Two-phase anaerobic co-digestion of dairy manure with swine manure

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Abstract: In order to solve the problems associated with high fiber content, and the ensuing lower biogas volume yield in anaerobic digestion of dairy manure, a study of the co-digestion of separated liquids from dairy manure combined with swine manure using a two-phase anaerobic digestion process was conducted. The influence of level of total solids (TS) and hydraulic retention time (HRT) of the mixed liquor on the specific methane production were studied. Three TS levels 8%, 10% and 12% were investigated. Analysis of the results show that a maximum specific methane yield of 132.99 L/kg volatile solids (VS), can be obtained with a TS of 9%, an inoculation rate of 30%, the duration of hydrolytic acidification phase of 5 d, and an HRT of the methanogenic phase of 10 d. These findings could provide directions for improving the biogas production by performing the co-digestion of dairy manure with swine manure.

Keywords: co-digestion, two-phase anaerobic digestion, dairy manure, swine manure, biogas
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1 Introduction

With the increasing demand for meat products, the animal husbandry industry has seen rapid development in China over the past decade. According to a recent report, the amount of animal manure generated in China in 2010 was estimated to be 2.2 billion tons per year, while the percentage of manure used for biogas production was less than 15%[1]. Concentrated animal feeding operations along with a corresponding absence of suitable manure disposal methods have been shown to cause significant environmental and public health problems, including unpleasant smell and pathogen contamination of surface and ground waters[2].

Anaerobic digestion of manure can offer substantial benefits to animal feeding operators and surrounding communities. Compared with pig and chicken manure, dairy manure presents a high content of crude fiber, resulting in a low raw gas production rate of about 0.20 m^3/kg of Total Solids (m^3/kg TS), which holds back engineering initiatives for the construction of large-scale biogas plants[3]. Approaches to improve the efficiency of anaerobic fermentation fall into three categories: increasing the feed organic loading rate; improving the

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gas production characteristics of raw materials; increasing the activity of methanogens[4]. Liu et al.[5] studied the rheological properties of several kinds of feed stocks for anaerobic fermentation, the result showed that dairy manure had the biggest fluid viscosity coefficient. Kaparaju et al.[6] studied the anaerobic digestion of solid-liquid separated solution of dairy manure to produce biogas and found that it can greatly reduce the viscosity and be helpful in the mass transfer of fermentation microorganisms. However, liquid separated from dairy manure contains low TS, resulting in a system with low organic loading when it was used as fermentation material alone, which may reduce the utilization efficiency of equipment. Co-digestion of different materials may enhance the anaerobic digestion process by providing a better system stability[7-12]. Swine manure is a common animal manure in rural areas of northeast China and is also a good material for anaerobic fermentation[13]. Therefore, we added a certain amount of swine manure to the solid-liquid separated solution of dairy manure, which can not only increase the organic load of the system and the volume gas production, but also solve the pollution problems resulted from piled manure in rural areas of the Northeast.

Methane fermentation is a complex process, which can be divided into four main phases: hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanation[14]. In conventional anaerobic digestion, the four phases are carried out in the same reactor without consideration of the different growth rates and pH optima for acidogenic and methanogenic organisms. Physical separation of the anaerobic digestion process into its acidogenesis and methanogenesis phases can improve fermentation. Compared with one-phase anaerobic fermentation, the two-phase anaerobic fermentation process can prevent the build-up of organic acids and improve the digestion efficiency of the entire reactor system[15,16].

Therefore, two-phase anaerobic co-digestion system can improve the fermentation characteristics of raw materials, improve the organic load of anaerobic fermentation and enhance the impact resistance of the anaerobic fermentation system.

The main objective of this research was to explore ways to improve the efficiency of anaerobic fermentation of dairy manure. This study was based on the research of effect of solid-liquid separation on utilization of dairy manure and two-phase anaerobic fermentation by solid-liquid separated solution of dairy manure[17,18]. Two-phase anaerobic co-digestion experiments were carried out and suitable fermentation concentration and flow rate for successful methane production from dairy manure treated with solid-liquid separation and swine manure were defined.

2 Materials and methods

2.1 Raw material

Dairy manure was collected from the experimental base of Northeast Agricultural University and was diluted with tap water in a mass ratio of 2:1. The mixture was then treated with a system developed in the laboratory (Patent number: ZL200920099135.2) which separates the solid and liquid components from one another. Experiments were conducted solely with separated liquids from dairy manure (SLDM). Swine manure was provided by a piggery in Harbin, China. We mixed SDLM with swine manure in a proportion of 70% and 30% respectively. Water was added to the mixture such that the measured TS were 8%, 10%, and 12% respectively for the three experimental conditions. The materials were stored in a refrigerator at 4°C until use. The inoculum was continuously and anaerobically digested slurry collected from a mesophilic anaerobic digest in the laboratory that was rich in methanogenic bacteria. The characteristics of the substrates tested in this study are shown in Table 1.

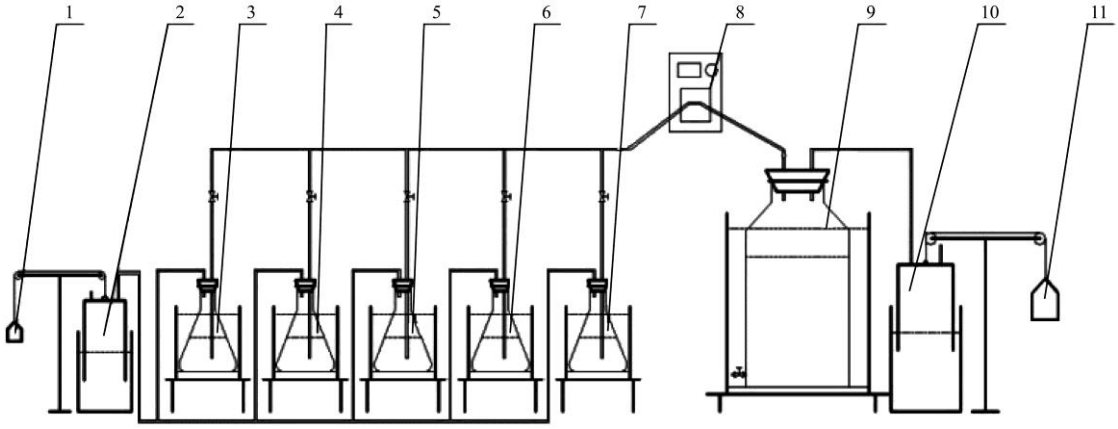
Table 1 The characteristics of substrates

Parameter	TS/%	VS/%	Lignocellulose/%	Viscosity/MPa s	C/N
SLDM	6.60±0.01	4.98±0.09	37.70±1.32	182±10	26.00
Swine manure	27.55±0.10	19.00±0.04	—	—	13.57
Inoculum	4.37±0.03	3.09±0.06	35.38±0.85	124±4	18.26
Mixture1	8.00±0.10	5.70±0.03	—	—	—
Mixture2	10.00±0.10	7.13±0.01	—	—	—
Mixture3	12.00±0.10	8.55±0.05	—	—	—

2.2 Experimental set-up

The test device for mesophilic anaerobic co-digestion used in this study is shown in Figure 1. It is composed

of a temperature control section, a fermentation section and a gas collection section. In the fermentation section, the number of acidification tanks was determined by the duration of the hydrolytic acidification phase. The thermostatic water bath was used to control the ferment temperature and the temperature fluctuations in the range of 1℃-2℃. The hydrolysis and acidification stages were performed in Erlenmeyer flasks (1 L). The methanogenic tank was a glass bottle with upper and lower exports, and a 4.5 L active volume and a 5 L total volume. The collection tanks and fermentation tanks were connected with latex tubes. A peristaltic pump was used to achieve the flow of material in the two fermentation tanks through the latex tube.



Note: 1, 11. Counterweights 2, 10. Collection tanks 3-7. Acidification tanks 8. Peristaltic pump 9. Methanogenic tank

Figure 1 Digester set-up

2.3 Experimental procedure

The acidification characteristics, start time of the methanogenic phase and gas production characteristics of the two-phase semi-continuous anaerobic fermentation process were studied. Before implementing the semi-continuous operation process, the duration of the hydrolytic acidification phase (Section 2.3.1) and the start time of the methanogenic phase (Section 2.3.2) were determined in batch experiments. Experiments were run as follows:

2.3.1 Hydrolysis and acidification stage

The acidification phase of mixtures of SLDM and swine manure was tested at 35℃ in three reactors (T1-T3) with 4.5 L active volume and 5 L total volume. The three reactors had a level of total solids mixtures (TS) of 8%, 10%, and 12%, respectively. The test lasted for 15 d. The performance of the reactors was monitored by measuring the pH and volatile fatty acids (VFA) daily at the same time every day.

2.3.2 Start-up of methanogenic phase experiments

Batch digestion tests were performed on mixtures of SLDM and swine manure at 35℃ in three reactors (T4-T6) with three levels. In each reactor, 1.35 L of bacterial inoculum was added (30% of the active volume). The test lasted for 10 d. Water displacement devices were used to monitor the gas production and the reactors were shaken manually once a day after gas production determination.

2.3.3 Semi-continuous co-digestion experiments

For the semi-continuous co-digestion experiments, one acidification tank (Figure 1) per day was filled with either 8%TS, 10%TS or 12%TS mixture such that the number of tanks corresponding to the acidification period would be ready for the methanogenic phase at the appropriate time for material transfer. The methanogenic tank was filled with 1.35 L of inocula and 3.15 L of 8%TS, 10%TS or 12%TS mixture. The time chosen to fill the methanogenic tank was such that the beginning of the methanogenic phase would coincide with the end of the acidification period in the first acidification tank. The semi-continuous co-digestion experiments started with the transfer of material from the acidification tanks to the methanogenic tank (Figure 1). Three different flows were tested with a total of 9 semi-continuous laboratory scale co-digestion experiments (L1-L9 experiments, 8%TS: L1-L3, 10%TS: L4-L6, 12%TS: L7-L9), all at 35℃. One acidification tank was used per day and the remaining content after the

transfer of material was discarded. One new tank was added such that the number of acidification tanks in the different stages of the acidification period remained constant. The equivalent volume added to the methanogenic phase was discarded prior to the addition of the thoroughly acidified material to the methanigenic tank. The material was added and withdrawn with flow rates of 0.36 L/d (L1, L4 and L7), 0.45 L/d (L2, L5 and L8) and 0.54 L/d (L3, L6 and L9), respectively, which were determined from preliminary experimental results. The test lasted for 15 d.

2.4 Analytical methods

The methane and carbon dioxide concentrations in the biogas were determined with a gas chromatograph (GC-6890N, Agilent Inc., USA) equipped with a stainless steel column (1 m × 3 mm i.d. carbon molecular sieve TDX-01: 1.5 nm to 2.0 nm) and a thermal conductivity detector (TCD). The injector, oven, and detector temperatures were 120 ℃, 190 ℃, and 220 ℃, respectively. Argon served as the carrier gas at a flow rate of 40 mL/min. VFA concentrations were determined by gas chromatography. An Agilent GC-6890N gas chromatograph with a flame ionization detector (FID) and a 30 m × 250 μm ID (Agilent 1909/N-133 HP-INNOWAX Polyethylene Glycol), 0.5 μm column was used. The carrier gas was He. The initial oven temperature was 70 ℃ was increased to 140 ℃ at a rate of 15 ℃/min, and then maintained for 2.5 min. The injector and detector temperatures were both 250 ℃.

The TS, VS, pH (Hanna basic pH meter HI9224, Italy), total organic carbon (TOC) of the feed and samples were measured according to the standard methods of American Public Health Association[19]. All reagents were of analytical grade. All measurements were conducted in triplicate, and the averaged results are presented here.

3 Results and discussion

3.1 Acidification phase

During the acidification phase the pH and total VFAs followed similar trends for all experiments (T1, T2 and T3). The pH decreased during the first 7 d dropping to a minimum value on day 7 (T1), 5 (T2) and 5 (T3), as shown in Figure 2. When comparing the pH with the range for normal growth of hydrolysis acidification bacteria and methanogenic bacteria, we can conclude that in the late acidification stage, the system had entered the symbiotic stage for both the acidification bacteria and the methanogenic bacteria. This observation can also be drawn from the gas composition measurements.

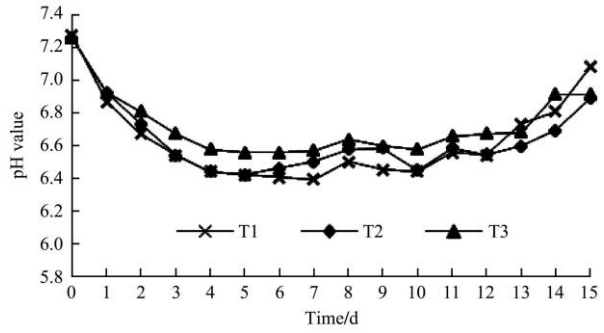


Figure 2 Variation of pH values in the hydrolysis-acidification phase

The variation of the total VFA concentration is shown in Figure 3. We can infer that at the beginning, easily degradable substances of separated liquids of dairy manure and swine manure transformed rapidly into simple organic compounds, resulting in a gradual increase of VFA content (0-7 d). It then increased at a slower rate due the content having less easily degradable substances and the accumulation of VFA.

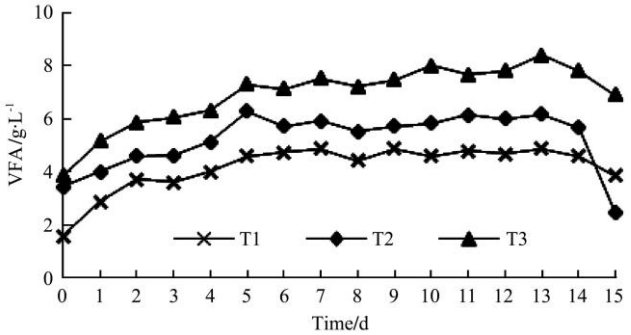


Figure 3 Variation of volatile acids content in the acidification phase

From the variation of pH and total VFA concentration, we determined that the durations of the hydrolytic acidification phase of T1, T2 and T3 were 7 d, 5 d and 5 d, respectively.

3.2 Methanogenic phase

3.2.1 Start-up of the methanogenic phase

The variation of biogas production during start-up of the methanogenic phase is shown in Figure 4. The

biogas production rate followed a similar trend for all three experiments; a double peak appeared in all of them. For all three groups, the first biogas production peak appeared on the third day of the methanogenic phase. Two main reasons can be proposed to explain this result. One reason is that the denitrifying bacteria can transform nitrite nitrogen and nitrate nitrogen into N_2 which then adds to gas production. This can be proven from the nitrogen content in the biogas during the start-up of the methanogenic phase. The other reason is that after inoculation, the rich nutrient substances in the substrate can enhance the activity of methanogeneic bacteria, resulting in an increase in biogas production. After the first peak, the biogas production of the three groups keeps declining until the fifth day, and then increases again. In T1, the gas production changed smoothly and reached its second peak on the sixth day, so we determined that the start time of the methanogenic phase was 6 d. T2 and T3 reached their second peak on the 8th day. It was then followed by a sharp decrease in production. We determined that the start time of the methanogenic phase for T2 and T3 was 8 d. The change in biogas production of T1 was stable whereas that of T2 had a large fluctuation, especially in the late startup process. The results showed that with the increase in fermentation concentration, the buffer capacity of the system decreased and making the system less stable.

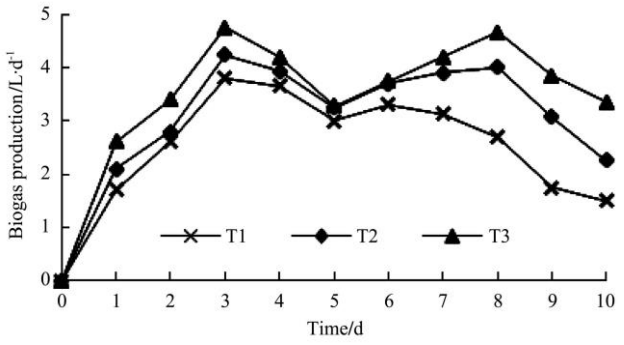


Figure 4 Variation of biogas production during the start-up of the methanogenic phase

3.2.2 Results of gas production characteristics

The two-factors semi-continuous co-digestion experiments (Factor A: 3 levels and Factor B: 3 levels) were carried out at (35±1)℃ for 15 d. Factor A was the fermentation concentration, factor B refers to the three different flow rates. An orthogonal experiment was used to investigate the effects of the two factors on VS methane yield based on a single factor experiment. The design and results of the experiment are shown in Table 2.

Table 2 Experimental arrangements and experimental data

Experiment	Factor A (TS)	Factor B (flow rate, L/d)	Methane yield /L·kg^{-1}VS collected
L1	1 (8%)	1 (0.36)	122.23
L2	1 (8%)	2 (0.45)	130.35
L3	1 (8%)	3 (0.54)	125.42
L4	2 (10%)	1 (0.36)	128.76
L5	2 (10%)	2 (0.45)	131.97
L6	2 (10%)	3 (0.54)	118.00
L7	3 (12%)	1 (0.36)	123.56
L8	3 (12%)	2 (0.45)	122.17
L9	3 (12%)	3 (0.54)	108.32

The general factorial parameter analysis method of the software Design-Expert Version 6.0 was used to establish the equation of the effect of the two factors on VS methane yield. The significance test was performed by the equation (1):

$$Y = -228.98 + 30.70A + 1004.29B - 25.60AB - 1.06A^2 - 878.4B^2 \quad (1)$$

The analysis of the variance of the effect of the two factors on VS methane yield is shown in Table 3.

Table 3 Anova for response surface quadratic model of methane production rate

Source	SS	DF	MS	*F* value	*p* value	
Model	404.35	5	80.87	25.07	0.0119	Significant
A	95.60	1	95.60	29.63	0.0122	
B	86.72	1	86.72	26.88	0.0139	
AB	84.92	1	84.92	26.32	0.0143	
A^2	35.87	1	35.87	11.12	0.0446	
B^2	101.25	1	101.23	31.38	0.0112	
Residual Error	9.68	3	3.23			

Note: A, B mean factor A (TS), factor B (flow rate).

In this model, the factors A, B, AB, A^2 and B^2 all had significant effect on the equation. After significant test, the *F* value of the model was 25.07, $p<0.05$, which indicates that the model is significant. The coefficient of determination R^2 was 97.66%, which means 97.66% response values change could be explained by this model.

The two-phase anaerobic fermentation tried to achieve the highest VS methane yield and transform raw materials into methane more efficiently, so we choose the maximum value in its range. This research used Design-Expert Version 6.0 to select optimum parameter

of fermentation concentration and flow rate so that the comparatively better technical parameters could be found. Considering the actual condition, the fermentation concentration of 9% and the flow rate of 0.45 L/d were chosen and validated by experiment. The optimization and validation of the results are shown in Table 4.

Table 4 Comprehensive optimization result and verification value

Parameter	TS/%	Flow rate /L d^{-1}	Methane yield /L kg^{-1} VS added
Optimization parameters	9.29	0.44	132.03
Authentication parameters	9	0.45	132.99

Based on the above experiments, three fermentations were carried out. Experiments showed that the predicted values were in agreement with the experiments. The model was proven to be accurate.

4 Conclusions

In the study, the acidification characteristics, the start time of the methanogenic phase and the gas production characteristics under mesophilic condition were studied. The duration of the acidification phase was determined to be 7 d, 5 d, 5 d, when the TS of mixed liquor was 8%, 10%, and 12%, respectively. Similarly, the start time of the methanogenic phase were determined to be 6 d, 8 d and 8 d. Analyses of the methane production rate results indicate that the optimum parameters of fermentation are a TS of 9% and a flow rate of 0.45 L/d. This was further validated by experiment. Under these conditions, the maximum specific methane yield was 132.99 L/kg. Compared to the value predicted by the model, the relative error was less than 1%.

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