Biogas Production from Chinese Herb-extraction Residues: Influence of Biomass Composition on Methane Yield

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The Chinese traditional medicine industry is developing quickly in China, and there is a growing demand for the reasonable treatment of Chinese herb-extraction residues (CHER) that are generated during the process of preparing such medicines. Different from other biomass materials, the nutrient composition of CHER discharged from different producers may vary widely, which makes the study of CHER recycling quite difficult. The present study concerns the effect of nutrient composition on the specific methane yield from the anaerobic digestion of CHER in batch trials under mesophilic temperatures. Large differences were found in the nutrient compositions of the six kinds of CHER, and the total fat and neutral detergent fiber contents affected the specific methane yield more significantly than did the total protein and total sugar contents. The specific methane yields of the six kinds of CHER were 199, 208, 211, 144, 151, and 201 mL CH_4 per gram of volatile solids. From the digestion experiments, a multiple linear regression equation, the Methane Energy Value Model (MEVM), was derived; this model estimates the methane yield from the nutrient composition of CHER. The model requires further validation and refinement.

Keywords: Chinese herb-extraction residues; Anaerobic digestion; Methane production; Nutrient composition; Methane energy value model

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INTRODUCTION

Chinese herb-extraction residues (CHER) are one of the major solid organic wastes generated in China. Approximately 1.5 million tons of CHER are produced each year (Wang *et al*. 2010). This solid waste is generally treated in sanitary landfills and by incineration; however, these methods of disposal cause secondary pollution of the groundwater, soil, and air. With the rapid development of the Chinese herbal medicine (CHM) industry, more and more herb-extraction residues will be generated in the future. Therefore, a new method to treat this solid waste that does not cause pollution is needed.

Anaerobic digestion is an effective method of decomposing high-concentration organic waste. During this process, a great deal of biogas is simultaneously generated and could be used as energy fuel (Gallert *et al*. 2003; Lastella *et al*. 2002; Vahini *et al*. 2010). Because of its abundance of starch, fat, cellulose, hemicelluloses, lignin, and protein, several research groups have tried to use herb-extraction residues as a substrate for anaerobic digestion. For instance, Cheng and Liu (2009) used the method of microwave-assisted alkaline pretreatment to improve biogas production from the anaerobic digestion of CHER, and Li *et al*. (2011) studied the feasibility of biogas production from the

anaerobic co-digestion of CHER with swine manure. These reports demonstrated that CHER is a suitable biomass material for producing biogas by anaerobic digestion.

According to previous reports, the nutrient composition of the substrate has a significant influence on methane production from anaerobic digestion (Amon *et al*. 2007; Teghammar *et al*. 2012). There may be differences in the nutrient composition of CHER discharged from different producers due to the special procedures of CHM, as illustrated in Fig. 1; therefore, it is necessary to study the relationship between the nutrient composition of CHER and methane production due to the diversity of herb materials. However, the effect of the nutrient composition of CHER on methane production has not been reported. In the present study, we randomly selected six kinds of CHER from three herbal medicine producers. The effect of the nutrient composition of CHER on the specific methane yield was investigated in batch experiments under mesophilic anaerobic conditions. Finally, to evaluate the potential methane yield of CHER, a model (MEVM) to estimate the methane yield from the nutrient composition of CHER was developed via regression analysis.

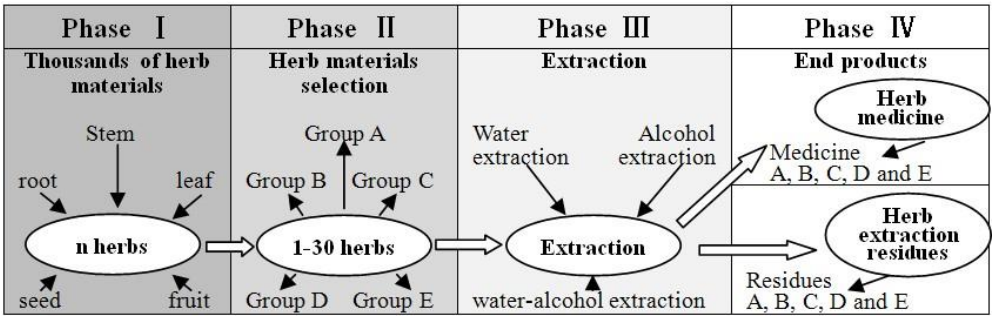

Fig. 1. The procedure flow diagram of Chinese herbal medicine and the herb-extraction residues

MATERIALS AND METHODS

Origin of CHER and Inocula

The six kinds of CHER investigated in this study were obtained from different CHM manufacturers. CHER-1 and -6 were obtained from the Heilongjiang Academy of Traditional Chinese Medicine, CHER-2 was obtained from a drugstore that manufactures CHM on a small scale, and CHER-3, -4, and -5 were obtained from the Heilongjiang University of Chinese Medicine; all of the CHER where produced in the city of Harbin (Heilongjiang, China). The residues were dried at 105 °C and then milled to 50- to 200-mesh powder before use.

The anaerobic sludge used as inocula was collected from an anaerobic digester that digested cattle manure at 35 °C; the pH and suspended solids (SS) were 7.35 ± 0.5 and 47.85 ± 1.5 g L^{-1}, respectively. On the basis of SS, the volatile suspended solids (VSS), TKN, TOC, and C/N ratio of the inocula were 81%, 2.08%, 34.83%, and 16.7, respectively. The substrates and inocula were stored at 4 °C until use. The characterization of each CHER is shown in Table 1. The values are averages of three determinations, with standard deviations lower than 5%.

Table 1. Characteristics of the Six Kinds of CHER Investigated in This Assay

CHER	Composition of CHER										CH_4-yield ml g^{-1} VS	
	TS	VS*	TOC*	TKN*	C/N	pH	TP*	TF*	TSUG*	NDF*	Real	Estimated (MEVM)
1	45.4	90.2	39.1	1.74	22.4	6.98	10.9	3.0	36.5	46.3	199	201
2	51.3	85.9	43.1	1.90	22.7	7.02	11.9	9.1	33.2	46.6	208	206
3	48.8	95.3	46.7	2.06	22.6	6.74	12.9	17.8	22.9	47.3	211	212
4	46.6	88.6	43.6	1.95	22.3	7.16	12.2	2.3	39.3	55.0	144	146
5	47.3	92.5	43.1	1.84	23.4	6.58	11.5	2.7	38.8	54.5	151	149
6	53.6	93.4	40.6	1.74	23.2	6.77	10.9	5.4	34.7	46.9	201	200

* % of TS; all data are averages of three replicates. TP = total protein; CF = total fat; TSUG = total sugar; NDF = neutral detergent fiber; MEVM = Methane Energy Value Model

Batch Experiments

Two-factor batch experiments (Factor A: 6 levels and Factor B: 3 levels) were carried out at 35 ± 1 °C for four weeks. More precisely, the substrate type was set as factor A containing six kinds of CHER, which were named as CHER-1, -2, -3, -4, -5, and -6; and the factor B refers to the three different inoculation ratios of 1.5, 2.5, and 3.5 (basis: dry matter), measured in terms of inoculum (g SS)/CHER (g TS). All of the treatments were performed in triplicate.

The anaerobic assays were conducted in 54 Erlenmeyer flasks (500-mL) with a working volume of 350 mL and a sample dry matter content of 7 to 8%, which corresponds to the concentration commonly found in commercial biogas plants. Two blanks containing 350 mL of inocula were also used to determine the endogenous biogas production of the anaerobic sludge. These bottles were closed with suitable rubber plugs, in the center of which were drilled a hole as a gas channel; a 1-L aluminium gas pack (Dalian Hede Technologies LTD. China) was connected with the channel using a glass tube, and a rubber tube was used for biogas collection. The headspace of the bottles was flushed with pure N_2 to remove oxygen before they were sealed, and the volume of the biogas produced was measured using the water displacement method. All of the bottles were placed in a large water bath (70 * 100 cm), which had a thermostat used to control the working temperature at a stable range of 34 to 36 °C. An analysis of variance and a multiple regression analysis for the data were performed using Design-Expert 8.0.6 trial software and SPSS 19.0 software, respectively.

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 to 2.0 nm) and a thermal conductivity detector (TCD). The injector, oven, and detector temperatures were 120 °C, 190 °C, and 220 °C, respectively. Argon served as the carrier gas at a flow rate of 40 mL min^{-1}.

The total solids (TS), volatile solids (VS), pH (Sartorius basic pH meter PB-10, Germany), total organic carbon (TOC), and total Kjeldahl nitrogen (TKN) were determined according to standard methods (APHA, 2004). The content of the total sugar (TSUG) was tested with a Fehling reagent (Lane and Eynon 1923). The total fat (TF) was

measured as the weight of the dried ethyl ether extract obtained by prolonged extraction at 45 °C for 12 h using a Soxhlet apparatus (Luque-García and Luque de Castro 2004), and the total protein was calculated as TKN×6.25. The neutral detergent fiber (NDF) was determined by the method described by Goering and Van Soest (Goering and Van Soest 1970). All reagents used were of analytical grade. All of the measurements were conducted in triplicate, and the averaged data are presented.

RESULTS AND DISCUSSION

Influence of the Inoculation Ratio

Table 2 shows that abnormal methane fermentation occurred in the T4 and T7 digesters. The low pH values of 5.26 and 5.45 that were detected at the end of the fermentation suggested that the failure may have been due to the excessive accumulation of volatile fatty acids (VFAs). After an analysis of its causes, the low inoculation ratio of 1.5 might be one of the reasons for the weaker buffer capacity compared to the VFAs in the failed digesters; anaerobic digestion of CHER failed at an inoculation ratio of 1.0 in our previous studies (data not shown).

Table 2. Composition, pH-value, and Responses of Each Treatment

Treatment	Factor A	Factor B	CH_4 yield*	pH		Treatment	Factor A	Factor B	CH_4 yield*	pH	
				Initial	Final					Initial	Final
T1	CHER-1	1.5	204	7.11	7.36	T10	CHER-4	1.5	141	7.08	7.56
T2		2	201	7.12	7.45	T11		2	145	7.22	7.49
T3		2.5	193	7.23	7.55	T12		2.5	147	7.25	7.55
T4	CHER-2	1.5	43	6.95	5.26	T13	CHER-5	1.5	161	6.85	7.38
T5		2	205	6.98	7.55	T14		2	143	7.23	7.56
T6		2.5	211	7.23	7.57	T15		2.5	149	7.34	7.49
T7	CHER-3	1.5	24	6.85	5.45	T16	CHER-6	1.5	199	6.95	7.39
T8		2	194	7.02	7.38	T17		2	198	7.02	7.45
T9		2.5	228	7.24	7.67	T18		2.5	206	7.15	7.55

* Units are mL g^{-1} VS, and data are averages of three replicates

A previous report showed that a high C/N ratio could accelerate the accumulation of VFAs, causing the pH value to decrease quickly (Wu *et al.* 2010). As a result, the anaerobic digestion would fail; however, all of the C/N ratios of CHER (22.3 to 23.4) reported in Table 1 were in the optimum range of 18 to 26 and proved to be the most suitable for anaerobic digestion (Wu *et al.* 2010). Furthermore, the weak buffer capacity might also be due to the low content of alkaline inorganic salts in CHER. This characteristic of CHER stems from the special production process of CHM, shown in Fig. 1, during which abundant inorganic salts are lost during the extraction of a medical ingredient from the CHM raw material. In addition, in comparison with other biomasses used as substrates of anaerobic digestion, the performance of the anaerobic digestion could work favorably with an inoculation ratio of 0.5 to 1.5, or even lower (Kim *et al.* 2006; Salminen and Rintala 2002). All of the findings mentioned above indicated that CHER was easier to acidify during the process of anaerobic digestion. Moreover, it is especially notable that a majority of digesters could operate normally at the inoculation ratio of 1.5 except for T4 and T7; therefore, according to the diverse nutrients constitute,

it could be speculated that the causes of failed digestion in T4 and T7 might be attributed to the higher total fat content in CHER-2 and -3 (Table 1).

The two blank trials containing 350 mL of inocula were not observed to produce biogas until the end of the fermentation, which was due to the fact that the inocula were collected after the period of biogas production from the previous adapted culture experiment. The averaged methane yield of all of the normal operation of digesters (48 bottles) was 186 mL CH_4 g^{-1} VS (volatile solids). That was slightly bigger than the methanogenesis ability of cattle manure (132 to 166 mL CH_4 g^{-1} VS) as a single substrate for anaerobic digestion (Amon *et al.* 2007); all of the methane contents were above 55% (data not shown). The highest specific methane yield was 228 mL CH_4 g^{-1} VS (T9), obtained from CHER-3 with an inoculation ratio of 2.5, and the lowest specific methane yield was 141 mL CH_4 g^{-1} VS (T14), obtained from CHER-4 with an inoculation ratio of 1.5.

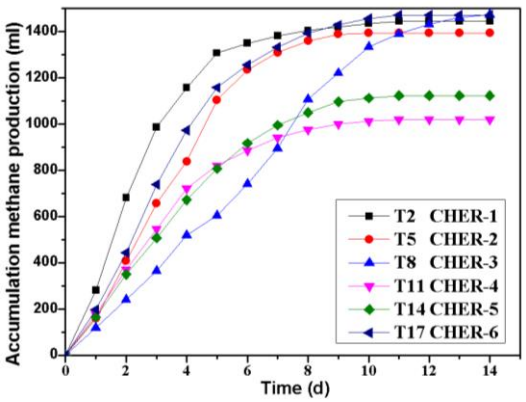

Fig. 1. Accumulated methane production from CHER with an inoculation ratio of 2.0.

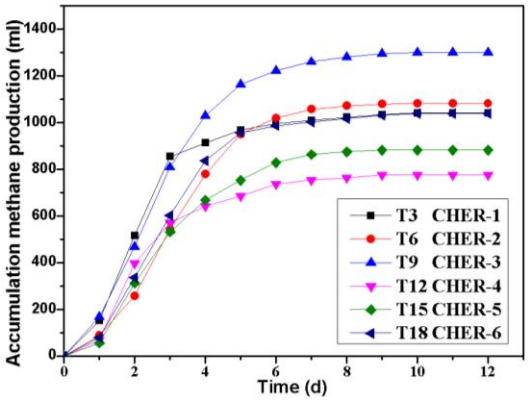

Fig. 2. Accumulated methane production from CHER with an inoculation ratio of 2.5.

Figure 1 illustrates the accumulated methane production throughout the digestion time for T2, T5, T8, T11, T14, and T17 with an inoculation ratio of 2.0 (basis: dry matter). All except T8 obtained the maximum accumulated methane production on day 11, and about 90 percent of the total methane yield had been produced by the seventh day. However, the maximum accumulated methane production from T8 (CHER-3) was delayed and observed on day 14, and 90 percent of the total was produced by day 10. The reason for the delay might be the high total fat content in CHER-3, as shown in Table 1. The phenomenon was not significant at an inoculation ratio of 2.5, as shown in Fig. 2, which might be due to the fact that the fat content was insignificant when more micro-organisms existed in the digesters.

Table 3. Analysis of Variance Results for Methane Production

Source	Sum of squares	Degrees of freedom	Mean Square	F Value	P-value	Significance
Model*	12371.77	7	1767.40	21.40	0.0001	Significant
A-A	11950.08	5	2390.02	28.94	0.0000	
B-B	224.67	2	112.33	1.36	0.3101	
Residual	660.67	8	82.58			
Cor. Total	13032.44	15				
* The data from the normal working digesters, T4 and T7, were ignored for the ANOVA						

The ANOVA results of the batch experiments for the methane production are shown in Table 3. It can be concluded that the model was significant because the Model F-value of 21.40 was greater than the calculated one (0.0001). The P-value of factor A was lower than 0.0000, while the P-value of factor B (0.3101) was much greater than 0.05, which suggests that factor A was more significant relative to the response values as compared to factor B. That is to say, the different inoculums ratios had no obvious effect on the specific methane yields, which could also be seen clearly from Fig. 3. This result suggested that the specific methane yields of all the CHER were not influenced by the nutrients from the inoculums used in this assay. Therefore, the mean methane production rate of each CHER was calculated according to the normal working digesters, and the values of CHER-1, -2, -3, -4, -5, and -6 were, respectively, 199, 208, 211, 144, 151, and 201 mL CH_4 g^{-1} VS.

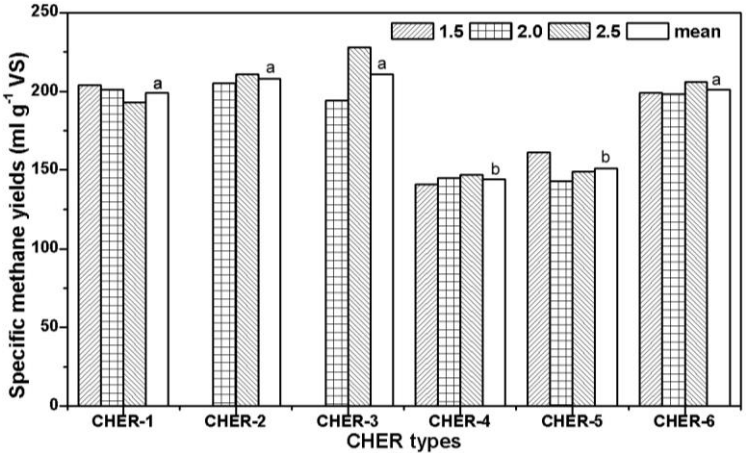

Fig. 3. The specific methane yields of all the CHER types at different inoculation ratios. Different letters indicate a significant difference at $p < 0.05$.

Methane Energy Value Model for CHER

Amon *et al.* proposed the Methane Energy Value Model (MEVM), which estimates the methane production during anaerobic digestion from the composition of maize; the model was further developed as Eq. (1) with subsequent research by the same group (Amon *et al.* 2007). The model shows that the percentage of total protein, total fat, cellulose, and hemi-cellulose (basis: dry matter) has a significant correlation with methane production from maize. In addition, the total protein and total fat contribute most to the net total methane yield, which can be determined from Eq. (1).

Methane Energy Value [mL CH_4 g^{-1} VS] (for Maize) =

19.05*(total protein %) + 27.73*(total fat %) + 1.80*(cellulose %) + 1.70*(hemi-cellulose %) (1)

Methane Energy Value [mL CH_4 g^{-1} VS] (for CHER) =

487.31 + 1.17*(total fat %) -6.26*(total cellulose %) (2)

It was hoped to obtain an equation similar to Eq. (1) that could be used to estimate the methane production from the nutrient composition of CHER. Therefore, a linear relation between the nutrient composition of CHER (TP, TF, TSUG, and NDF) and the methane production was simply deduced by means of a stepwise regression analysis, and the results were obtained using a bivariate regression equation, Eq. (2). Table 4 shows the regression coefficients, standard error, and level of significance of the regression model for the estimation of the methane yield from the anaerobic digestion of CHER. The regression coefficients are highly significant, which demonstrates that the percentages of the total fat and neutral detergent fiber had a significant influence on the methane yield from CHER. Furthermore, the standardized coefficients of TF and NDF were, respectively, 0.233 and -0.864, suggesting that the former variable (TF) had a positive correlation with the methane yield from CHER and the latter variable (NDF) had a negative correlation. However, unlike Eq. (1), the total protein is not significant in the regression model, Eq. (b), which may be due to the small differences in total protein content between the CHER investigated in the present assay. The total sugar content was not significant in either Eq. (1) or Eq. (2).

Table 4. Analysis of Regression Coefficients for Eq. (2)

Nutrient [% of TS]	Regression coefficient	Standard error	Standard coefficient	Level of Significance (P)
Constant	487.31	14.29	---	0.000
Total fat	1.17	0.19	0.233	0.009
NDF*	-6.26	0.28	-0.864	0.000
* NDF= neutral detergent fiber				

The specific methane yields obtained in this experiment were compared to the values estimated with the Methane Energy Value Model (Eq. 2) shown in Table 1. The estimated values are quite close to the measured values with a small difference in a range of 1 to 2 mL CH_4 g^{-1} VS. The good agreement suggests that the model validity could be verified by the present six kinds of CHER. However, because of the species diversity of CHER, there may be great differences on the nutrients composition between the CHER of the present assay and other CHER types. Therefore, it is important to further verify the validity and improve the accuracy of the Methane Energy Value Model by using more CHER materials.

Generally, many studies have shown that there are great effects of total sugar, total protein, and carbohydrate contents on methane generation. Obviously, neither Eq. (1) nor Eq. (2) could be used to estimate the specific methane yields from all organic substrates; rather, they can be applied strictly to the samples tested in the present work. It follows that in order to obtain a universal model for estimating the methane yield of any organic substrates from their nutrients composition, it would be necessary to conduct a much wider range of similar experiments and to investigate more realistic effects of variations in nutrient composition on specific methane yield using as many different kinds of organic substrates as possible.

CONCLUSIONS

1. Batch experiments showed that the anaerobic digestion of CHER could be carried out steadily with a suitable inoculation ratio, and the average specific methane yield reached 186 $mL \cdot g^{-1}$. A higher inoculation ratio guaranteed the anaerobic digestion of CHER due to the easy acidification under a low inoculation ratio.
2. A high total fat content of CHER might be beneficial to produce more VFAs and thereby obtain a higher methane yield. However, it might also lead to the rapid accumulation of acidification, leading to anaerobic digestion failure.
3. The total fat and NDF contents were more significant than the total protein and total sugar contents. On the basis of this finding, the Methane Energy Value Model was developed. This model could be used to estimate the methane yield of CHER from its nutrient composition. Because of the species diversity of CHER, the accuracy of the Methane Energy Value Model needs to be improved by adopting more data from more species of CHER.
4. Comparing the MEVM for maize and the MEVM for CHER, it is clear that to obtain a widely applicable model to estimate the methane production of organic substrates from their compositions, an investigation of the relationship between the methane production and composition of more kinds of biomass is needed.

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