Methane Yield Predictive Model Based on the Composition of Biomass: Focus on the Anaerobic Digestion Mode and Regression Method

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Nine kinds of biomass were used to investigate the correlation of organic composition with the methane yield in continuous anaerobic digestion (AD) mode with different organic loading rates (OLRs). The experimental results showed that the methane yields were different with the change of OLR; thus, only one model was unable to satisfy the prediction accuracy for all the operation conditions. A stepwise regression model and two self-defined models were used to determine the prediction accuracy for different operation OLRs in the present assay. The results showed that the self-defined models constituted by biodegradable components (protein, fat, and readily degradable sugars) obtained a higher determination coefficient (R^2) than the model fitted by the traditional stepwise regression method. Biomass with a higher content of easily degradable matter had a lower predictive deviation. Based on this, it is recommended that the various biomasses be divided into two groups to obtain better model fitting and prediction accuracy. The biomass with a content of readily degradable section that was more than 47% or the content of lignin was less than 8% can be classified into one group, and the others can be classified into another group according to the present test results.

Keywords: Methane yield predicative model; Continuous anaerobic digestion; Organic compositions; Lignocellulosic biomass

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INTRODUCTION

Biogas can be produced from organic wastes *via* an anaerobic digestion (AD) process, which has attracted great attention in past years. This attention has been mainly attributed to the double benefits of protecting the environment and reducing consumption of fossil energy. Many types of biomass materials have been shown to be reusable for biogas production, such as agricultural waste, animal waste, municipal solid waste, *etc.* Due to a wide range of sources, the organic composition and distribution of these biomass types are usually different, leading to a different methane potential.

Proteins, fats, and carbohydrates are the main organic parts of biomass. Previous studies have found that the methane potential of the three compounds is different. The theoretical methane yields of proteins, fats, and carbohydrates are 496, 1014, and 415 $mL \cdot g^{-1}$, respectively (Møller *et al.* 2004). However, in the practical AD process, the biological methanation potential (BMP) of one biomass type is hard to equate to the algebraic sum of theoretical methane yield of each component. This can be attributed to two reasons. One is the synergistic fermentation among different components, such as different nutritive proportion (C/N) that is able to lead a different microbial metabolic efficiency. The other reason is that carbohydrates usually are divided into two types according to their biodegradability in the AD process, namely, the easily degradable part and the recalcitrant part. The latter is mainly composed of the lignocellulosic compounds that are difficult to decompose *via* the AD process with a shorter fermentation period. Therefore, the distribution of various organic compounds in biomass is also a key factor that affects the methane yield. Many previous studies have focused on the investigation of the correlation between organic composition and BMP of biomass. The methane yield predication models (MYPM) found in these studies are summarized in Table 1.

Table 1. Correlations Found in the Literatures between the Organic Compositions of Biomass and Methane Production

Biomass Used	Methane Production Model
Maize straw with different mature period (n=12) (Amon *et al.* 2007)	BMP=19.05Pro+27.73Fat+1.80Cel+1.70Hcel
Chinese herb-extraction residue (n=6) (Wang *et al.* 2013)	BMP=487.31+1.17Fat-6.26NDF
Lignocellulosic residue (n=7) (Gunaseelan 2007)	BMP=0.18+0.48Soluble Car +2.8Pro+0.2ADF-0.003Lig/ADF-0.83Ash
Lignocellulosic residue (n=12) (Gunaseelan 2009)	BMP=0.045+1.23Soluble Car +0.24Pro+1.51Fat-0.68ADF-0.81Cel-6.1Ash
Domestic and agricultural waste, *etc.* (n=14) (Buffiere *et al.* 2006)	Biodegradability (%)=0.87−1.03(Lig +Cel)
Lignocellulosic biomass (n=20) (Monlau *et al.* 2012)	BMP=303.14-4.53Lig+0.77Soluble Sug+1.28Pro-1.59Crystalline Cel +0.61Amorphous Cel+1.33Aluronic acid
Mushroom residue (n=10) (Li *et al.*2017)	BMP=69.992+5.220Total Sug−4.881Lig
NDF: Neutral detergent fiber; ADF: Acid detergent fiber; Car: Carbohydrate; Pro: proteins; Sug: sugar; Hcel: hemicellulose; Cel: cellulose; Lig: lignin	

Amon *et al.* (2007) found that four components of crude protein, crude fat, cellulose, and hemicellulose are positively related to the methane production. The factor coefficients showed that the contributions of crude protein and crude fat to BMP are significantly greater than the latter two. Wang *et al.* (2013) selected six Chinese herb-extraction residue (CHER) types and found the contents of crude fat and neutral detergent fiber present a significant positive and negative correlation to the BMP of CHER, respectively, and the contents of protein and starch have no significant influence on the BMP. Gunaseelan *et al.* (2007) used a variety of lignocellulosic materials to investigate the correlation of organic composition and BMP. The results showed that all the components present a positive correlation to the BMP except for lignin. In another study of Gunaseelan *et al.* (2009), both lignin and cellulose are negatively correlated to the BMP. According to the mode established by Buffiere *et al.* (2006), both lignin and cellulose have also been proved to be difficult to use by anaerobic microbes. There is a great difference among these models, namely, one model may only apply to the biomass in the self-studied samples, while it might be lack of accuracy to evaluate other biomass out of the studied samples.

According to previous studies, stepwise regression usually has been used for the model fitting of MYPM. A component having a significant influence ($P<0.05$) on the ultimate methane yield will be retained in the model, while one without significant influence will be eliminated from the model. As a result, many models reported by previous literatures are different. Table 1 displays that the same organic component might show a different influence on the methane yield in different models, as well as a difference relative to the type and quantity of factors in these models. Therefore, it can be concluded that the MYPM built by linear regression analysis has a large variation, which might be due to a great deal of dependence on the studied samples. In addition, the models reported in previous studies were established using the data of methane yields obtained from batch experiments, while lacking investigation on the continuous AD model. However, most of the biogas engineering is operated in continuous mode, and the methane yield would be different with the batch test and depending on the change of organic loading rates (OLRs). In acknowledgement of this, the main objective of the present work is to build a MYPM based on more variation of biomass materials, thereby improving the model universality on the evaluation of biogas production, especially for the prediction of continuous AD mode. Therefore, a series of continuous AD tests were conducted with different OLRs and multiple biomass materials found in the present work to investigate correlation of methane yields with biomass compositions and operating OLR. The obtained methane yields in different OLRs would be used to fit the MYPM.

EXPERIMENTAL

Substrate and Materials

Nine biogas source materials were used in this study, including three livestock manures (diary manure, swine manure, and chicken manure), two agricultural wastes (corn straw and soybean straw), and four other materials (food waste, cassava residue, Chinese herb-extraction residue, and corn flour).

Table 2. Characteristics and Organic Compositions of the Nine Biogas Materials and Feedstock

Organic Component	DM	SM	CM	FW	CR	CHER	CS	SS	CF
TS (%)	22.20	31.40	11.50	21.80	88.10	45.50	87.40	88.30	87.20
VS (%)	83.32	79.85	75.40	94.62	97.54	85.92	94.77	93.55	98.00
Pro (%)	9.00	16.50	17.06	11.31	3.06	11.81	5.88	5.13	8.13
Fat (%)	5.61	8.06	8.41	33.51	2.92	9.16	3.57	1.17	2.67
TSug (%)	20.06	23.33	22.14	42.27	64.23	25.99	40.15	22.46	88.00
Hcel (%)	23.93	22.8	21.88	2.04	10.4	28.75	25.74	18.25	1.20
Cel (%)	20.98	13.83	10.45	3.22	17.17	12.03	23.4	39.96	0.78
Lig (%)	10.80	4.01	7.67	2.48	4.68	8.97	2.33	15.85	0.02

Note: All data based on dry matter of biomass; TS: total solid content; VS: volatile solid content; DM: dairy manure; SM: swine manure; CM: chicken manure; FW: food waste; CR: cassava residue; CHER: Chinese herb-extraction residue; CS: corn straw; SS: soybean straw; CF: corn flour; Pro: proteins; TSug: total sugar; Hcel: hemicellulose; Cel: cellulose; Lig: lignin

All the biomass specimens were collected from Heilongjiang Province, China. The anaerobic sludge used as inoculum was collected from a pilot anaerobic digester of the authors' laboratory (working volume: 500 L) for cattle manure at 35 °C. The total solid (TS) of the inoculum was 4.48%, and based on TS, the measurements of the volatile solid (VS), total Kjeldahl nitrogen (TKN), and total organic carbon (TOC), were 81%, 2.08%, and 34.8%, respectively. The C/N of the inoculum and the ratio of inoculum to the mass of fresh biomass were 16.7 and 2, respectively. The organic compositions of all the samples are shown in Table 2. The samples were collected in triplicate, and averaged data of the measurements are presented. All the materials were diluted to a TS of 6% before being fed into the reactors except for chicken manure, which was diluted to below 3% to prevent an ammonia inhibition due to its high ammonia production capacity.

Experimental Design and Procedure

Nine biogas materials were used in the present work, and each one was fermented alone in a self-made continuous digester. The detailed construction of this digester was introduced in a previous article (Wang *et al.* 2017). The total volume and working volume of each digester were 2 L and 1.5 L, respectively. The generated biogas was collected daily by an aluminum gas pack (Dalian Hede Technologies Ltd., Liaoning, China), and the volume was measured based on the downward displacement of water. All the digesters were evenly placed in two constant-temperature shakers (35±1 °C, 80 rpm), and a time switch within the shaker was able to control the shaking at a specified time (running 10 min in 1 h). All digesters were initially loaded with 1.5 L of inocula; when they started to run, the OLR was set to a lower level for acclimation of microorganism. After running for a month, the OLR was raised gradually to 2.4, 3, and 3.6 $g{\cdot}L^{-1}{\cdot}L^{-1}$, and the schedule of the digester fed with chicken manure was 1.2, 1.5, and 1.8 $g{\cdot}L^{-1}{\cdot}L^{-1}$. The duration time of each OLR was kept at 30 days. During this time the methane yield was investigated. The biogas volume, methane content, and pH of the effluent liquid were monitored every two days.

Experimental Parameters and Analytical Methods

Basic characteristics

The methane and carbon dioxide concentrations in the biogas were determined with a gas chromatograph (GC-6890N; Agilent Inc., Santa Clara, CA, USA) equipped with a stainless-steel column (1.5 m × 3 mm i.d. carbon molecular sieve TDX-01: 1.5 to 2.0 nm) and a thermal conductivity detector using argon as the carrier gas. The TS, VS, pH (PB-10; Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany), total organic carbon (TOC), and TKN were determined according to standard methods (APHA 2004).

Organic component

Crude protein (CP) was calculated as TKN×6.25 (Girolamo *et al.* 2013). Crude starch (CS) was calculated as the total sugar×0.9, and the total sugar was tested with the method of Fehling reagent (Phygene Life Sciences Company, Fuzhou, China). Crude fat (CF) 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 (Luquegarcía and Castro 2004). The contents of hemicellulose, cellulose, and lignin were determined with an automatic fiber analyzer (ANKOM A2000i;ANKOM Technology, New York, NY, USA) according to the method previously reported (Van Soest *et al.* 1991). All reagents used were of analytical grade, all the measurements were conducted in triplicate, and the averaged data are presented.

Model building

According to the previous literatures, stepwise regression (SR) is usually used for the fitting of a methane yield predication model (MYPM). The components that have a significant influence on the methane yield are retained in the model, while any without significant influence are eliminated from the model. However, the final obtained models are different from each other and are largely dependent on the biomass samples. As a result, a universal model has not been built yet. Thus, two models were first defined in the present work according to the conversion efficiency of each organic compound in the AD process. The models are expressed as Eqs. 1 and 2,

$$y = a\times\mathrm{Pro}+b\times\mathrm{Fat}+c\times\mathrm{TSug} \tag{1}$$

$$y=a\times\mathrm{Pro}+b\times\mathrm{Fat}+c\times(\mathrm{TSug}-d\times\mathrm{HCel}) \tag{2}$$

where Pro, Fat, Tsug, and Hcel represent protein, fat, total sugar, and hemicellulose, respectively, and are given as a percentage (based on dry matter).

The factors in the two models were selected according to the reduction rate of each component in the AD process. The protein, fat, and total sugar are the largest amounts of components digested in the AD process; however, the hemicellulose and cellulose have a smaller degradability. Additionally, the test process of total sugar (TSug) is generally conducted within a rigorous environment of hot acid liquid (100 °C, 30 min, 6 $\mathrm{mol{\cdot}L^{-1}}$ HCl). This will result in a partial hydrolysis of hemi-cellulose and amorphous cellulose, which will lead to an increase of TSug value. Therefore, Eq. 2 was built from the hypothesis that the detected total sugar contains some hemicellulose. All model fitting processes were performed using SPSS 19.0 software (IBM Corporation, Armonk, NY, USA).

Theoretical Methane Potential

The theoretical methane potential (y_{th}, $\mathrm{mL{\cdot}g^{-1}}$) of untreated substrate was calculated based on the stoichiometric conversion of organic matter (Girolamo *et al* .2013) as Eq. 3,

$$y_{th} = 415\times\mathrm{Car} + 496\times\mathrm{Pro} + 1014\times\mathrm{Fat} \tag{3}$$

where Car, Pro, and Fat represent the contents of total carbohydrates, protein, and fat, respectively, and are given as a percentage (based on dry matter), and the content of total carbohydrates is equal to 1 minus the sum of contents of protein, fat, and ash.

RESULTS AND DISCUSSION

Methane Yield

The methane yields of the nine biomass materials used in present assay were investigated by semi-continuous AD mode with three levels of OLRs, respectively. All the obtained methane yields are shown in Table 3. The mean methane yields gradually decreased as the OLR increased for all biomass samples. This was mainly because the method used in the present assay to increase the OLR was to fix the feedstock TS content and gradually decrease the HRT. In the continuous AD mode, the feedstock fed into the digester on the day before was excreted from the digester in a proportion of 1/HRT the next day due to its specific operating mode. The shorter HRT will result in a decrease of biomass utilization degree. This was also an important reason that caused a lower methane yield in the continuous AD process. Thus, it was not hard to explain that the minimum methane yields were obtained at OLR of 3.6 g·L^{-1}·L^{-1} with a HRT of 16.7 days.

Table 3. Methane Yields by Actual Test and the Defined-Model II Prediction

Bio-mass	Mean Methane Yield (mL·g^{-1} TS)						y_{th} (mL·g^{-1} TS)
	OLR of 2.4g·L^{-1}·L^{-1}		OLR of 3g·L^{-1}·L^{-1}		OLR of 3.6g·L^{-1}·L^{-1}		
	Actual*	Model y_3	Actual	Model y_6	Actual	Model y_9	
DM	91.65	151.1(59.4)	81.8	125.0(43.2)	70.3	101.5(31.2)	371.2
SM	248.8	247.5(-1.3)	224.5	218.2(-6.3)	188.2	183.0(-5.2)	412.4
CM	269.9	252.9(-17)	236.3	223.8(-12.5)	195	188.1(-6.9)	395.9
FW	531.8	529.6(-2.2)	449.2	449.9(0.7)	405.6	406.7(1.1)	593.1
CR	252.4	244.0(-8.4)	230.1	213.6(-16.5)	203.6	193.7(-9.9)	425.8
CHER	155.1	217.7(62.6)	136.1	181.1(45)	117.1	149.7(32.6)	428.6
MS	189.6	169.5(-20.1)	151.6	139.4(-12.2)	129.8	117.0(-12.8)	435.9
SS	73.42	96.3(22.9)	55	79.9(24.9)	48.5	64.7(16.2)	372.1
CF	365.5	376.0(10.5)	330.8	344.3(13.5)	297.6	315.2(17.6)	440.8

Note: *The data shown refer to the authors' previous report (Wang *et al.* 2017); values in parenthesis are the deviation between actual and predictive value

The methane yield of food waste (FW) was noticeably greater than other biomass materials used in the present assay, while the soybean straw (SS) showed the weakest gas production capacity. This is precisely because FW is rich in fat and starch, and the SS is high in recalcitrant compounds (lignocelluloses). Liu *et al.* (2017) studied the AD process of FW and also obtained a higher methane yield that varied at the range of 371 to 541 mL·g^{-1}. It also can be seen from the previous studies shown in Table 1, that although there is a great difference among the BMP evaluation models found by the previous studies, lignin, hemicellulose, and cellulose have minimal contribution to the BMP, while protein, fat, and starch (soluble carbohydrate) have a positive correlation to the BMP with different degrees.

Regression Analysis

In the present work, all types of biomass materials were digested in continuous AD mode with three OLRs. All the obtained methane yields are shown in Table 3, and because the results of components analysis were shown based on the dry matter of each biomass, the methane yields used in regression analysis were also calculated as the dry matter. Three types of linear regression models were tested in the present work, namely, a stepwise regression model and two self-defined models of Eqs. 1 and 2. All the regression results are shown in Table 4.

As shown in Table 4, the MYPMs obtained from stepwise regression contained only one or two factors, fat and cellulose were included in the model with the lower OLR of 2.4 g·L^{-1}·L^{-1}, and only cellulose had a significant influence ($P<0.05$) on the methane yield in the other two stepwise regressions models with higher OLRs. The components not included in the models, such as protein and total sugar, were judged to be insignificant with respect to the methane yield. This is similar to some previous reports in which the MYPMs also only contained a factor of lignin, and the other ingredients were ignored (Triolo *et al.* 2011; Monlau *et al.* 2012). Additionally, the factors are often different in these models. This is mainly due to the fact that the result of stepwise regression depends heavily on the variance values of factor levels, while the factor levels of actual biomass are uncertain and not adjustable. Therefore, it is necessary to select the factors according to the AD characteristics of components to fit the more reasonable and universal model. The factors are fixed in the present two self-defined models and predefined before regression analysis according to the AD performance. Although Table 4 shows that all the determination coefficients (R^2) of the models are almost the same in the group with a lower OLR of 2.4 $g \cdot L^{-1} \cdot L^{-1}$,the R^2 of the two self-defined models were greater than that of the stepwise regression model at higher OLRs, and all the highest R^2 were observed at the self-defined model II. Thus, the self-defined models showed a better accuracy on the methane yield than the model fitted by the method of stepwise regressions, especially in the case with a higher OLR.

Table 4. Methane Yield Evaluation Models Fitted by Regression Analysis

OLR ($g \cdot L^{-1} \cdot L^{-1}$)	Operation Condition	Regression Model	R^2
2.4	Stepwise regression	y_1=313.4+6.57Fat-6.76Cel	0.969
	Self-defined model I	y_2=5.29Pro+9.94Fat+3.23TSug	0.969
	Self-defined model II	y_3=8.24Pro+9.01Fat+3.26(TSug-0.50HCel)	0.970
3.0	Stepwise regression	y_4=363.2-8.60Cel	0.865
	Self-defined model I	y_5=4.81Pro+8.01Fat+2.90TSug	0.953
	Self-defined model II	y_6=8.55Pro+6.96Fat+2.94(TSug - 0.71HCel)	0.982
3.6	Stepwise regression	y_7=328.2-8.22Cel	0.879
	Self-defined model I	y_8=3.15Pro+7.57Fat+2.71TSug	0.952
	Self-defined model II	y_9=7.25Pro+6.36Fat+2.75(TSug -0.83HCel)	0.990

Note: y_x is the methane yield, and the self-defined model I and II were obtained from Eq. 1 and Eq. 2, respectively

Model Verification

According to the results of regression (Table 4), the self-defined model II obtained a higher fitting degree to the methane yield in semi-continuous mode; thus it was used to verify the prediction accuracy. As shown in Table 3, except for dairy manure, CHER, and soybean straw, the variation between the measured value and the predictive value was less than 12% at all levels of OLR. It was not hard to find that the sum of easily degradable matters (protein, fat, and total sugar) of the six biomasses were all over 47% and the content of lignin were lower than 8% (Table 2). In contrast, in the group with a lower prediction accuracy of dairy manure, CHER, and soybean straw, the content of the more easily degraded section were generally lower 47% and the lignin contents were higher than 8%. Thus, it can be concluded that the model predication will be more accurate if the content of easily degraded matters is more or the content of lignin is less. Moreover, from the prediction results (Table 3), it can be also found that all the predictive values of dairy manure, CHER, and soybean straw were higher than the actual values. This was possibly because the recalcitrant matter (lignin, *etc.*) may cause some hindrance to the degradation of readily degradable matter. For the investigation of correlation between organic compositions and methane yield, it can be recommended that all the biomass materials are divided to two groups according to the contents of readily degradable matters or lignin. As shown in the current results, the biomass with more than 47% of readily degradable section or less than 8% of lignin can be classified as a group, and the others can be classified as a group, which may not only simplify the model, but also improve the prediction practicality. The obtained models were fitted on the AD performance based on the present nine biomass materials. The selected samples still have some limitations, so a more extensive validation for the present models is needed.

CONCLUSIONS

1. For the continuous AD mode, the methane yield would be varied as the change of OLR. Therefore, one model can hardly satisfy the predication accuracy for all the operation conditions. Therefore, the variation coefficients in a model need to be retrieved as the change of OLR.
2. Both the two defined models obtained a higher determination coefficient (R^2) than the model obtained from the traditional stepwise regression method, especially in the operation with a higher OLR. This suggested that the biodegradable components should be fixed in the model, which can improve the prediction accuracy.
3. It is recommended that the various biomass materials be divided into two groups according to the contents of easily degradable matters or lignin. This might be favorable to simplify the model, improve the model accuracy, and improve practicality to predict the methane yield. According to the present experimental results, the biomasses with a content of readily degradable section of more than 47% or the content of lignin was less than 8% can be classified as a group, and the others can be classified as a group.

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