Biogas production of Chicken Manure by Two-stage fermentation process

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Abstract. This paper performs a batch experiment for pre-acidification treatment and methane production from chicken manure by the two-stage anaerobic fermentation process. Results shows that the acetate was the main component in volatile fatty acids produced at the end of pre-acidification stage, accounting for 68% of the total amount. The daily biogas production experienced three peak period in methane production stage, and the methane content reached 60% in the second period and then slowly reduced to 44.5% in the third period. The cumulative methane production was fitted by modified Gompertz equation, and the kinetic parameters of the methane production potential, the maximum methane production rate and lag phase time were 345.2 ml, 0.948 ml/h and 343.5 h, respectively. The methane yield of 183 ml-CH_4/g-$VS_{removed}$ during the methane production stage and VS removal efficiency of 52.7% for the whole fermentation process were achieved.

1 Introduction

As the amount of the large scale livestock and poultry farms increased in China, the discharged manure increased accordingly and the environment pollution caused by manure became prominent gradually in recent years. Statistics suggested that the chemical oxygen demand (COD) and the ammonium nitrogen (NH_4^+-N) produced from livestock and poultry manure in China reached to 10.99 and 0.63 million ton, respectively, accounting for 45% and 25% of the summation discharged into environment nationwide, and also being equivalent to 95% and 78% of the summation produced by agricultural resources [1]. As one of the dominant manures in China, the chicken manure had the features of huge amount, complex component, high content of organic matters, and high concentration of nitrogen and phosphorus, etc. [2]. The centralized discharge of the chicken manure from large scale farms would directly get the surrounding soil, atmosphere and water polluted, and further bring down the local environment level in the rural area. However, the organic matters and nitrogen in chicken manure are the valuable biomass resource, and they could be reused and transferred into resources and energy materials by some recycle technologies. Anaerobic fermentation technology could produce biogas from the organic matters derived from manure by the metabolic activity of microorganisms, and it was recommended as the recycling method for manure treatment in China [3]. Besides, the NH_4^+-N in manure could be released greatly under the degradation of protein-based substrates, which would be benefit for increasing the fertilizer efficiency in the agricultural reuse.

However, the high content of free ammonia (NH3, main part of total NH_4^+-N) in the fermentation liquid would inhibit methanogens and reduce the methane production [4]. Among all kinds of livestock and poultry manure, chicken manure contains relatively high content of nitrogen as well as the low carbon to nitrogen ratio (C/N), which is more likely to cause severe inhibition effect as the fermentation substrate [5]. The simplest measure to reduce the reverse effects of NH_4^+-N on methanogens is diluting chicken manure with water by the application of wet anaerobic fermentation process. Two-stage fermentation process with pre-acidification stage and subsequence methane production stage was utilized in this study, and the component of volatile fatty acids (VFAs), methane yield and volatile solid (VS) removal efficiency were investigated to estimate the fermentation efficiency of chicken manure.

2 Materials and Methods

2.1. Seed sludge

The seed sludge was collected from sewage sludge digestion tank in Jingu wastewater treatment plant located in Tianjin, China. However, the seed sludge for pre-acidification was heat-treated at 100ºC for 30 min to eliminate the methanogens and enrich acidate bacteria, and its VS/TS (total solid) was 44.9%. The collected seed sludge with VS/TS of 49.5% was directly used as methane-producing seed sludge.

2.2 Chicken Manure

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The chicken manure collected from household farms in the surrounding of west campus of Tianjin agricultural University located in Xiqing District of Tianjin, China. It was used as fermentation substrate and its main component was analyzed.

2.3 Experimental conditions

Batch experiments were conducted using 300 ml serum bottles filled with 150 ml of substrates. The substrate concentration was 10 g-VS/l in each bottle. Batch experiments were conducted in duplicate. The initial pH was 5.5 and the seed sludge for pre-acidification was 1.20 g-VS. Both bottles were purged by nitrogen gas for 3 min and then sealed by rubber stoppers. The bottles were put in a shaker with rotating speed of 120 rpm and the temperature of 37°C. Three day later, the methane-producing seed sludge with the VS weight of 0.8 g was added into each bottle. The pH of the substrate was adjusted to 7.0 and then the K_2HPO_4/KH_2PO_4 buffer solution (pH 7.0) was added. All the bottles were purged by nitrogen gas for 3 min again before sealing and then put in the incubator for methane fermentation at 37°C. The produced gas and methane content was measured periodically.

2.4 Analytical methods

Production of biogas was measured by a glass syringe. Methane content in biogas produced in methane production stage were analyzed by a gas chromatograph (Clarus 680 PerkinElmer, USA) equipped with a flame ionization detector and a capillary column (Elite-5, 30m×0.25mm×0.25μm). Nitrogen was used as the carrier gas at a flow rate of 2 ml/min. The operation temperatures of the injection port, oven and detector were 200°C, 150°C and 250°C, respectively. VFA including acetate, propionate, butyrate, *i*-butyrate and *i*-valerate in the mixed liquor were analyzed by another gas chromatograph (Agilent 7890B, USA) equipped with a flame ionization detector and a fused-silica capillary column (HP-5, 30mm×320μm×0.25 μm). Nitrogen was used as the carrier gas with a flow rate of 30 ml/min and the split ratio of 20:1. The temperature of injection port and detector were 250°C and 270°C, respectively. The temperature program for oven was as following: 80°C for 1 min, and then rise to 220°C with the rate of 20°C/min, 220°C for 1 min, further increased to 240°C with the rate of 20°C/min, finally maintained at 240°C for 5 min. The soluble carbohydrate was analyzed using anthrone-sulfuric acid method with glucose as standard [6], and the soluble protein was analyzed by Lowry method [7]. The samples for analysis of soluble carbohydrate and protein were filtrated by 0.45 μm membrane before detecting. TS, VS and NH_4^+-N were determined according to Standard Methods [8].

2.5 Calculation for cumulative methane production

In the batch experiment, the cumulative methane production was calculated described as the equation (1) [9].

$$V_2 = V_1 + V_G \cdot C_2 + V_0 \cdot (C_2 \cdot C_1) \qquad （1）$$

Where V_1, V_2 represents the methane production (ml) at the time of t_1 and t_2, respectively; C_1, C_2 the methane content in the gas space of anaerobic bottle (%) at the time of t_1 and t_2, respectively; V_0 the volume of the gas space of anaerobic bottle (ml); V_G the produced biogas (ml) during the time gap between t_1 and t_2.

2.6. Kinetic analysis

The cumulative methane volume in batch experiments followed the modified Gompertz equation [10], as shown in Equation (2).

$$H = P \cdot \exp\{-\exp[\frac{R_m \cdot e}{P}(\lambda - t) + 1]\} \qquad (2)$$

where H represents the cumulative methane production (ml), the lag phase time (h), P the methane production potential (ml), and R_m the maximum methane production rate (ml/h). The values of P, R_m and λ were determined by best fitting the methane production data for Eq. (2) using Microsoft's software Excel 2010.

2.7 Calculation for VS removal efficiency

The VS removal efficiency in the overall fermentation process was calculated according to the equation (3), as following.

$$RE = (VS_f + VS_{S1} + VS_{S2} - VS_e)/(VS_f + VS_{S1} + VS_{S2}) \qquad (3)$$

Where RE represent the VS removal efficiencies in the whole process, respectively (%). VS_f, VS_{S1} and VS_{S2} were defined as the VS of initial feedstock, pre-acidification and methane-producing seed sludge (g). VS_e was the VS at the end of methane production (g).

3 Results and Discussion

3.1 Characteristic of chicken manure

The main component was shown in Table 1. Table 1 suggested that chicken manure contained 25.64% of TS in wet weight (ww) and 59.28% of TS was organic matters. The NH_4^+-N concentration was as high as 11.32 mg/g-ww in this study, which might be caused by the degradation of nitrogen-contain materials during the storage of chicken manure. Table 1 also shows that the sum of total carbohydrate and total protein account for 16.5% of VS, suggesting that great amount of other organic matters in chicken manure were not detected. The soluble component that was easily to be utilized by microorganisms was also measured. However, results

show only 4.5% and 5.7% of total carbohydrate and protein were soluble, implying the chicken manure was hard to be hydrolyzed to some extent.

Table 1. Component of Chicken Manure based on wet weight (ww).

Program	Unit	Value
Total carbohydrate	mg/g-ww	18.0
Soluble carbohyrate	mg/g-ww	0.81
Total protein	mg/g-ww	7.17
Soluble protein	mg/g-ww	0.4
NH_4^+-N	mg/g-ww	11.32
TS	%	25.64
VS	%	15.20
VS/TS	%	59.28

### 3.2 VFAs produced in pre-acidification stage

The pre-acidification stage was performed for nearly three days. The VFAs including acetate, propionate, butyrate, i-butyrate, i-valerate were detectable in the digestate after pre-acidification stage, as shown in Figure 1. Due to the hydrolysis and acidification of the organic matters, a sum of 1588 mg-VFAs/l was produced in the end of pre-acidification stage. The acetate was the dominant among all kinds of VFAs, accounting for 68% of the total amount. The acetate could be directly utilized by the pathway of aceticlastic methanogenesis, which was the main pathway for *Methanosarcina*, *Methanosaeta*, and etc [11]. Followed by acetate, the propionate with the percentage of 19% was the second main component, which was not popular with methanogens and would inhibit the methanogens activity under the high concentrations.
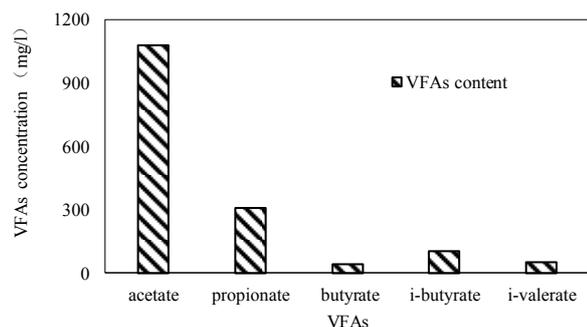
Fig. 1. VFAs component detected in the digestate in the end of pre-acidification stage.

3.3 Biogas production and methane content in methane production stage

After pre-acidification stage, the methane-producing seed was added into the anaerobic bottles, and pH was adjusted to 7.0, which is suitable for methane fermentation. The phosphate buffered saline was benefit for pH maintenance and the pH was 6.8-7.0 during the whole fermentation process. The daily biogas production and methane content in methane production stage was shown in Figure 2.
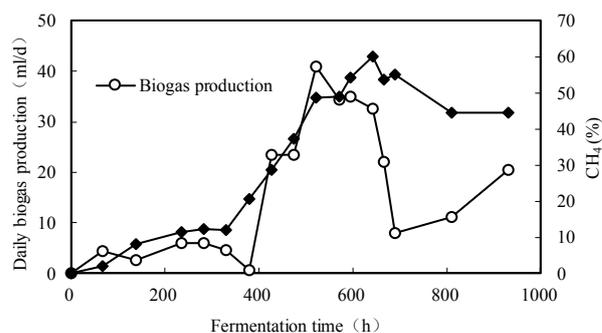
Fig. 2. The daily biogas production and methane content in biogas during the methane production stage.

As shown in Figure 2, the daily biogas production had experienced three peak periods during the total fermentation time of 930 h. In the first peak period (0-378.5 h), the daily biogas production was lower than 6 ml and even reduced to 1ml at 378.5 h, but the methane content increased smoothly to 20.7%. It could be speculated that a large percentage of the biogas produced by the biochemical reactions must be methane in the first peak period. In the second peak period (378.5-666.5 h), the daily biogas production increased greatly with the highest value of 40.8 ml/d and the average value of 32.9 ml/d. The methane content increased sharply to 60% with the increase rate of 3.3 ml/d. After the rapid increase stage, the daily biogas production slowly increased to 20.4 ml/d during the final peak period (666.5-930.5 h) and the methane content was slightly decreased to 44.5%. The tendency of the biogas production curve with three peak period during methane production stage might be caused by the insufficient of direct substrates for methanogens and the inhibition of metabolic products on the microorganisms.

3.4 Kinetic fitting of the cumulative methane production

Figure 3 shows the cumulative methane production calculated by Equation (1) by chicken manure digestion and the corresponding curves of Equation (2) using the best-fitted kinetic parameters. The results showed that the values of P, R_m and λ were 345.2 ml, 0.948 ml/h and 343.5 h, respectively.
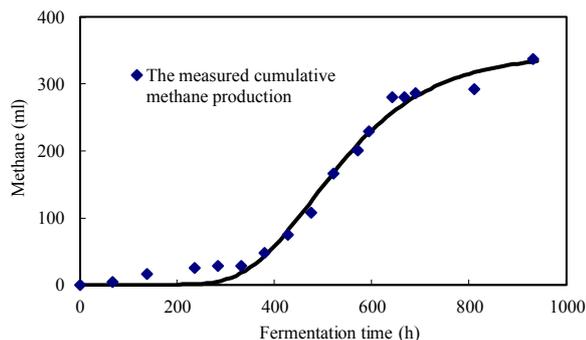
Fig. 3. The cumulative methane production in the methane production stage.

3.5 VS removal efficiency and methane yield

The VS removal efficiency during the whole fermentation process calculated according to equation (3) reached a value of 52.7%, implying that nearly half of the organic matters were converted into biogas. The methane yield was calculated through dividing the produced cumulative methane by the removed VS, and the value was 183 ml-CH_4/g-$VS_{removed}$.

4 Conclusion

The two-stage anaerobic digestion process with separated pre-acidification and methane production was applied in chicken manure treatment. Batch experiment results shows that the VFAs was produced in the pre-acidification stage with a total amount of 1588 mg-VFAs/l and the acetate was the main component. The methane yield reached 183 ml-CH_4/g-$VS_{removed}$ during the methane production stage and VS removal efficiency was 52.7% for the whole fermentation process.

Acknowledgments

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