Biochemical Methane Potential (BMP) of Vinegar Residue and the Influence of Feed to Inoculum Ratios on Biogas Production

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Vinegar residue, a typical agro-industrial by-product in the vinegar production process, constitutes a huge environmental problem in China. Though utilization of vinegar residue has drawn much attention, there is still no effective, economical, and environmentally friendly method to deal with it. Anaerobic digestion is an effective method widely used in organic waste processing which might be an alternative to convert this acidic waste into biogas energy. A biochemical methane potential assay was conducted, and the influence of different feed to inoculum ratios (F/I) was determined. The highest methane yield of 242.69 mL g VS^{-1} was achieved at a F/I of 1, while the lowest methane yield of 182.94 mL g VS^{-1} was obtained at a F/I of 6. The TVFA/TA ratio was higher than the limiting value (0.4) at F/I ratios of 5 and 6, which demonstrated destabilization during the anaerobic digestion process. The modified Gompertz equation was developed to calculate the cumulative methane yields from different F/I ratios. The results suggested that the vinegar residue had extensive potential in biogas production and anaerobic digestion as a promising method that may be applied to deal with such waste, thus it is worth doing further research in the future.

Keywords: Biochemical methane potential; Vinegar residue; Anaerobic digestion; Feed to inoculum ratio

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INTRODUCTION

Vinegar residue is a by-product of solid-state fermentation of cereals and their bran to make vinegar (Song *et al.* 2012). In China, about three million tons per year of vinegar residue is produced. Serious environmental problems have been created by this waste because of its high acidity (pH 4) (Zhong *et al.* 2012) and the difficulties in processing. Although the disposition of vinegar residue has drawn much attention in recent years, the traditional treatment methods such as incineration, landfilling, and open discharge, are still the main ways for the management of this waste in China. These methods often lead to the pollution of groundwater, land, and air (Song 2011), and also are inefficient and costly. Converting vinegar residue into animal feed or fertilizer has also been investigated; the amount consumed was limited and intensive energy was needed in these processes (Zhong *et al.* 2012). Thus, it is imperative to find environmentally friendly alternatives to treat and reutilize the vinegar residue in order to minimize the pollution.

Anaerobic digestion (AD) technology has been widely used for the conversion of organic wastes such as sewage, food waste, energy crops, and other biomasses to biogas. With the increasing application of anaerobic digestion processing, it is important to find a method to evaluate the biogas production performance and biodegradability of a chosen feedstock. In recent years, the biochemical methane potential (BMP) assay has been widely applied as a standard protocol to estimate the biodegradability and methane potential of different feedstocks in anaerobic digestion (Labatut *et al.* 2011; Raposo *et al.* 2011). There are still few reports on the BMP of vinegar residue so far.

The F/I ratio is defined to be the initial ratio of volatile solids (VS) in the feedstock (vinegar residue) to the VS in the inoculum at the beginning of each batch digestion test. In a start-up batch digester, a certain amount of inoculum should be added together with the feedstock to provide the required microorganisms to start the reaction. The F/I ratio has been found to be one of the most important parameters in batch anaerobic digestion (Chudoba *et al.* 1991). Each feedstock has its suitable F/I ratio because of the amount of volatile fatty acids (VFA) and the capacity to buffer the VFA cumulative in the anaerobic process (Lesteur *et al.* 2010). It was reported that a higher F/I ratio may be toxic, while a lower F/I ratio may prevent induction of the enzyme necessary for biodegradation (Prashanth *et al.* 2006). The lag phase of anaerobic digestion could also be affected by this factor (Chen and Hashimoto 1996). In addition, the determination of the optimum F/I ratio for a specific residue could also help to establish a stable start-up protocol for continuous anaerobic digesters (Fernandez *et al.* 2001). Therefore, it is necessary to determine an optimum F/I ratio for the anaerobic digestion of vinegar residue.

The objectives of this study were (1) to evaluate the biochemical methane potential and theoretical biochemical methane potential of vinegar residue under mesophilic conditions and (2) to determine the suitable F/I ratio for the biogas production from the vinegar residue under mesophilic conditions.

EXPERIMENTAL

Feedstock and Inoculum

The vinegar residue used in this study was provided by a vinegar factory in Jincheng, Shanxi province, China. The vinegar residue was air-dried at room temperature and then sealed in plastic bags for later use. The sludge used as the inoculum was the effluent from a manure-based anaerobic digester at a chicken manure plant in Deqingyuan Company, Beijing, China. After 24 h of quiescent settling and the removal of supernatant, the sludge was stored at 4 ^{o}C in a refrigerator prior to use.

Composition Analysis

The contents of C, H, N, and S in the vinegar residue and inoculum were determined with an elemental analyzer (Vario ELcube, Germany). The content of O was analyzed by a 2400 Ⅱ oxygen analyzer (Perkin Elmer Instruments, USA). All of the data were used to calculate the C/N ratio and the theoretical methane yield. The total solids (TS) and volatile solids (VS) of the inoculum sludge and vinegar residue were measured using the standard methods (APHA 1998).

The pH value was measured with an le438 pH electrode (Mettler Toledo, USA). The contents of cellulose, hemicellulose, and lignin were determined according to the

Van Soest method (Van Soest 1963) using a A2000 fiber analyzer (ANKOM Technology, USA).

Theoretical Biochemical Methane Potential (TBMP)

The theoretical biochemical methane potential (TBMP) of the vinegar residue was calculated based on the reaction Formula 1 and Equation 2, as reported by Sosnowski *et al.* (2003):

$$C_nH_aO_bN_c + \left(n - {}^{a}/_{4} - {}^{b}/_{2} + {}^{3c}/_{4}\right)H_2O \rightarrow \left({}^{n}/_{2} - {}^{a}/_{8} - {}^{b}/_{4} + {}^{3z}/_{8}\right)CO_2 + \left({}^{n}/_{2} + {}^{a}/_{8} - {}^{b}/_{4} - {}^{3z}/_{8}\right)CH_4 + cNH_3 \quad (1)$$

$$\text{TBMP (ml CH}_4\text{ g VS}^{-1}) = \frac{22.4\times\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{3c}{8}\right)}{12n+a+16b+14c} \quad (2)$$

Anaerobic Digestion

A series of anaerobic digestion batch tests were conducted to investigate the biogas production of the vinegar residue in 1 L digesters with a working volume of 500 mL. The methane production due to biomass decay and the possible presence of residual feedstock in the inoculum was subtracted by performing blank controls. The vinegar residue was digested at six initial volatile solid (VS) loadings: 6, 12, 18, 24, 30, and 36 (g VS L^{-1}). The corresponding F/I ratio for the six initial VS loadings were 1, 2, 3, 4, 5, and 6, respectively. Based on the VS contents of inoculum, a certain amount of the inoculum was added to start the batch digester. After adding the required amounts of the inoculum and feedstock, each digester was filled up to 500 mL with deionized water and closed with a rubber stopper. N_2 was flushed to assure anaerobic conditions before the screw cap was sealed. The reactors were placed in 37 ^{o}C for 60 days. All of the digesters were manually shaken once a day for one minute. The effluents after digestion were centrifuged at 9000 rpm for 10 min, and the supernatant was used for determination of the alkalinity and total volatile fatty acids (VFA).

Determination of Biogas Content and Yield

The daily biogas yield from each anaerobic digester was calculated by the measurement of the pressure in the headspace using a WAL-BMP-Test system pressure gauge (Type 3151, Wal, Germany) with an accuracy of 0.1%. The daily biogas yield was calculated according to the equation reported by El-Mashad and Zhang 2010. Biogas samples were taken once every 3 days and analyzed for the contents of methane using gas chromatography (7890A, Agilent Company, USA) equipped with a thermal conductivity detector.

A TDX-01 column was used with argon as the carrier gas. The temperatures of the oven, injector, and detector were 120, 150, and 150 ^{o}C, respectively. A standard gas, consisting of 34.9% (V/V) CO_2, 10.06% (V/V) H_2, 5.07% (V/V) N_2, and 49.97% (V/V) CH_4 was used for the gas chromatography (GC) calibration. The VFA concentration was determined by the gas chromatography (7890A, Agilent Company, USA) equipped with a flame ionization detector (FID). A DB-WAX column was used with nitrogen as the carrier gas.

Kinetic Modeling

The modified Gompertz equation (Eq. 3), which has been extensively used by many researchers (Zwietering *et al.* 1990), was fitted to the observed cumulative methane yield to determine the maximum methane production potential in this research,

$$y = A\,exp\left\{-exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (3)$$

where y represents the cumulative methane production (mL CH_4 g VS^{-1}), t means the time (d) over the digestion period, A is the methane production potential (mL CH_4 g VS^{-1}), μ_m represents the maximal methane production rate (mL CH_4 g VS^{-1} d^{-1}), λ stands for the lag phase time (d), and e is equal to 2.71.

Methane-based Degradability

The methane-based degradability of the vinegar residue could be calculated using Eq. 4 (Raposo *et al.* 2011; Zhou *et al.* 2011),

$$\textit{Methane-based degradability} = BMP/TBMP \times 100\% \quad (4)$$

where BMP represents the biochemical methane potential (mL CH_4 g VS^{-1}) and $TBMP$ is the theoretical biochemical methane potential (mL CH_4 g VS^{-1}).

Statistical Analysis

All of the experiments were performed three times, and the results were expressed as mean values and standard deviations. All of the graphs and regression models were completed by Origin 8.0 (OriginLab, USA). The t-test of different F/I ratios on the biogas yield, methane content, and biodegradability of experimental data were analyzed with SPSS 20 (IBM, USA). Differences were considered statistically significant when $p<0.05$ for statistical tests.

RESULTS AND DISCUSSION

Characteristics of Vinegar Residue and Inoculum

The characteristics of the vinegar residue and inoculum are shown in Table 1. The fiber compositions of the vinegar residue accounted for more than 70% of the total dry matter (28.15±0.41% of cellulose, 32.56±0.37% of hemicellulose, and 9.73±0.27% of lignin).

The C/N ratio for the vinegar residue (22.68) was in the suggested range (22 to 35) for stable operation of a digester (Habiba *et al.* 2009).

According to the elemental contents of the vinegar residue shown in Table 1, the organic matters in the vinegar residue could be expressed as a formulation of $C_{26.49}H_{44.74}O_{17.88}N$.

The theoretical biochemical methane potential for the vinegar residue were estimated to be 473.34 mL CH_4 g VS^{-1} based on Eq. 2.

Table 1. Characteristics of Vinegar Residue and Inoculum

Parameter	Vinegar residue	Inoculum
Total Solids (%)	92.37±0.03	10.51±0.01
Volatile Solids (%)	84.59±0.60	4.91±0.02
VS/TS(%)	91.57±0.63	46.76±0.11
Cellulose (%)	28.15±0.41	ND
Hemicellulose (%)	32.56±0.37	ND
Lignin (%)	9.73±0.27	ND
N (%)	1.92±0.21	2.34±0.12
C (%)	43.56±0.76	23.00±0.88
H (%)	6.13±0.10	3.32±0.21
S (%)	0.38±0.01	ND
O (%)	39.22±0.31	ND
C/N	22.68±1.02	9.82±0.77
ND: not detected		

Effect of F/I Ratios on Biogas Yield

The results of daily biogas yields of the vinegar residue at different F/I ratios were shown in Figure 1. The biogas production started after inoculation and kept increasing until reaching a peak. Then it began to decline and was stable for the last 20 days. For all of the F/I ratios, the daily biogas yield was less than 15 mL g VS^{-1} within the initial five days. The highest daily biogas yield of 35 mL g VS^{-1} was obtained at an F/I ratio of one, which was about two times higher than that of an F/I of six (15 mL g VS^{-1}).

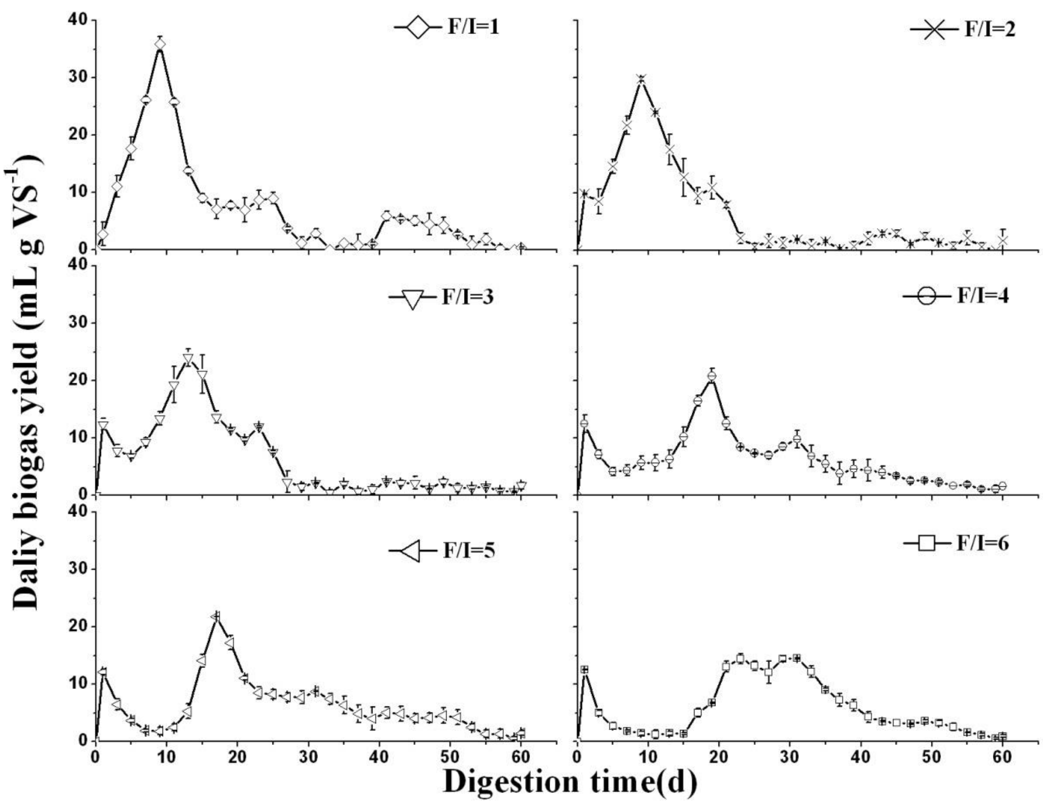

Fig. 1. Daily biogas yield of vinegar residue at six different F/I ratios

An obvious decrease of the daily biogas yield from the beginning was observed at the F/I ratio of 3, 4, 5, and 6. At the F/I ratio of 6, the daily biogas yield was kept in a relatively low level during the 60 days of anaerobic digestion. These results collectively suggested that the F/I ratio had significant influences on daily biogas yield over 60 days of digestion under mesophilic conditions.

As shown in Figure 2, the cumulative biogas yield decreased with the increasing of the F/I ratios. After 60 days of digestion, the highest cumulative biogas yield of 455.77 mL g VS^{-1} was achieved at the F/I ratio of 1 and the lowest biogas yield of 361.91 mL g VS^{-1} was obtained at an F/I ratio of 6, which was 20% less than that of one ($p < 0.05$).

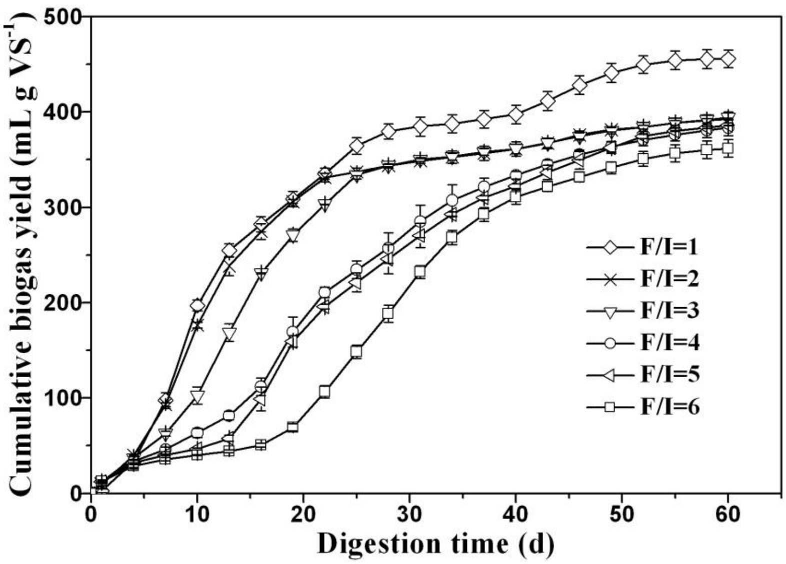

Fig. 2. Cumulative biogas yields of vinegar residue at 6 different F/I ratios

However, there were no significant differences when the F/I ratio was in the range of 2 to 5. The average daily cumulative biogas yield at different F/I ratios were 7.60, 6.54, 6.57, 6.39, 6.43, and 6.03 mL, respectively. The results showed that the cumulative biogas yield was influenced by the F/I ratio, and a negative correlation between the biogas yield and the F/I ratio was found. A similar trend was obtained by Liu's research (Liu *et al.* 2009) during the digestion of food and green wastes. According to Neves' report, the increase in the F/I ratios probably caused an overloading due to the VFAs accumulation (Neves *et al.* 2004), thus leading to a lower biogas yield.

Effect of F/I Ratios on Methane Content

As shown in Fig. 3, the methane content in the biogas produced by the vinegar residue was increased for the first 25 days and stabilized during the last period. At the F/I ratio of 1 and 2, the methane content in the biogas was raised to more than 50% rapidly, after 9 days of digestion. An obvious delay of the methane content increasing from the beginning was observed as the F/I ratio increased from 3 to 6. After 25 days of digestion, there was no significant difference between the methane contents obtained at each F/I ratio, and the final methane content ranged from approximately 61% to 67% at different F/I ratios, implying that the F/I ratios had no significant effects on the final methane content after 25 days of digestion.

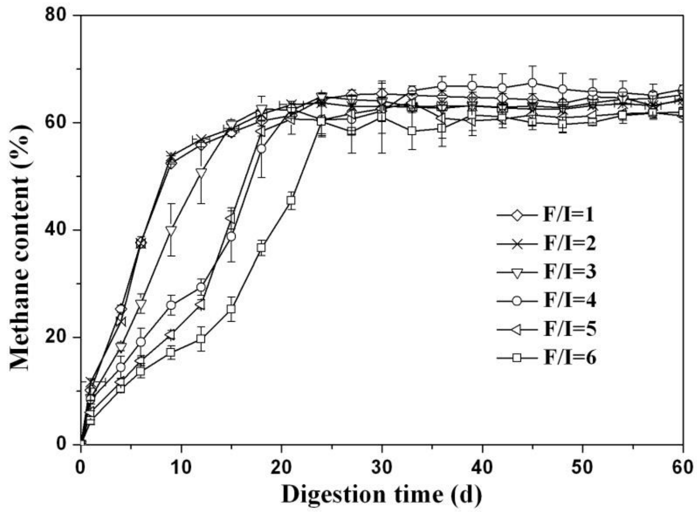

Fig. 3. Methane contents in the biogas from vinegar residue at six different F/I ratios

Biochemical Methane Potential

As shown in Table 2, the maximum methane yield of 242.69 mL g VS^{-1} was achieved at a F/I ratio of 1, while the lowest yield of 182.94 mL g VS^{-1} was obtained at a F/I ratio 6. The regression results showed that *A*, μ_m, and λ were all dependent on the F/I ratio. The *A* value predicted from the modified Gompertz equation seemed to be slightly lower than those of the experimental cumulative methane yield. Figure 4 describes the cumulative methane yields and modeling at six different F/I ratios. With the increase of the F/I ratio from 1 to 6, the lag phase increased implying that the methanogens were inhibited at high F/I ratios. A good fitting of the experimental data could be verified with R^2. Compared with other results of lignocellulosic biomass, the BMP obtained from vinegar residue was relatively lower. Amon *et al*. (2007) reported the BMP of wheat ranged between 140 and 343 mL g VS^{-1}. Triolo *et al.* (2012) determined the BMP of an herbaceous garden plant as 332.7 mL g VS^{-1} and herbaceous wild plants of 214.0 mL g VS^{-1}. The relatively lower BMP of vinegar residue could be attributed to the mass lost in the vinegar produced process. However, as a by-product of agro-industry, the result obtained in this research has already shown us the extensive potential of vinegar residue in the view of energy recovery and waste reutilization.

Table 2. Cumulative Methane Yield and Kinetic Parameters Estimated by the Modified Gompertz Model

F/I	*A* (mL CH_4 g VS^{-1})	μ_m (mL CH_4 g VS^{-1})	λ (days)	R^2	Cumulative methane yield (mL CH_4 g VS^{-1})
1	230.93	9.15	2.15	0.979	242.69
2	191.66	10.90	3.73	0.990	203.42
3	192.69	10.49	6.96	0.996	202.33
4	198.10	6.89	10.82	0.997	196.52
5	202.31	6.91	11.32	0.995	200.36
6	183.46	8.37	18.42	0.999	182.94

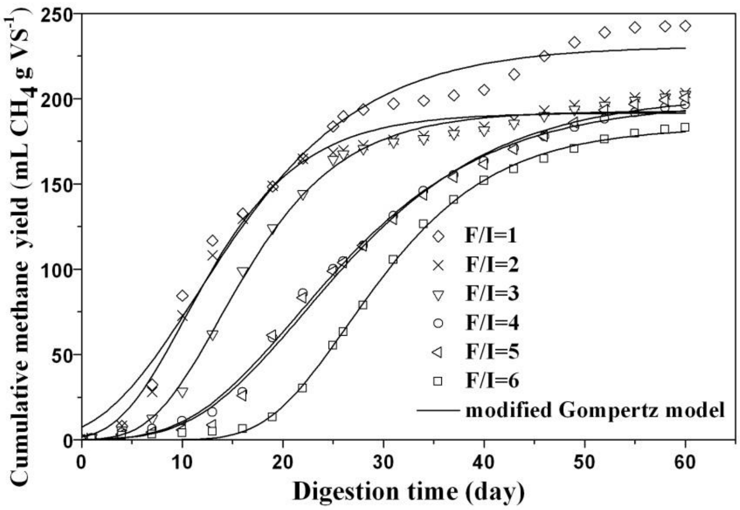

Fig. 4. Cumulative methane yield and modified Gompertz plots at six different F/I ratios

VFA Accumulation

The anaerobic digestion process can be inhibited at low pH value, which can be attributed to the accumulation of volatile fatty acids (VFA) (Callaghan *et al.* 2002). The concentration of total VFA and pH value after digestion were detected and are shown in Table 3. The final pH values were below 6.5 when the F/I ratio was 5 or 6.

The VFA (especially acetic acid) is the main source of methane production. It is also a major inhibitor of anaerobic digestion at higher concentration. As shown in Table 3, less VFA accumulation was observed at the end of the digestion time at the F/I ratio of 1 to 4. However, for F/I ratio higher than 5, remarkable VFA accumulation in the digester was observed, increasing the total VFA concentration up to the values of 479.34±70.90 and 866.38±103.69 mg L^{-1} for F/I ratio of 5 and 6, respectively. The results show that higher F/I ratio led to the VFA accumulation in the anaerobic process. These observations are consistent with the results of other previous studies (Zhou *et al.* 2011).

Table 3. Volatile Fatty Acid Concentration, PH Value, and Total Alkalinity After Digestion

F/I	Volatile fatty acid (mg L^{-1})	pH Value	Total alkalinity (g $CaCO_3$ L^{-1})
1	115.59±2.93	6.69±0.03	1084.56±155.59
2	234.25±3.82	6.60±0.03	959.56±35.07
3	231.65±1.44	6.59±0.67	1043.06±37.35
4	289.23±6.89	6.52±0.21	989.74±215.57
5	479.34±70.90	6.49±0.03	924.60±6.87
6	866.38±103.69	6.43±0.14	1049.145±200.91

Another indicator for determining the stability of anaerobic digestion is the VFA/alkalinity ratio. There are three critical levels for this: 1. <0.4 stable; 2. 0.4-0.8, some instability will occur; 3. >0.8, significant instability (Callaghan *et al.* 2002). As shown in Fig. 5, the ratio values were lower than 0.4 for F/I ratios below than 4, which demonstrated the high stability in these conditions. An obvious higher ratio was observed at F/I of 6, which showed destabilization in that digester.

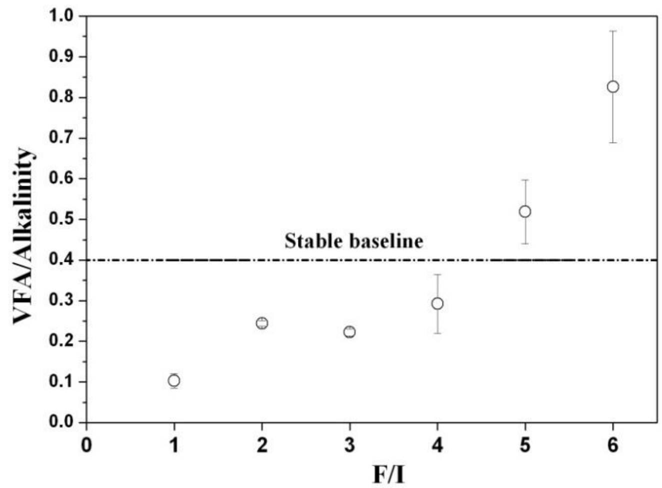

Fig. 5. VFA/Alkalinity ratio at different F/I ratios

Technical Digestion Time and Biodegradability

Technical digestion time, which is defined as the time required to reach 80% of the maximum cumulative biogas yield, is another indicator of the feedstock biodegradability and was thus investigated in this study. The maximum cumulative biogas yield of a feedstock is defined as the anaerobic biogas yield reached by the most productive sample at the end of digestion (455.77 mL g VS^{-1} at F/I of 1 in this study). As shown in Fig. 6, the technical digestion times at different F/I ratios were 28, 44, 43, 52, 52, and 60 days, respectively. The F/I ratios had an obvious influence on the digestion time, and a higher F/I ratio led to a significant increase of the digestion time for the vinegar residue (F/I ratio of 1 vs 6, $p < 0.05$).

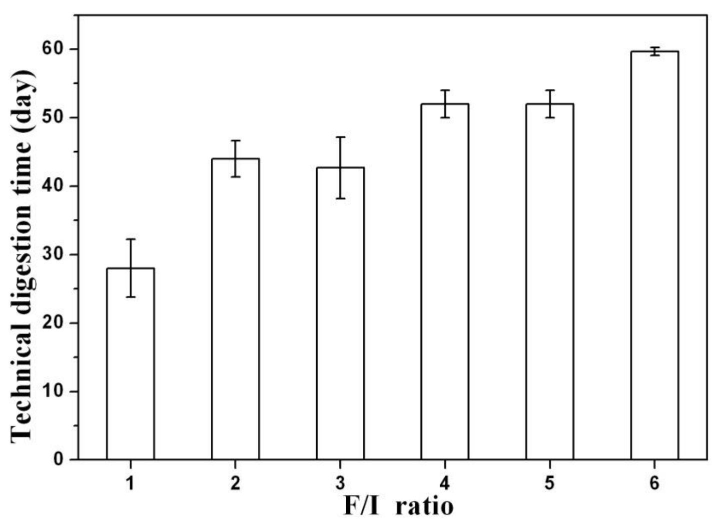

Fig. 6. Effect of F/I ratio on technical digestion time for vinegar residue

As shown in Table 4, with the increase of the F/I ratios, the methane-based degradability decreased, implying that a low F/I ratio was beneficial for the vinegar residue digestion. The methane-based biodegradability of the vinegar residue was 51.27%, 42.98%, 42.75%, 41.52%, 42.33%, and 38.64% for F/I ratios from 1 to 6, respectively. The methane-based biodegradability at a F/I ratio of one was 13% more than that of six ($p < 0.05$), which indicated a significant difference between them.

Table 4. Comparison between Experimental and Theoretical Methane Yield

F/I	Experimental CH_4 yield (mL CH_4 g VS^{-1})	Theoretical CH_4 yield (mL CH_4 g VS^{-1})	Methane-based degradability (%)	VS reduction (%)
1	242.69	473.34	51.27	58.4
2	203.42	473.34	42.98	53.9
3	202.33	473.34	42.75	47.1
4	196.52	473.34	41.52	51.6
5	200.36	473.34	42.33	55.4
6	182.94	473.34	38.64	55.1

The results for the VS reduction of the vinegar residue during the digestion process are also shown in Table 4. Considering the higher lignocelluloses contents in the vinegar residue, an efficient pretreatment technology might be helpful to increase the biogas yield of vinegar residue in further applications.

CONCLUSIONS

1. The results obtained in this study clearly demonstrated that anaerobic digestion is a promising method to convert vinegar residue into biogas energy. Anaerobic digestion also provides an alternative and a possibility in the comprehensive utilization of vinegar residue. Further studies of pretreatment methods and a large-scale continuous experiment are in progress in our laboratory now.
2. The biochemical methane potential of vinegar residue and the influence of different F/I ratios were studied using batch anaerobic digestion in this research. The results showed that the vinegar residue is feasible for the production of biogas. A negative correlation was found between the cumulative biogas yield and F/I ratios ranging from 1 to 6, indicating that adjusting the F/I ratio to a proper level was critical to enhance the biogas yield. A higher F/I ratio led to the VFA accumulation in the digester, which caused lower biogas yields, implying that imbalance in the digester occurred.
3. The highest cumulative biogas (455.77 mL g VS^{-1}) and methane yield (242.69 mL g VS^{-1}) were obtained at the F/I ratio of 1, while the lowest cumulative biogas (361.91 mL g VS^{-1}) and methane yield (182.94 mL g VS^{-1}) were achieved at an F/I ratio of 6.

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