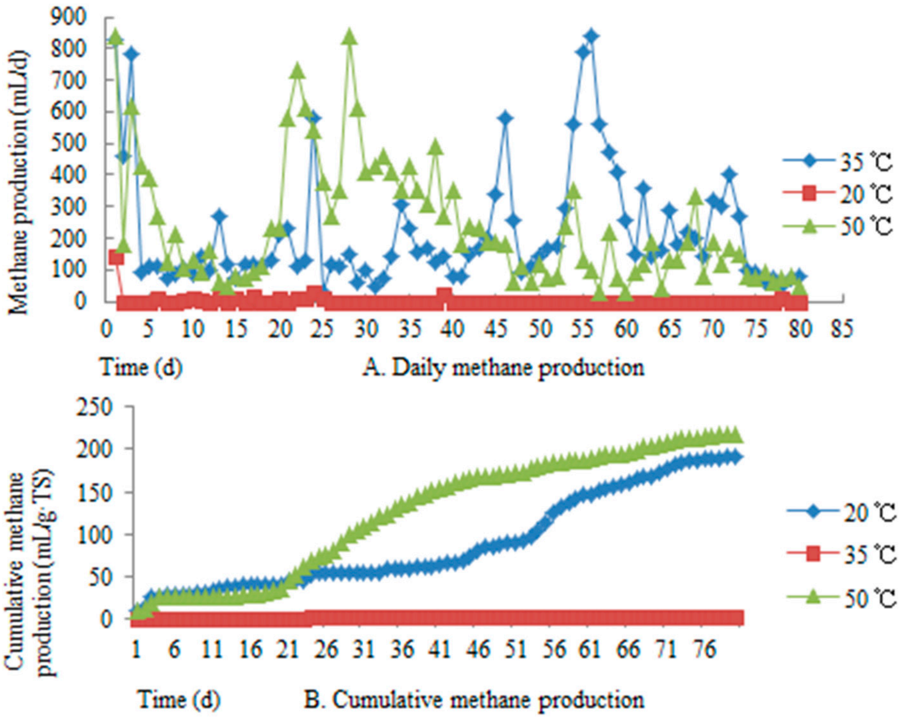

Figure 3. The change in methane production at different temperatures.

The results showed that the AD experiments, at different temperatures, all underwent acidification, without an acid–base adjustment (Figure 4). At 50 °C, the pH was more than 7.0 on the ninth day. At 35 °C, the pH was less than 7.0 for the first 45 days, and then the pH was approximately 8.0. At 20 °C, the pH was below 7.0.

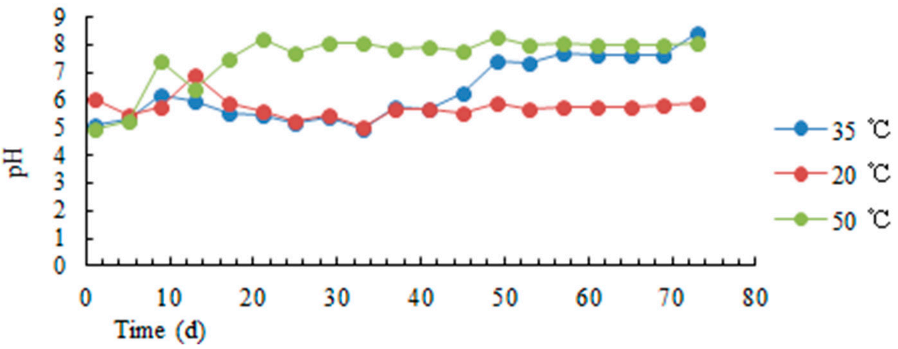

Figure 4. The change in pH at different temperatures.

3.1.2. Changes in VFAs and Ammonia Nitrogen Content during AD

In the process of AD, methane is mainly produced in two ways—namely, the conversion of VFAs to methane and the interaction between CO_2 and H_2 generating methane—of which the conversion of VFAs to methane is the main pathway for methane production. The initial VFA content for the three groups was basically maintained between 1.0 and 2.0 g/L. At 50 °C, the VFA content increased and was approximately 8.0 g/L after the 20th day. From the 10th to the 40th day, the VFA content at 20 °C and 35 °C was similar. At 35 °C, the VFA content initially increased and then fluctuated in the range of

4–6 g/L. As shown in Figures 3a and 5a, during the 8th to the 60th day of high methane production, the variation in VFAs was small and was basically maintained between 3 and 5 g/L. Acetic acid had the highest content of VFAs of the three groups of experiments, but the propionic acid content was small.

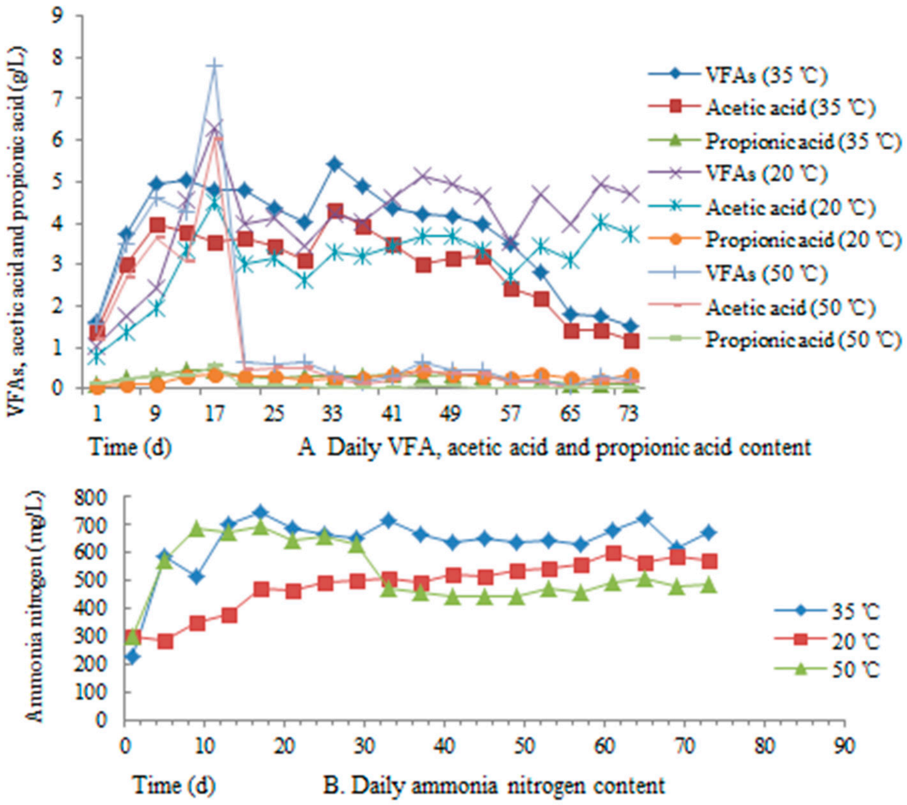

Figure 5. The change in the volatile fatty acids (VFA) and ammonia nitrogen content at different temperatures.

Ammonia nitrogen is an important source of nitrogen and is required for microbial flora growth and reproduction during AD, which can reflect the stability of the anaerobic fermentation system. As shown in Figure 5b, at 35 °C, the ammonia nitrogen content remained above 600 mg/L after 12 days. At 20 °C, the ammonia nitrogen content showed an increasing trend. At 50 °C, the ammonia nitrogen content changed slightly from the 10th to the 30th day and after the 33rd day, but overall, it initially increased and then decreased, finally reaching a steady state.

3.2. The Effect of Different Amounts of Mushroom Residue on the AD of Deer Manure

3.2.1. Change in the Amount of Methane Production during the Fermentation Period

The quantity and activity of the microbe per unit volume in the deer manure fermentation mixture restrained the generation of CH_4 and influenced the deer manure resource utilization level. Figure 6 shows that when the additive amount of deer manure was 35 g in the experimental process, the highest amount of methane production appeared on the 22nd day during AD. The experimental results and analysis showed that the amount of cumulative methane production reached 231 mL/g·TS for the whole AD process. Based on the experimental data and analysis results, for the experimental group with 50 g of mushroom residue, the cumulative methane production reached 192 mL/g·TS for the whole AD process. For the experimental groups with 20 g and 80 g of mushroom residue, the methane production was low during AD. When 35 g of mushroom residue was used, the methane production of the deer manure fermentation mixture was the largest, and the methane production period was relatively short.

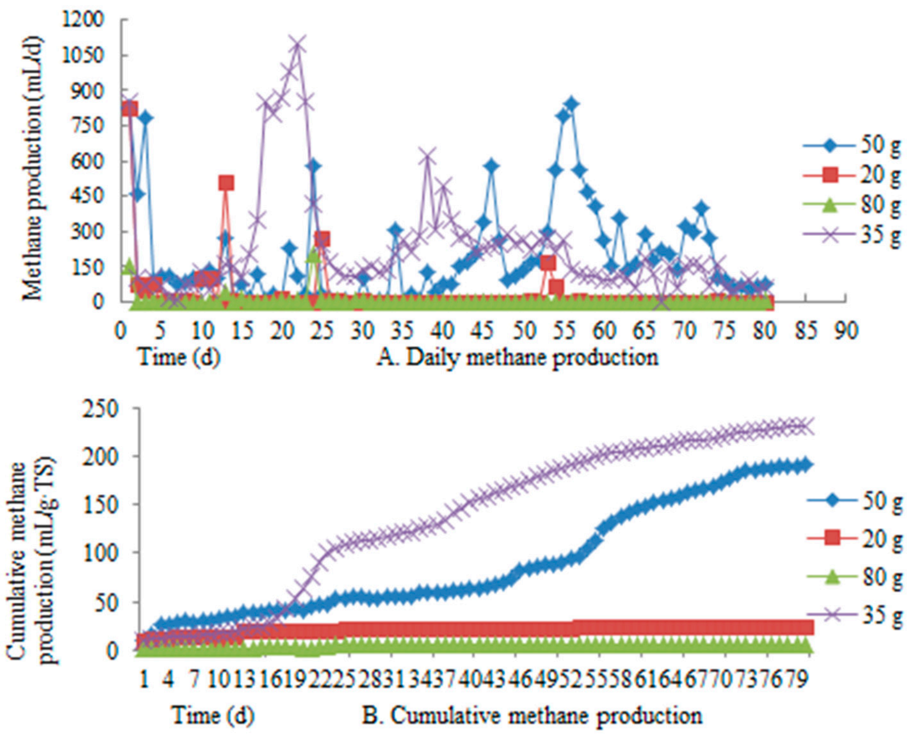

Figure 6. The change in methane production with different dosages of the mushroom residue.

3.2.2. Change in the pH, VFA Content, and Ammonia Nitrogen Content

By constantly monitoring the pH for these four experimental groups, it was determined that there was no significant difference in the first 17 days of the experiment, with a pH of approximately 5~6. In the experimental group with 35 g of mushroom residue, the changing pH trend generally increased after 22 days. In the experimental group with 80 g of mushroom residue, the pH returned to 7.0 on the 25th day. The pH of the experimental group with 20 g of mushroom residue did not exceed 7.0 until the 37th day. The pH of the experimental group with 50 g of mushroom residue was over 7.0 after 40 days.

As can be seen in Figure 7a, the VFA content of the four experimental groups increased at first and then decreased. The VFA of the experimental group with 35 g of mushroom residue reached a peak value of 10.05 g/L on the 17th day, and then the VFA content decreased. During the stable phase period of methane production, the VFA value did not change significantly. In the experimental group with 20 g of mushroom residue, the VFA content reached a peak value on the 17th day. Compared to the experimental group with 35 g of mushroom residue, the VFA content of the experimental group with 50 g of mushroom residue changed more smoothly and reached a peak value on the 33rd day. Compared to the other three experimental groups, the experimental group with 80 g of mushroom residue reached a peak VFA value on the ninth day. Acetic acid had the highest content of VFAs of the four groups of experiments, but the propionic acid content was small.

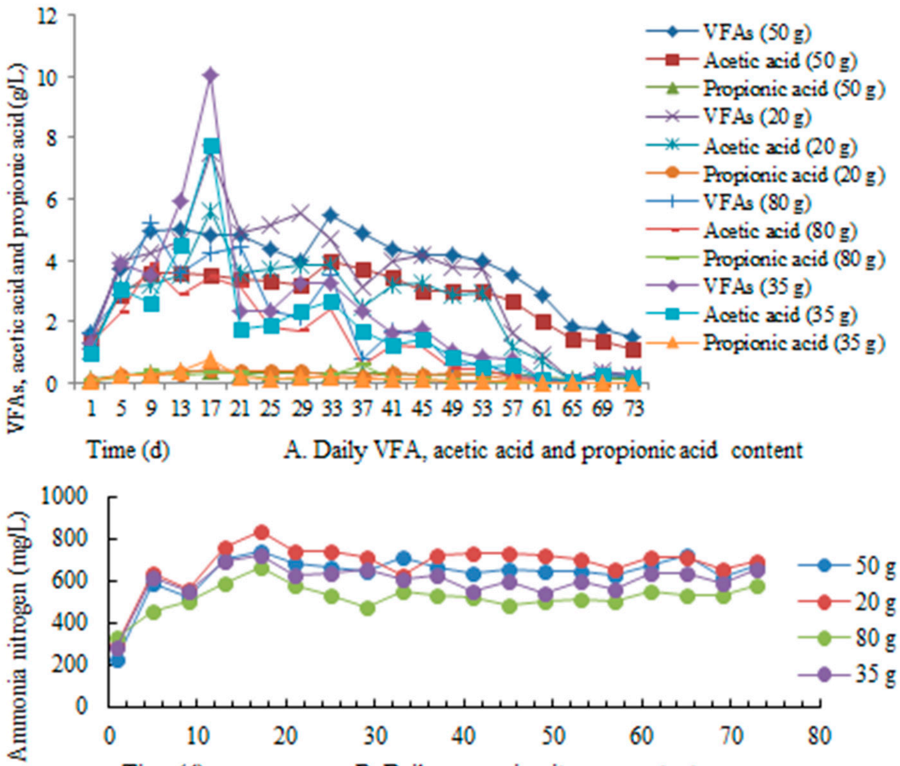

Figure 7. The change in the VFA and ammonia nitrogen content with different mushroom residue dosages.

The experimental results showed that the peak ammonia nitrogen content, for all four experimental groups, appeared on the 17th day, with peak values of 837.5 mg/L, 716.67 mg/L, 741.67 mg/L, and 666.67 mg/L. The ammonia nitrogen contents for these four experimental groups generally increased before decreasing (Figure 7b). When the additive amount of mushroom residue was 20 g, the ammonia nitrogen content was higher than that of other groups.

3.3. The Effects of TSes on the AD of Deer Manure

3.3.1. Changes in Methane Content and pH During the Fermentation Period

The change in daily methane production is shown in Figure 8. Compared to the other two groups, TSes (6%) for the experimental groups were higher in the first 25–50 days, while TSes (8%) for the experimental groups were higher than the other two groups after the 55th day. A methane production of 10% for the experimental groups was low for the whole AD process. Cumulative methane production reached 24 mL/g·TS, with TSes of 10%, for the entire experiment cycle.

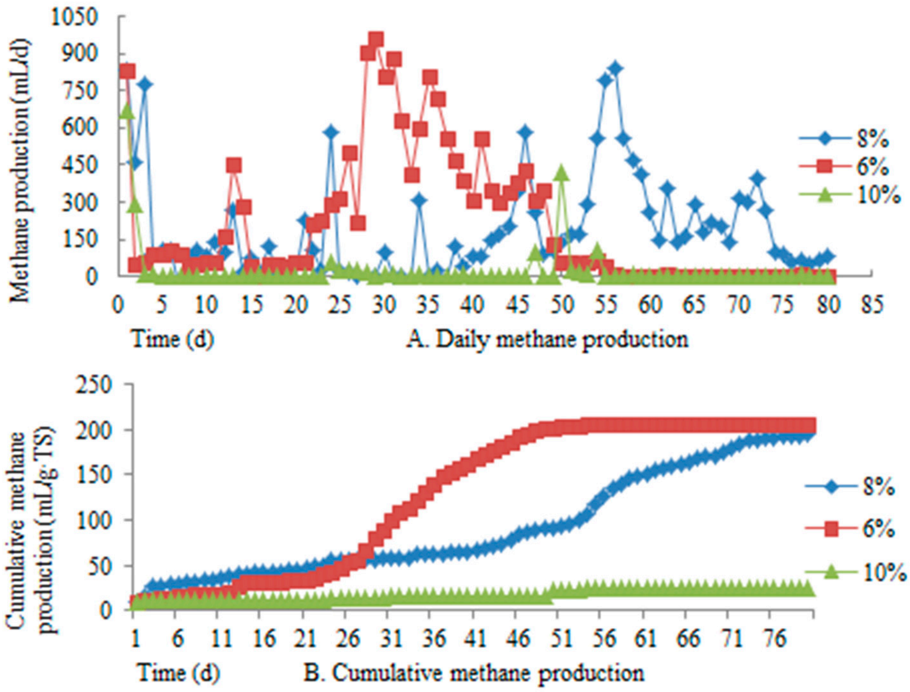

Figure 8. The change in methane production with different total solids (TSes).

The statistical results showed that cumulative methane production was the highest with TSes of 8% in the early AD stage (0–5 d). Cumulative methane production in experimental conditions with TSes of 6% began to show a rapid increase after the 20th day, and then nearly stopped increasing after the 50th day. Cumulative methane production reached 206 mL/g·TS, with TSes of 6%, which was considered to be a shorter production cycle compared to other experimental groups. Cumulative methane production was about 200 mL/g·TS, with TSes of 8%. Comparatively speaking, the experimental group with TSes of 6% generated more methane in a relatively short period of time, which was more conducive to the optimal use of deer manure.

3.3.2. Changes in the pH, VFA Content, and Ammonia Nitrogen Content

The pH of the experimental group with TSes of 6% and 10% was more than 7 and tended to increase steadily after 30 days. The pH of the experimental group with TSes of 8% was more than 7 and tended to increase steadily after 50 days. Compared to the experimental group with TSes of 6%, the experimental group with TSes of 10% had a higher pH after 30 days.

VFAs reflect the capability of the microbial metabolism. Figure 9a shows that the change in the index in these two experimental groups with TSes of 6% and 10% was similar, and both of them increased and then decreased rapidly. In the experimental group with TSes of 8%, the population had an upward trend. After 25 days, the VFAs of the experimental group with TSes of 8% were higher than the other two groups. This phenomenon was likely due to the low production of VFAs and the volatilization of VFAs in the experimental group with TSes of 10%.

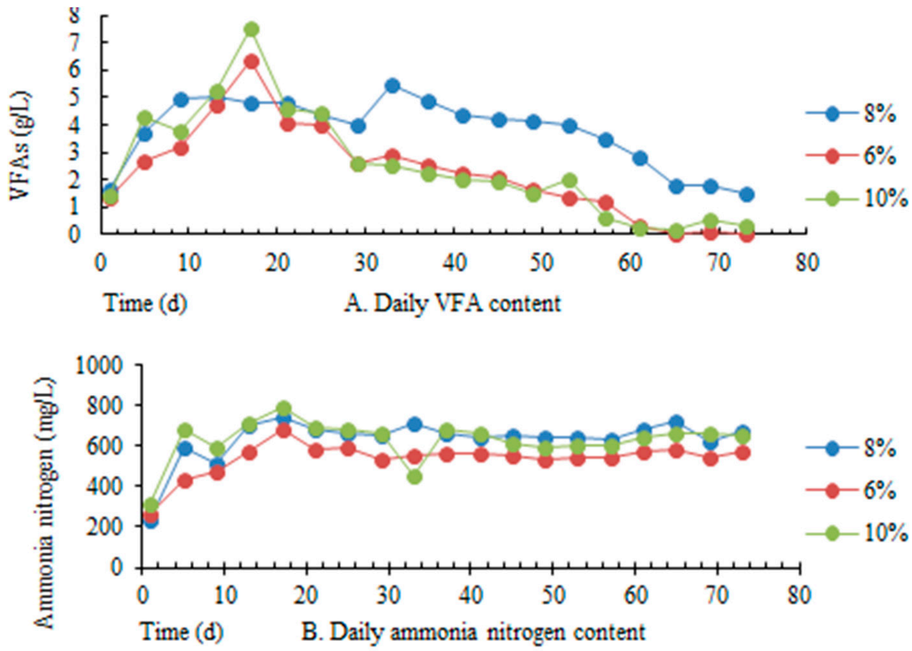

Figure 9. The changes in the VFA and ammonia nitrogen content with different TSes.

The content change is shown in Figure 9b. In these three experimental groups, the ammonia nitrogen content in the whole AD process initially increased and then tended to be stable. The peak value of the experimental group with TSes of 10% appeared on the 17th day, which was higher than the other two groups. The peak values of the experimental groups with TSes of 6% and 8% also appeared on the 17th day.

3.4. Effects of OW on the AD of Deer Manure

3.4.1. Changes in Methane Production during the Fermentation Period

In order to study the effects of OW on the AD system, two groups of comparative experiments on OW and DW were set up. The change in daily methane production, after different treatments, is shown in Figure 10a. In the initial phase of AD, the experimental OW generated more methane than DW did. This demonstrated that OW positively affected the AD of deer manure. Because of the absence of microbial flora, the amount of methane production in the experiments with DW and OW was low and unstable during the AD experiment (Figure 10b).

3.4.2. Changes in the pH, VFA Content, and Ammonia Nitrogen Content

The pH of the experimental DW fluctuated between 7.2 and 8.1 for the whole AD process. In the experimental OW, the acidification period was relatively short, since by the 25th day, the pH exceeded 7.0. The VFAs of the experimental DW were relatively low among the three groups. The peak value for the experimental OW was achieved on the 17th day and then decreased (Figure 11a). The VFA contents in the experiments with OW and DW were generally low, which made it difficult to generate CH_4. As seen in Figure 11b, the ammonia nitrogen contents of the two groups rose before a gradual change in the stability trend. The ammonia nitrogen content for the experimental OW remained at a level of 700–800 mg/L, and the ammonia nitrogen content for the experimental DW remained at a level of 300–400 mg/L (Figure 11b).

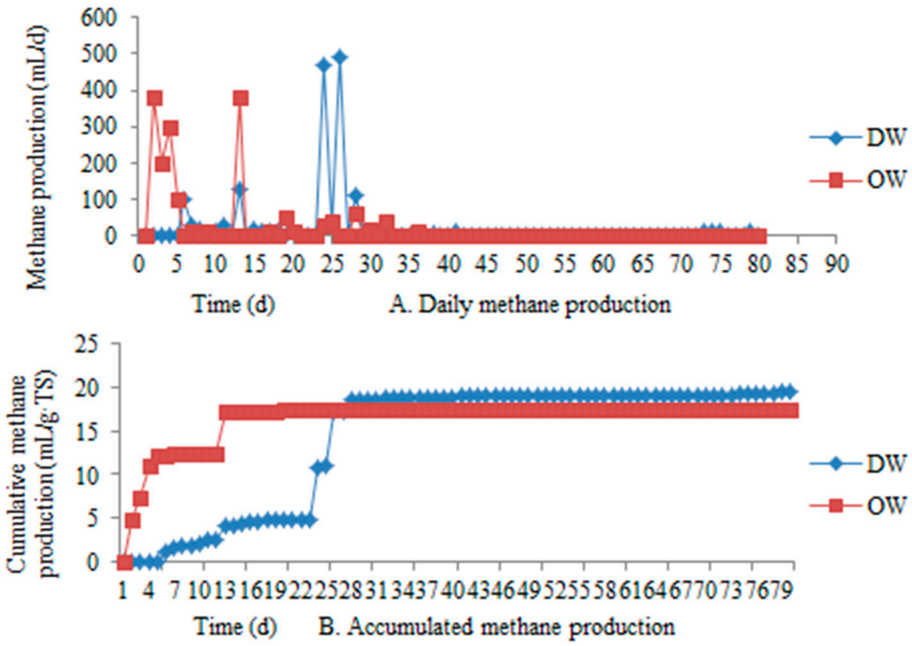

Figure 10. The change in methane production under different conditions.

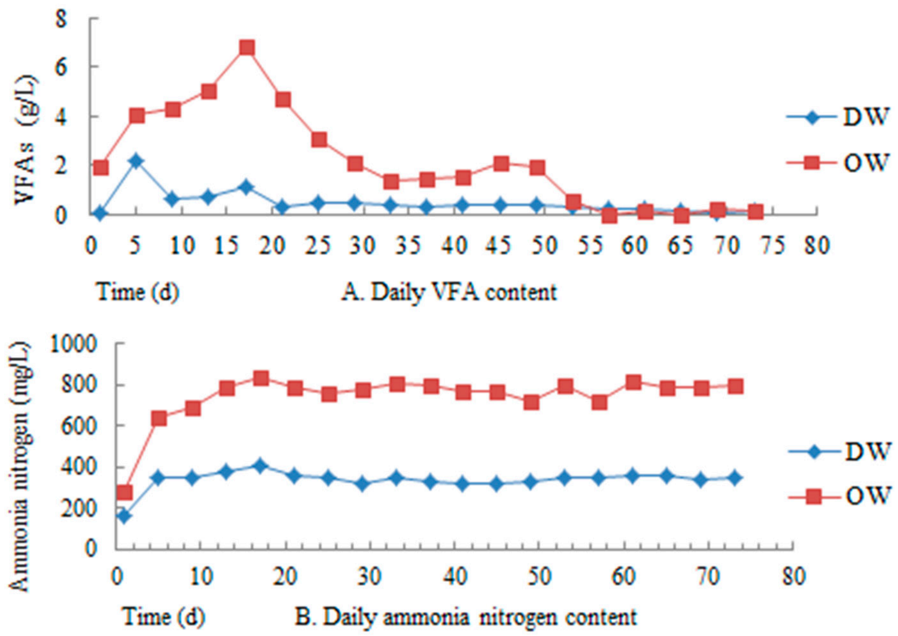

Figure 11. The change in VFA and ammonia nitrogen content under different conditions.

3.5. A Method for Improving the Methane Production Rate

3.5.1. Improving the Methane Production Rate by Adding Zeolite

The improved experiment of deer manure for AD indicated that adding 6.25% zeolite could effectively improve biogas production efficiency, and total biogas production increased by 10%. The methane content in the total biogas production was improved by 6.3%, and the methane production time was early. The mushroom residue additive amount started at 35 g, TSes were 6%, the temperature was 35 °C, and the total biogas production was 246 mL/g·TS. The total methane production was 70 mL/g·TS. It can be seen that, when the zeolite dosage was over 8%, the biogas production increased, but the rate decreased, and the average methane content decreased (Table 3).

Table 3. Methane increments with different zeolite dosages.

Zeolite Content / Biogas Production	0	2%	4%	6.25%	8%	10%	12%
Average methane content (%)	28.5	30.5	32.9	34.8	35.1	34.9	35.4
Methane increment (mL)	0	1069	1507	2000	2816	2880	2942
Methane increment percentage (%)	0	19.0	26.8	35.6	50.2	51.3	52.4

Note: The percentage of methane increments and the methane increment percentage are relative to the blank experiment.

3.5.2. Improving the Methane Production Rate by Enhancing the Magnetic Field Strength

Magnetic field strength can influence living and multiplying creatures. A proper magnetic range strength can promote the fermentation of anaerobic bacteria. A change in the methane production rate can be determined by biogas production in different magnetic field environments and the methane content in the biogas in order to calculate the average biogas content, total biogas, and relative background value in the methane-producing increment. The results are shown in Table 4. The average methane content was 28.5%, without the use of a magnetic field.

Table 4. Total biogas production and methane production at different magnetic field strengths.

Item	Magnetic Field Strength (mT)							
	0	5	10	20	50	70	90	120
CH_4 average content (%)	28.5	28.9	31.6	33.7	36.7	29.3	27.3	22.6
CH_4 average content increment (%)	0	0.4	3.1	5.2	8.2	0.8	−1.2	−5.9
CH_4 increment (mL)	0	369	1187	1268	1299	925	123	−56

The results showed that, when the measured magnetic field strength was 10–50 mT, the methane content and methane production increased. When the magnetic field strength was over 90 mT, the increase in the system's biogas production showed a downward trend.

4. Discussion

4.1. Four Single-Factor Experimental Conditions: Temperature, Mushroom Residue Addition, TSes, and OW

Temperature is an important index for the AD of livestock manure, and the effect of temperature on anaerobic degradation is greater than that of acid concentration [51–53]. The thermal energy loss of biogas AD mainly includes the heat consumption required for the temperature rise of the newly invested raw materials for fermentation and the heat transfer consumption of the anaerobic fermentation reactor [54]. Therefore, a low temperature is very disadvantageous for AD. High-temperature digestion has many advantages, but it needs more calories. Moderate-temperature AD is often used in livestock manure. It has been found that a moderate-temperature fermentation system is more stable than a high-temperature fermentation system [55]. Given equal masses of organic matter, net methane generation is higher in the mesophilic range [56].

The AD of deer manure showed that total methane production was only 4.3% different at 35 °C and 50 °C, and there was little difference between them in the methane production cycle time. The acidification time at 35 °C was longer than at 50 °C, and both were alkaline at the end of the reaction. The acidification at 20 °C was serious, and was the main factor affecting methane production. As shown in Figure 5a, the VFA content increased first and then decreased rapidly at 50 °C, which corresponded with the methane production peak (20–40 d) shown in Figure 3 indicating that VFAs were converted into methane. At 50 °C, VFAs were produced by the decomposition of raw materials in the initial AD stage, so the content of VFAs increased. Because methanogens are active under a high temperature, VFAs decomposed continuously, and the content of VFA decreased. After a period of time, the amount of VFAs produced by raw material decomposition was basically the same as that produced by decomposition: VFAs were maintained at a low level. VFAs began to decline sharply after 50 days at 35 °C, which was also relative to peak methane production. When the VFA content increased at 20 °C, the VFA content was maintained in the high-content area (4–6 g/L), so less methane was produced. Because the initial stages of 20 °C and 35 °C were acidified, the VFA content was basically the same because of the small difference in temperature. Because of the low methane production at 20 °C, VFAs basically did not decompose, so VFAs were maintained at a high content. However, at 35 °C, the methane production in the initial stage was small, and VFAs began to decompose. At the same time, the VFAs produced by the decomposition of raw materials were continuously added to the solution, and the content of VFAs remained basically unchanged. Because of the large amount of biogas produced in the later stage, VFAs were heavily decomposed, there were not enough VFAs from raw materials to add to the solution, and the content of VFAs showed a significant downward trend. As can be seen from Figure 5b, the relative change in the ammonia nitrogen content was the smallest at 35 °C, which indicated that the AD system was the most stable at this temperature. Under a condition of 50 °C, with an increase in gas production, fatty acids decomposed, the pH increased, and the ammonia in the fermentation broth was released. As can be seen from Figure 3, methane production was at its peak on the 30th day, and the consumption of fatty acids and acetic acid was the largest. Therefore, the release of ammonia significantly increased, and the ammonia nitrogen content in the corresponding fermentation mixture system was significantly reduced.

The results showed municipal sewage sludge as a common inoculum (used in anaerobic fermentation to produce biogas) [57]. Biogas slurry is also an ideal additional source of bacteria [58,59]. Spent mushroom substrate can be used as an important choice in studying fermentation inocula [60]. With developments in the economy, the demand for edible mushrooms is increasing, and consequently, more and more waste mushroom residue is produced. As mushroom residue contains a large number of mixed bacteria [61,62], it is of great significance as an anaerobic digestion inoculant. The results showed that when mushroom residue was added at 35 g, the methane production of the deer manure fermentation mixture was the largest, the methane production cycle was relatively short, and methane production was relatively stable (Figure 6). The pH of 35 g of mushroom residue showed an upward trend. After 22 days, it was basically alkaline, which was the best condition for the experiment. The experimental group with 35 g of mushroom residue had the highest peak value of VFA content and the most obvious decrease after the peak value. The VFAs converted into methane were the most favorable for AD (Figure 7a). Among the four groups of experiments, the experimental group with 35 g of mushroom residue was the most stable and most favorable for AD (Figure 7b). Mushroom residue mainly played the role of adding bacteria, which could make the system start quickly. Because the organic carbon and C/N ratios of mushroom residue were lower than those of deer manure, the excessive addition of mushroom residue was harmful to methane production. In addition, when the total solid content was unchanged, adding too much mushroom residue reduced the content of deer manure in the fermentation system. The demand for methane bacteria in the AD system was limited, so the demand for mushroom residue was also limited. When the amount of mushroom residue was 35 g, methane bacteria could be effectively supplemented [63,64], the organic matter content was high, the C/N ratio was reasonable, and thus the decomposition efficiency of VFAs was the highest.

TS content is an important indicator of the AD of livestock manure [65]. With TSes of 15% as the limit, AD can be divided into dry AD and wet AD [66]. In general, wet AD produces more biogas than dry AD does [67–70]. In the process of AD, the TSes of the fermentation material also change with degradation [71]. In the AD of deer manure, it was found that, under the condition of 6% TSes, methane production was high, and the methane production time was short (Figure 8). By inference, the methane production time with 4% TSes would be shorter than 6%. Under the condition of 6% and 10% TSes, the acidification stage was short, and the change was basically the same. The change in VFAs was basically same when the TSes were 6% and 10%, and the change showed ta trend of first increasing and then decreasing (Figure 9a). The experimental results showed that the VFAs with 8% TSes were the highest after 25 days. As shown in Figure 8, in the experimental group with TSes of 10%, the amount of methane was the smallest, and the decomposition of VFAs was smallest. After the 25th day, two experimental groups with TSes of 6% and 8% entered a higher methane production stage, and the VFAs in the fermentation mixture were continuously produced and decomposed. Because VFA production was lower than decomposition, the VFA content was in a downward trend. Due to the low methane production in the experimental groups with TSes of 10%, the VFAs in the mixed fermentation materials almost did not decompose and were in a near saturated state, so the new VFAs were less or were even not produced. In this experiment, a drainage method was used to collect biogas. Therefore, the pressure of the liquid level in the fermentation vessel in these two experimental groups with TSes of 6% and 8% was higher than in the experimental group with TSes of 10%. Since there was almost no biogas production after 25 days in the experimental groups with TSes of 10%, the volatilization of VFAs was unavoidable at 35 °C. Therefore, this phenomenon should have been due to the low production of VFAs and volatilization of VFAs after the 25th day. In addition, the concentration of 6% TSes was small, and VFA production was low. By inference, VFA production with 4% TSes would be less than with 6%. In terms of changes in the ammonia nitrogen index, the experimental group with 6% TSes showed a more stable trend, indicating that the system was the most stable at this time (Figure 9b). In short, the system was in a relatively optimal state with 6% TSes.

In order to adjust the TSes, the usual method is to add straw when the water content is high [72]. One study found that rice wash helped to improve the rate of the degradation of carbohydrates, the yield of VFAs, and the acidification in peanut residue during AD [73]. Slaughterhouse wastewater and kitchen wastewater are also effective media for adjusting TSes [74,75].

Because OW contains organic matter, it can theoretically promote the AD of livestock manure. From the point of view of waste utilization, it is of great significance to carry out an experiment involving the replacement of DW with OW. The experimental study found that methane production started ahead of time (after the OW was replaced with DW), but total methane production did not change much. The main reason was that the organic matter content in the OW was low. According to the results concerning the system pH measurement, the acidification stage of OW was obvious after DW was replaced. During the reaction, the VFA content and ammonia nitrogen content were significantly higher than in the experimental group after DW was replaced. The VFA content first increased and then decreased, and the effect was obvious, while the ammonia nitrogen content was basically maintained between 700 and 800 mg/L. It can be seen that OW, replacing DW, had the effect of inducing the AD system to produce methane, but the effect was limited.

From the above analysis, it can be seen that 35 °C was the ideal temperature condition. When mushroom residue was used as an inoculant, the optimum dosage was 35 g. Taking TS content as the analysis factor, the optimum condition was a TS content of 6%. While the addition of OW could not significantly improve the biogas production effect, it was of great significance in studying the water content of the fermentation broth using OW (instead of tap water) from the perspective of waste utilization. While adding OW could not significantly improve the biogas production effect, it was of great significance in adjusting the water content of the anaerobic fermentation material using OW (instead of tap water) from the perspective of waste utilization.

4.2. Effect of Zeolite on Biogas Production by AD

Zeolite, as an additive for anaerobic digestion, can promote gas production. Zeolite can provide a variety of trace elements for the system, so it is widely used to strengthen the AD process [76]. Zeolite was added to improve the bacterial flora and biogas production in anaerobic fermentation [77–81]. The introduction of the iron oxide−zeolite system also produced a more stable pH condition, higher total VFAs, and a lower ammonia concentration [82]. The biozeolite fixed-bed strategy contributed to higher efficiency and stability of the ammonium-rich AD process [83]. Therefore, adding zeolite to the AD mixture to improve AD was of great significance.

The beneficial effect of zeolite on AD in producing biogas has been recognized. Because previous studies were carried out under composite conditions, the exact amount of zeolite added when the conditions changed was unknown. In the single-factor AD of deer manure with zeolite, it was found that biogas production increased with an increase in zeolite content. The analysis showed that adding zeolite could not only supplement trace elements (such as Ca, Na, K, and Ba) needed by the AD system, but could also improve the activity environment of methane bacteria and promote the reproduction and activity of methane bacteria. This was beneficial to the methane production of the system. When the zeolite content was over 8%, the methane increase was not obvious (Table 3). This indicated that the demand for zeolite in the AD system was limited. The addition of 8% zeolite was more advantageous from an economic point of view.

4.3. Effect of a Magnetic Field on Biogas Production through AD

Magnetic fields play an important role in the utilization of solid waste resources. Research has suggested that a magnetic field can accelerate the decomposition of AD sludge [84]. The maximum increments in methane production, with an addition of 3 g of magnetite, were over 50% for the co-digestion of wheat straw, sheep manure, and chicken manure [85]. The study found that high specific biogas and methane production were attained with Fe_3O_4 magnetic nanoparticles [86]. Therefore, magnetic fields play an important role in improving the biogas utilization technology of AD.

The effect of magnetic fields on AD has been confirmed by experiments, but detailed studies on magnetic field intensity are lacking. This experimental study found that a magnetic field intensity that was either too high or too low was harmful to the AD of livestock manure. With an increase in magnetic field intensity, the increase rate of methane production first increased and then decreased (Table 4). This was most advantageous to biogas production when the magnetic field intensity was 70 mT. When the magnetic field intensity was 10–70 mT, the average content of methane in total biogas and total methane production could be significantly increased. The analysis showed that certain magnetic field conditions were beneficial to the activity of methane bacteria. However, the activity of methane bacteria could be inhibited if the magnetic field intensity exceeded a certain value.

5. Conclusions

The best experimental conditions for deer manure AD were explored by developing research on the effects of an additive amount of mushroom residue, TSes, and temperature conditions on gas production. We drew the following conclusions from the experiments, as shown below:

1. The best temperature for the reaction was 35 °C. Under this condition, the VFA and ammonia nitrogen contents changed less during the stable gas production stage, and the pH increased;
2. The best TS content for deer manure AD was 6%. Under this condition, the reaction produced more biogas in less time. Before the stable gas production stage, the pH increased, and the VFA stably changed. This was beneficial for taking full advantage of the deer manure;
3. With the addition of mushroom residue, the deer manure AD produced a lot of gas, and the acidification stage was obvious. The ammonia nitrogen content changed to a lesser extent. Comparing the reaction to the addition of OW to that of the addition of DW, OW was found to promote the process, but not obviously;
4. Under the condition of adding 35 g of mushroom residue, the reaction produced large amounts of gas, and the gas production process was shorter. The acidification stage was obvious, but during the stable gas production stage, the VFA content (except for the peak value and ammonia nitrogen content) changed less to a lesser extent;
5. Zeolite was added in the reaction process, and its influence on the gas production rate was discussed. It was revealed that adding zeolite to the reaction process could promote the gas production rate. When the added amount of zeolite resulted in TSes over 8%, gas production improved, but the rate decreased;
6. Different magnetic field strengths were set for the AD in order to improve the gas production rate and production. When the distance between the magnetic field and the reactor was 50 mm,

and the magnetic field strength was 10–50 mT, the methane production increment and the methane content of the biogas obviously increased.

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