Comparative Analysis of Single-stage and Two-stage Fermentation Systems under Various Process Conditions

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A comparative study of single-stage fermentation (wet or dry) and two-stage (wet-dry and dry-wet) fermentation systems was carried out under medium temperature conditions. The effect of the length of the first wet or dry fermentation stage (5-, 10-, 15-, 20-, and 25-d) in the two-stage anaerobic fermentation was investigated. The results showed that the gas production of wet fermentation and two-stage wet-dry fermentation was better than that of the dry fermentation and two-stage dry-wet fermentation. The cumulative gas production increased gradually with increased stage conversion times for the two-stage wet-dry fermentation. The gas production for the 20-d experimental group of the two-stage wet-dry fermentation system was the best. The cumulative biogas production in the anaerobic fermentation of straw correlated significantly with the changes in the degradation rates of volatile solids, cellulose, and hemicellulose ($P < 0.01$). The kinetic fitting analysis showed that the Reaction Curve (RC) model was more suitable for data modeling of the single-stage wet fermentation and two-stage wet-dry fermentation with straw than the Modified Gompertz (GM) and Modified Logistic (LM) models. The results of this study provided a theoretical basis for choosing a fermentation process for large-scale biogas production with straw.

Keywords: Straw; Fermentation process; Single-stage fermentation; Two-stage fermentation; Wet-dry; Kinetic fitting model

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INTRODUCTION

In addition to the increasing need for fuels and energy in industrial uses, the demand for the production of bio-fuels from lignocellulosic biomass and agro-industrial waste is increasing. Anaerobic fermentation that produces biogas from crop straw is a potential solution to meet the demands and can help to solve environmental pollution (such as burning), adjust the energy structure, and reduce emissions of greenhouse gases (Weiland 2010; Lian *et al.* 2014; Khor *et al.* 2015). Therefore, to promote fermentation technology and industry development of straw biogas, a sizeable amount of research into the collection, storage, pretreatment, fermentation process, and comprehensive utilization of fermentation straw residue was carried out by domestic and foreign researchers.

On the policy level, to accelerate the transformation and upgrading of rural biogas production, the National Development and Reform Commission and the Ministry of Agriculture implemented twenty-five pilot-scale biogas projects using the central budget in China. This measure brings about new opportunities for the industry to develop renewable energy. The factor of collection radius for livestock manure and other conventional fermentation raw material was considered, and straw has henceforth become one of the main fermentation materials for large-scale natural biogas production. Therefore, the initiative also provides policy support for the development of energy utilization technology that uses crop straw.

The process of biogas fermentation can be divided into batch, semi-continuous, or continuous from the point of import and export mode. The concentration of fermentation substrate that is needed depends on whether it is wet fermentation, high concentration fermentation, or dry fermentation. Thus, the choice of fermentation process is vital for the operational efficiency and stability of biogas production. Research has shown that the concentration in anaerobic fermentation significantly affects the start-up performance, the retention time, biogas yield, and the conversion rate of total solids (TS) and volatile solids (VS) (Wilms *et al.* 2007; Du *et al.* 2011; Li *et al.* 2011b; Du *et al.* 2016). Wet fermentation has the advantages of good start-up performance and uniformity of mass, but the amount of material treated per unit volume of the reactor is less than for dry fermentation, and floating crust is easy to produce when straw is used as the raw material. In contrast, the start-up performance is poor for dry fermentation, but it still has a lot of advantages, such as a higher biogas production rate per unit volume, and the amount of biogas slurry generated is lower. In addition, dry fermentation compared to wet fermentation has other advantages, including lower energy consumption, lower water consumption, and lower investment cost (Cheng *et al.* 2012). However, dry fermentation also has the disadvantages of easy acidification in the early stage of fermentation, and it is difficult to start.

In addition, the fermentation process can be divided into single-stage, two-stage, and multistage fermentation based on the fermentation reactor series. The biogas fermentation process involves multistage joint action with many kinds of microbes because of the partition function. The multistage fermentation process has obvious advantages, including fermentation speed, degradation rate, shorter fermentation period, *etc.* Therefore, recently, more attention has been given to experts and scholars in China and abroad to the application of biogas engineering. Cheng *et al.* (2012) carried out research on straw biogas production, using a combination process of Continuous Stirred Tank Reactor (CSTR) and Up-flow Anaerobic Sludge Bed (UASB). The methane production was 200.9 mL/g, and the energy recovery rate was 67.1%. With this research in mind, two-stage dry-wet and wet-dry fermentation processes were gradually created (Li *et al*. 2011c; Xi and Wang 2014; Chen *et al*. 2015; Guo *et al*. 2015; Qu *et al*. 2015).

The period of anaerobic fermentation is usually 40 to 50 days for fibrous raw material under medium temperature conditions when the TS concentration of the raw material is 20%, and the period of high solid anaerobic fermentation is usually longer, 2 to 3 months, or even upwards of 4 months or more. The anaerobic biological conversion rate of wet fermentation is higher compared to high solid fermentation, but the load of raw material for the reactor per unit volume is low, which results in a low utilization rate of the reactor. Although the volume load for high solid anaerobic fermentation unit reactor is high, the initial fermentation reactor is easily acidified. Other drawbacks are that the initial fermentation reactor generally does not have the raw material mixing device because of low water content, a low anaerobic biological conversion rate, inconsistent fermentation, and longer fermentation times (Chen *et al*. 2015). Accordingly, the TS fermentation concentration (dry or wet) and reactor series (single-stage or two-stage) were studied, a comparative study on the development of the fermentation process was conducted, and the optimal process for fermentation type with straw was determined. This was all done to provide a theoretical basis for the selection of large scale biogas production with straw.

EXPERIMENTAL

Chemicals and Materials

All chemicals were of reagent grade and were purchased from either Sinochem (Shanghai, P. R. China) or Fluka Chemical (Buchs, Switzerland). CO_2, H_2, and N_2 were obtained from Nanjing Special Gases Factory (Nanjing, P. R. China).

Feedstock and Inoculums

The rice straw was freshly collected from an experiment field in Nanjing, Jiangsu Province, China. It was cut into approximately 5-mm-sized particles using a grinder (Hummer 900, Taisete, Tianjing, China). After being air-dried, the straw particles were stored at 4 °C ± 0.5 °C until use. The TS content, VS content, and carbon/nitrogen ratio (C/N) of the rice straw in this study was 87.62% ± 0.07%, 87.99% ± 0.13% (related to dry mass), and 73.20 ± 2.35, respectively.

The inoculum of anaerobically digested sewage sludge was taken from a wastewater treatment plant in Yangzi Petrochemical Co., Ltd, Nanjing, China. The amount of glucose that was fed into the sludge was 1.5 g/L per d at 35 °C ± 1 °C for 1 month, and then the feeding of glucose was stopped. When no biogas production was observed for 1 week, the seed culture was then thoroughly mixed and filtered through a screen with a pore size of 833 μm (20 mesh). This was carried out to ensure the removal of easily degradable organic matter still present in the inoculum and to remove the dissolved methane. The pH value, TS content, and VS content of the mesophilic inoculums used were 7.62 ± 0.07, 4.88% ± 0.01%, and 65.06% ± 0.09% (related to dry mass), respectively.

Experimental Design

The anaerobic fermentation experiments in this study were divided into single-stage fermentation (dry and wet) and two-stage fermentation (dry-wet and wet-dry). A total of 12 treatment groups were set up, where CK1 was the single-stage wet fermentation (TS = 6%), and CK2 was the single-stage dry fermentation (TS = 16%). In the first stage of the two-stage with dry-wet or wet-dry fermentation, the addition of total solids with straw and the inoculums was kept uniform (45 g and 250 g respectively), but the difference is that distilled water of 250 mL needed to be added in the first stage of the two stage with wet-dry fermentation, which was mixed in wide mouth glass bottles with volumes of 500 mL and 1000 mL (the work volumes were 375 mL and 750 mL, respectively). The bottle of anaerobic fermentation was aerated for 5 min with a mixed gas of CO_2 (20%) and N_2 (80%), and then sealed with a rubber plug and placed in a water bath culture (37 °C ±1 °C). The first stage of the wet-dry fermentation was set for 5, 10, 15, 20, and 25 d. The material was transferred into a wide mouth glass bottle with a volume of 1000 mL after the fermentation period, and a porous separator was arranged to achieve solid-liquid separation of the fermentation material in the middle of the bottle. The first stage of the dry-wet fermentation was also set for 5, 10, 15, 20, and 25 d. Fermentation inoculums were continually added into the material after the fermentation period, which was material for the second stage of the dry-wet fermentation. The bottle for the second stage of the dry-wet fermentation was placed in a constant temperature culture box, where the temperature was maintained at 37 °C. In this experiment, the reactors containing only inoculums were used as control to measure the background gas production, the experimental group of the single-stage wet fermentation and dry fermentation were also carried out as control groups. The gas production and methane content were determined every day. Three replicates were provided for each experimental group.

Analytical Methods

The daily biogas production was directly determined by the volume of displaced saturated $NaHCO_3$ solution in the graduated cylinder after the mixture was stirred manually. The methane concentration in the biogas was analyzed using a gas chromatograph (GC 9890A, Renhua, Nanjing, China) equipped with a TDC-01 column (ϕ 4 mm × 1 m, Shimadzu, Kyoto, Japan), using hydrogen as the carrier gas. The injector, oven, and detector temperatures were 100, 150, and 120 °C, respectively. The flow rate of the carrier gas was 50 mL/min, and the injection volume of the samples was 0.5 mL. The detection of TS and VS was performed in accordance with the standard methods of APHA (American Public Health Association 1998). The total carbon (TC) and total nitrogen (TN) were analyzed by a CHN (carbon, hydrogen, nitrogen) analyzer vario EL (Perkin Elmer, Foster, USA). The pH value was directly measured from the liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Greifensee, Switzerland). The cellulose, hemicellulose, and lignin contents were determined by sequential fiber analysis using Goehring and Van Soest's method with a FIWE Cellulose Analyzer (Velp Scientifica Company, Rome, Italy) (Van Soest 1963).

Statistical Analysis

All of the analytical results were conducted in triplicate, at a minimum. The values of the different parameters were expressed as the mean and standard deviation. The standard deviations and fitting curve were analyzed using Microsoft Excel 2003 (Redmond, USA) for Windows and Origin 9.0 (Hampton, USA) for Windows, respectively.

RESULTS AND DISCUSSION

Characteristics of Biogas Production for Single-Stage and Two-Stage Fermentation Processes

Daily biogas production and cumulative biogas production of single-stage wet fermentation and two-stage wet-dry fermentation

The daily biogas production and cumulative biogas production of single-stage wet fermentation and two-stage wet-dry fermentation can be seen in Figs. 1 and 2, respectively. Figure 1 shows the curve for the daily biogas production from the single-stage wet fermentation and two-stage wet-dry fermentation with rice straw after the control group was deducted. The total period of the single-stage and two-stage fermentation was 40 days. The biogas production was significantly higher early on in the process than it was for later on in the fermentation process, regardless of whether it was the single-stage or two-stage fermentation, as shown in Fig. 1. The peak of the daily biogas production occurred on the fourth day for the single-stage wet fermentation and the first stage of the two-stage fermentation, and the peak values were 17.50, 15.96, 17.98, 15.26, and 16.05 mL/gVS for the 5-, 10-, 15-, 20-, and 25-d wet fermentation times, respectively. Because of the impact of conversion from the first stage to the second stage of the wet-dry fermentation, the results showed that there was a trend of increased biogas production first, which was followed by slowly decreased production for the rest of the fermentation time for the two-stage wet-dry fermentation experimental groups. This occurred for all of the experimental groups, except for the 5- and 10-d experimental groups, where there was a rapid decline in the biogas production. For the single-stage wet fermentation, on the 18^{th} day there was a second peak in the gas production, which was followed by slowly decreased production. These results were consistent with the research results from Zhao *et al.* (2012) and Lian *et al.* (2014).

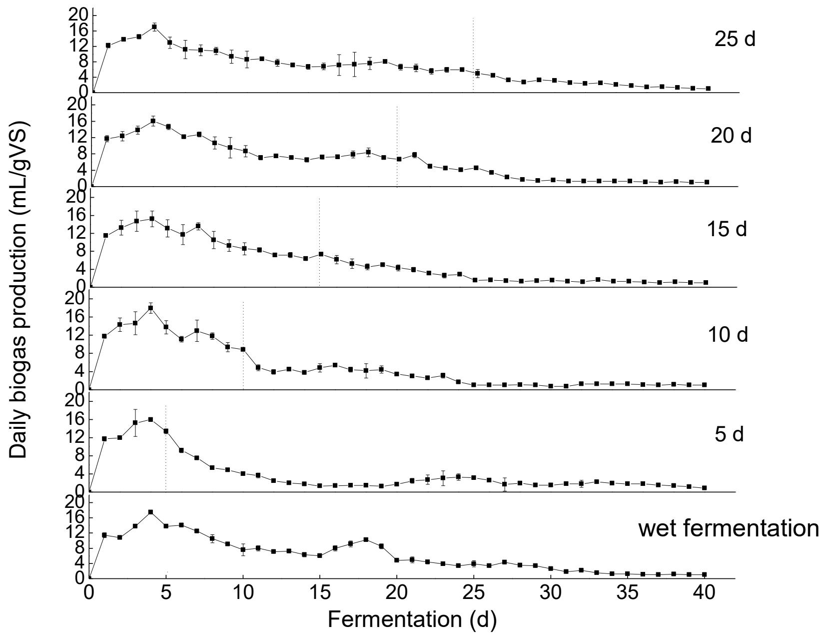

Fig. 1. The daily biogas production of the single-stage wet fermentation and two-stage wet-dry fermentation. The dotted lines indicate where the first stage ended for each two-stage fermentation experimental group.

The cumulative biogas production for the two-stage wet-dry fermentation was the sum of the biogas production from both the wet and dry stages, and the results are shown in Fig. 2. As can be seen in Fig. 2, the different stage conversion times for the two-stage wet-dry fermentation had a large influence on the cumulative biogas production. The cumulative biogas production increased at first, which coincided with the longer periods of wet fermentation, and then there was a slight downward trend. Additionally, the 5- and 10-d experimental groups had relatively low biogas production. The rest of the experimental groups for the two-stage wet-dry fermentation and the single-stage wet fermentation all had similar biogas production values, and the 20-d experimental group had the highest production. The cumulative biogas production values from the 15-, 20-, and 25-d two-stage wet-dry fermentation experimental groups and single-stage wet fermentation were 289.3, 313.8, 298.0, and 295.7 mL/gVS, which increased by 35.04%, 46.48%, 39.10%, and 38.03% compared to the 5-d experimental group, respectively. These results were consistent with the research results from Lian *et al.* (2014).

The biogas production from the different stages of the two-stage wet-dry fermentation was analyzed. The cumulative biogas production for the first stage of the wet-dry fermentation gradually increased as the length of wet fermentation increased, but the growth rate gradually decreased. The biogas production that occurred in the first stage of fermentation for the 5- to 25-d experimental groups was 23.12% to 78.49% of the biogas production that occurred in the single-stage wet fermentation. When the conversion from wet fermentation to dry fermentation occurred after 20 d, the biogas production from the first stage was more than 70% compared to the biogas production from single-stage wet fermentation, which indicated that the conversion occurred too early for wet fermentation to have the greatest influence on the biogas production. The reason may have been that without the slurry recirculation from the dry stage of the fermentation in this experiment, the acid accumulated in this process, and the effect was that there was less biogas production. However, if the conversion period was appropriately extended, that may have the advantage of mass transfer during wet fermentation and cause the consumption of the organic matter, which are easily degraded. In that case, the acidification phenomenon and the problem of low volume production are avoided. There are also the advantages of high processing capacity of raw materials, saving reaction space, and exchange for a long fermentation time. Although the biogas production of the single-stage wet fermentation and two-stage wet-dry fermentation was similar, the rule was followed that the early stage of fermentation is fast and the late stage is slow. This made the fermentation system more stable compared to the single-stage wet fermentation.

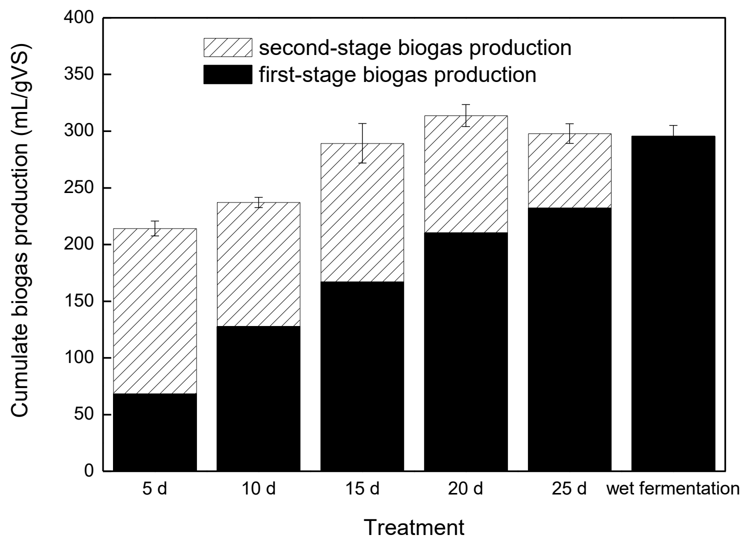

Fig. 2. Cumulative biogas production of the single-stage wet fermentation and two-stage wet-dry fermentation

Daily biogas production and cumulative biogas production of single-stage dry fermentation and two-stage dry-wet fermentation

Figure 3 shows the curve for the daily biogas production of the single-stage dry fermentation and two-stage dry-wet fermentation with rice straw after the control group was removed. The length of the single-stage and two-stage fermentation was 40 d. The daily biogas production of the single-stage dry fermentation was different from the first stage of the two-stage dry-wet fermentation. The reason was that perhaps the mass transfer effect was poor in the dry fermentation, and there was poor uniformity of mass for the easy decomposition of organic matter in the dry fermentation process. For the two-stage dry-wet fermentation, the results showed there was a trend of significant increase in production after dry fermentation converted into wet fermentation for all of the experimental groups, except for the 25-d experimental group where only a slight increase was seen. There was a peak in the biogas production between 2- to 4-d, which indicated that the small molecular organics produced from hydrolysis acidification during dry fermentation were more conducive to the rapid conversion into biogas after the wet fermentation stage began. In addition, the period of high gas production was reduced gradually with later stage conversion times, and the gas production was also reduced. This may have been due to more inhibitory substances being produced due to the poor mass transfer in the dry fermentation stage. The adverse effects on the gas production gradually increased for the wet fermentation stage with later stage conversion times.

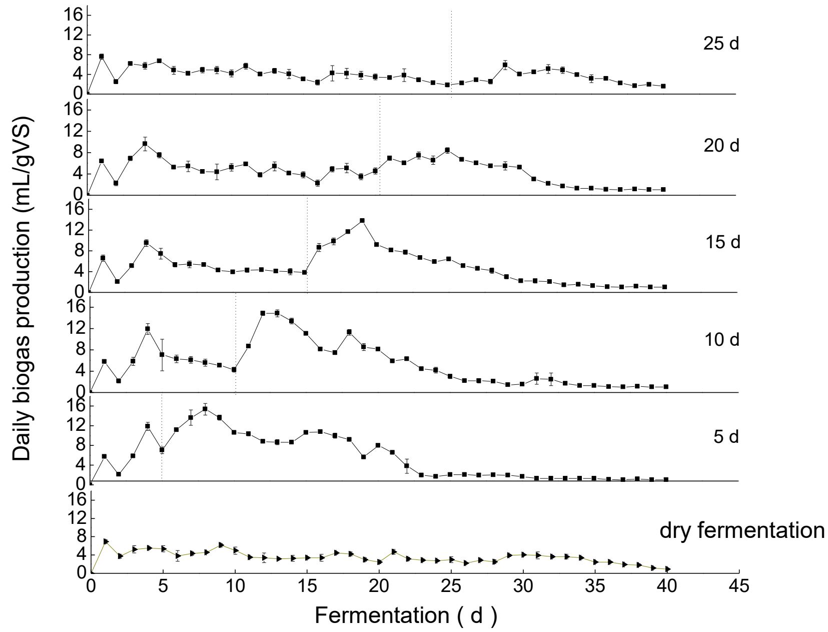

Fig. 3. The daily biogas production of the single-stage dry fermentation and two-stage dry-wet fermentation. The dotted lines indicate where the first stage ended for each two-stage dry-wet fermentation experimental group.

The cumulative biogas production of the two-stage dry-wet fermentation was the sum of the biogas production from both the dry and wet stages, and the results are shown in Fig. 4. As can be seen in Fig. 4, the different stage conversion times for the two-stage dry-wet fermentation had a large influence on the cumulative biogas production.

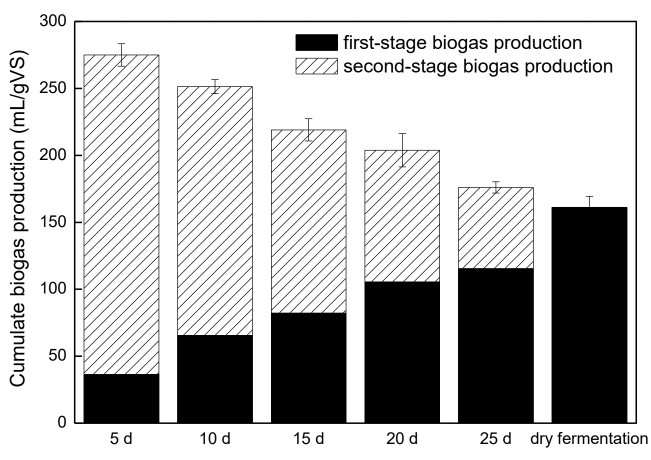

Fig. 4. Cumulative biogas production of the single-stage dry fermentation and two-stage dry-wet fermentation

The cumulative biogas production gradually decreased with later stage conversion times, but the decline rate increased first and then decreased. The cumulative biogas production values of the 5-, 10-, 15-, 20-, and 25-d experimental groups for the two-stage dry-wet fermentation were 274.9, 251.4, 219.0, 203.8, and 176.1 mL/gVS, respectively, which when compared with the single-stage dry fermentation experimental group (161.3 mL/gVS), were higher by 9.16% to

70.45%. This indicated that the cumulative biogas production was poor when the stage conversion time was later.

The biogas production from the different stages in the two-stage dry-wet fermentation was analyzed. The cumulative biogas production for the first stage of dry-wet fermentation gradually increased with later stage conversion times, but the growth range was different. The dry stage fermentation gas production from the 5- to 25-d experimental groups was 22.51% to 71.51% of the biogas production that occurred in the single-stage dry fermentation. The ratio of the gas production of the first stage and second stage for the two-stage dry-wet fermentation was 1:6.6, 1:2.8, 1:1.7, 1:0.9, and 1:0.5 for the 5-, 10-, 15-, 20-, and 25-d experimental groups, respectively. This showed that the contribution of biogas production from the wet stage of the dry-wet fermentation was higher, and the later stage conversion times weakened the biogas production in the wet stage of the dry-wet fermentation.

Comparative Analysis of the Characteristics of Single-stage Fermentation and Two-Stage Fermentation

It can be seen from Figs. 2 and 4 that the cumulative biogas production of the single-stage wet fermentation was significantly higher than that of the single-stage dry fermentation. The biogas production from the single-stage wet fermentation was 83.35% higher compared with the single-stage dry fermentation. This showed that the wet fermentation was more conducive in improving the production of biogas. The stage conversion time that appropriately extended the two-stage fermentation of wet-dry, or appropriately shorten the two-stage fermentation of dry-wet, can improve the gas production. The best experimental group was the 20-d experimental group from the wet-dry fermentation, which had a 14.12% higher biogas production compared to the best experimental group (5 d) from the two-stage dry-wet fermentation. In addition, the biogas slurry can be filtered during the stage conversion in the wet-dry fermentation process and can be used to modulate the new material, which is an operation that can be done by the drainage engineer. The biogas slurry can be added again during the stage conversion in the dry-wet process, which belongs to the replenishment operation, can achieve reuse of mixed liquid slurry after fermentation solid-liquid separation. Obviously, the transformation process for the conversion of wet stage to dry stage was reduced and easy to operate, and solved the problem with dry fermentation, so the two-stage wet-dry fermentation has the advantage of cost efficiency.

Optimum Fermentation Process Analysis by Mathematical Model Fitting

The model of modified Gompertz (GM) (1,1) (Li *et al.* 2011a), modified logistic (LM), and the model of RC (Redzwan and Banks 2004; Donoso-Bravo *et al.* 2010) were adopted for the influence of the different stage conversion times on the two-stage dry-wet and wet-dry fermentation processes and to be more scientific. The cumulative gas production data from each experimental group was simulated with nonlinear regression. In order to find the most suitable fitting model for the cumulative methane production, this study compared three kinds of model fitting. The data fitting and curve fitting is shown in Table 1, Table 2, Fig. 5, and Fig. 6. The equation of the modified GM (1,1) model is as follows,

$$B = P\left\{-\exp\left[\frac{R_{\mathrm{m}}e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where P is the maximum cumulative methane production per gram of VS straw (mL/gVS), R_m is the maximum methane production rate (mL/dgVS), R^2 is the coefficient of the goodness of fit, λ is the retention period (d), and t is the first stage fermentation time (d).

The equation for the modified LM model is as follows,

$$B = P \Big/ \left\{ 1 + \exp\left[\frac{4R_m}{P} (\lambda - t) + 2 \right] \right\} \tag{2}$$

The RC model for the study of the kinetics of the cumulative methane production is derived from the following model,

$$B = P \left\{ 1 - \exp\left[-\frac{R_m}{P} (t - \lambda) \right] \right\} \tag{3}$$

Table 1 shows the model parameters for the methane production from the two-stage wet-dry fermentation with the change in the first stage fermentation time. It was found that it could be modeled by the LM, GM, and RC models, but the GM model was not suitable for the 15-d and 20-d experimental groups from the two-stage wet-dry fermentation. The RC model was determined to be more suitable for the analysis of the single-stage wet fermentation and two-stage wet-dry fermentation by the comparison of the R^2 values obtained for the three models.

As can be seen by the R^2 values of the LM model, the trend of cumulative methane production from the two-stage wet-dry fermentation can be characterized, but the correlation was low compared to the RC model.

The relevant parameters of index in the RC model were analyzed. The R^2 values for each experimental group were above 0.99. The P-values were prone to increase at first and then decrease slightly for higher t values. This was consistent with the actual trend. In addition, the cumulative methane production of the 20-d and 25-d experimental groups for the two-stage wet-dry fermentation was similar to the methane production of the single-stage wet fermentation. The absolute value of λ was between 0 and 1. This indicated that the start-up time of the experiment was fast because in 1 d gas production could start.

The R_m value decreased gradually, except for the 5-d experimental group. The 10-d experimental group had the highest methane production rate, at 8.81 mL/dgVS. As a result, the maximum methane production rate of the fermentation system and the higher cumulative methane production could be ensured by having a stage conversion time of more than 15-d, which corresponded with the data because the best experimental group was 20-d.

The methane production of the two-stage dry-wet fermentation could be modeled with the LM, GM, and RC models, as shown in Table 2. The RC model was not suitable for the 5-d and 10-d experimental groups for the two-stage dry-wet fermentation. It was found that the GM model was the most suitable model, and the LM model was the next most suitable model for the single-stage dry fermentation and two-stage dry-wet fermentation through the comparison of the R^2 values of the three models. As can be seen from the R^2 values, the LM model could characterize the trend of the cumulative methane production for the two-stage dry-wet fermentation, but the correlation was low compared to the GM model.

The GM model fitting analysis of the relevant parameters showed that the P-values were stable for 5 to 20 d. The absolute value of the λ was approximately 0 to 3 days, which showed that the start-up time was slightly slower compared to the two-stage wet-dry fermentation.

Table 1. Kinetic Parameters of Model Fitting for Methane Production from Two-Stage Wet-Dry Fermentation and Wet Fermentation

t (d)	Methane production (Exp.) (mL/g VS)	LM				GM				RC			
		P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2	P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2	P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2
5	122.1	121.7	-2.82	4.05	0.9705	127.2	-2.67	4.24	0.9820	144.6	-0.72	6.67	0.9947
10	135.2	130.5	-2.45	5.01	0.9632	134.5	-2.04	5.38	0.9787	147.1	-0.24	8.81	0.9947
15	164.9	161.3	-1.10	5.70	0.9765	/				201.5	-0.05	8.54	0.9988
20	178.8	178.3	-0.47	6.05	0.9821	/				240.5	-0.05	8.36	0.9993
25	169.8	169.5	-1.25	5.58	0.9774	179.9	-1.54	5.68	0.9880	221.2	-0.29	8.10	0.9982
Wet fermentation	168.5	169.1	-0.29	5.92	0.9832	179.5	-0.75	5.95	0.9919	223.3	0.08	8.25	0.9986

Table 2. Kinetic Parameters of Model Fitting for Methane Production from Two-Stage Dry-Wet Fermentation and Dry Fermentation

t (d)	Methane production (Exp.) (mL/g VS)	LM				GM				RC			
		P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2	P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2	P (mL/g VS)	λ (d)	R_m (mL/dg VS)	R^2
5	151.2	145.4	2.40	6.22	0.9844	153.4	1.77	6.15	0.9949	/			
10	138.3	135.3	3.88	5.89	0.9932	144.6	2.89	5.63	0.9979	/			
15	120.5	124.4	4.64	4.73	0.9952	138.9	3.13	4.36	0.9949	297.0	1.29	4.38	0.9861
20	112.1	124.3	3.17	3.62	0.9896	147.6	1.58	3.37	0.9930	603.1	-0.02	3.28	0.9949
25	96.8	104.9	2.04	2.95	0.9876	122.6	0.67	2.79	0.9940	554.0	-1.49	2.44	0.9970
Dry fermentation	88.7	88.99	-1.27	2.76	0.9699	95.1	-1.57	2.80	0.9821	405.3	-5.16	2.13	0.9676

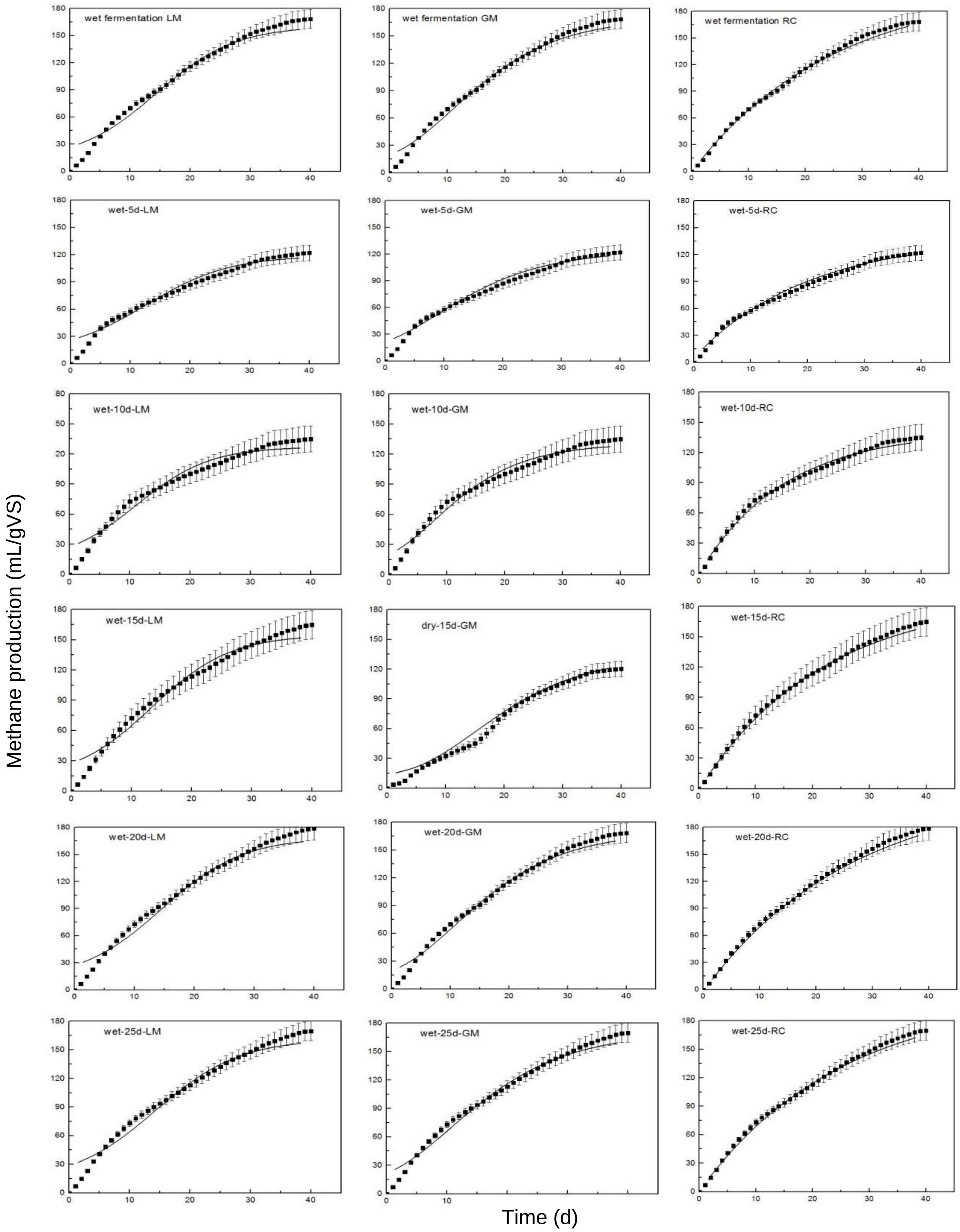

Fig. 5. LM, GM, and RC models for the cumulative methane production from the two-stage wet-dry fermentation and wet fermentation. The curve of black squares is the model for the experimental values.

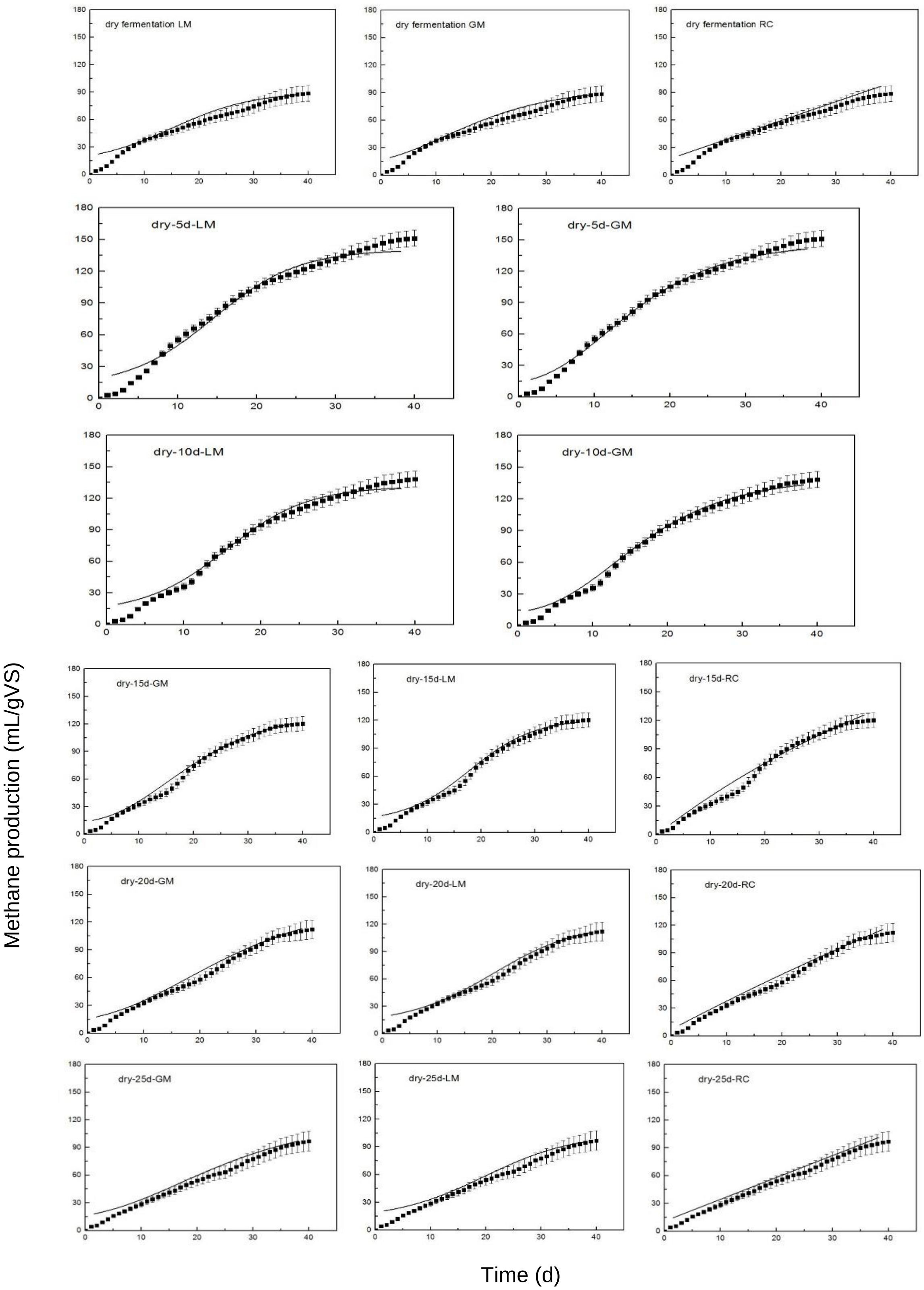

Fig. 6. LM, GM, and RC models for the cumulative methane production from the two-stage dry-wet fermentation and dry fermentation. The curve of black squares is the model for the experimental values.

The R_m gradually decreased as the t-value increased, and the 5-d experimental group had the highest value, at 6.15 mL/dgVS. The fitting parameters of the LM model were similar to the GM model, and the λ was between 2- to 5-d. The absolute values were significantly higher than the GM model. Comprehensive analysis of the fitting data of the integrated LM model and GM model showed that the largest methane production rate and the highest cumulative methane production can be ensured with a 5-d stage conversion time for the two-stage dry-wet fermentation.

A comparison of the fitting data in Tables 1 and 2 shows that the R_m values and the P-values of the two-stage wet-dry fermentation were significantly higher than those of the two-stage dry-wet fermentation, and the λ value was less than 1 d. This showed that the wet fermentation can shorten the start-up time, and increase the methane production rate and cumulative methane production.

Comparison of Degradation Rates in Single-stage and Two-Stage Fermentation Process

VS degradation rate

The anaerobic fermentation process is the combination of multiple anaerobic microbes to decompose organic matter, so the analysis of the degradation rate of VS before and after the single-stage and two-stage fermentation processes can explain the best fermentation process and determine the most efficient two-stage fermentation conversion cycle. The degradation rate of VS of the single-stage of fermentation system and the two-stage fermentation with straw is shown in Fig. 7. The degradation rate of VS in the first stage for the two-stage systems gradually increased for both wet and dry fermentation, but the degradation rate of VS for the first stage of the wet-dry fermentation system was significantly higher than the first stage of the dry-wet fermentation. This showed that wet fermentation was more conducive to the degradation of VS than dry fermentation. In addition, the degradation rate of VS for the wet fermentation gradually increased and stabilized before and after the fermentation process, whereas the degradation rate for the dry fermentation gradually decreased, which was consistent with the single-stage wet and dry fermentation results ($P < 0.01$).

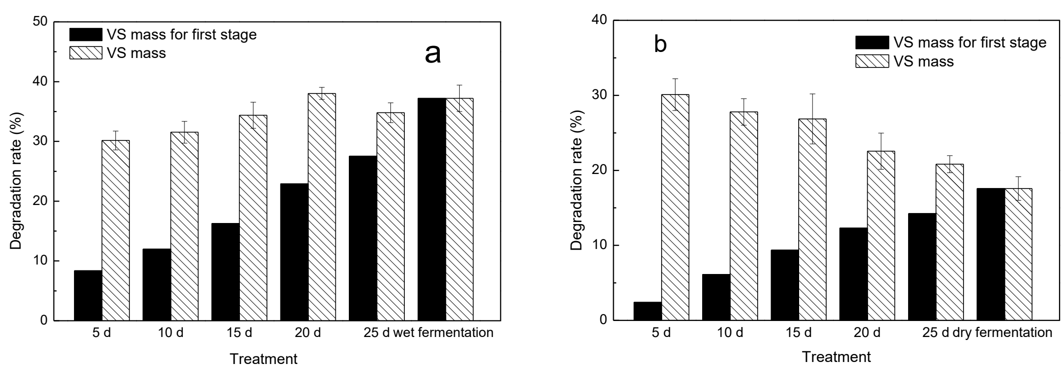

Fig. 7. Degradation rate of the VS in the single-stage and two-stage fermentation systems (a. the two-stage wet-dry fermentation and the single stage of wet fermentation; b. the two-stage dry-wet fermentation and the single stage of dry fermentation)

By difference analysis between the VS degradation rate of the two-stage fermentation process and the single-stage fermentation, the variation scope of the degradation rate of VS was determined to gradually decrease with longer dry fermentation times. The 5-d experimental group of the two-stage dry-wet fermentation had the highest degradation rate. This showed that it was beneficial to the improvement of the degradation rate of VS when the stage conversion time from dry to wet fermentation was earlier, which meant the wet fermentation was longer. From another point of view, it can be concluded that the wet fermentation was more conducive to the degradation of VS than the dry fermentation.

Degradation rate of straw fiber

For this kind of plant material, the biodegradability depends on the extent to which lignin is covered by cellulose and hemicellulose. Cellulose and hemicellulose can be biodegraded, but the component of lignin is difficult to degrade, especially when lignin is wrapped by cellulose and hemicellulose on the surface under anaerobic conditions (Komilis and Ham 2003; Yang *et al.* 2009). It is difficult for the enzyme to come into contact with the cellulose and hemicellulose, which then results in slow degradation.

In biomass with high fiber content, hydrolysis is the rate limiting step for the majority of anaerobic biological treatments (Kübler *et al.* 2000; Mata-Alvarez *et al.* 2000; Pavlostathis *et al.* 2007). Therefore, the study of the relationship between dry or wet fermentation period and the change of degradation rate for straw lignocellulose in order to determine the most efficient conversion period for two-stage fermentation has a good theoretical basis and value in engineering applications.

To obtain the degradation trend of the cellulose and hemicellulose in the straw from the process of anaerobic fermentation, the content of cellulose and hemicellulose in the fermented materials was determined by measuring each period (5-, 10-, 15-, 20-, and 25-d) of the first stage for the two-stage fermentation processes, and to determine the optimal first stage length for two-stage fermentation from the angle of the degradation of straw lignin. The change in the degradation rate of cellulose and hemicellulose for the single-stage fermentation and two-stage fermentation is shown in Fig. 8. The hemicelluloses were more susceptible to degradation compared to the cellulose in the straw, as can be seen from Fig. 8a and 8b. There were great differences in the degradation rates of the cellulose and hemi-cellulose between the single-stage wet fermentation or two-stage wet-dry fermentation and dry fermentation. The degradation rate of cellulose and hemicellulose for all of the groups in the wet-dry fermentation were 22.89% to 38.19% and 35.97% to 47.59%, respectively. For the dry fermentation, the degradation rates of cellulose and hemi-cellulose were 14.49% to 29.53% and 17.88% to 40.60%, respectively. This showed that the degradation rate of lignocellulose in straw for single-stage wet fermentation and two-stage wet-dry fermentation was better than for the dry fermentation process.

The difference between each treatment can be seen in Fig. 8a and 8b. The degradation rate of cellulose and hemicellulose first increased and then stabilized after the wet-dry fermentation process as the wet stage length increased, which corresponded significantly ($P < 0.01$, $R^2 = 0.952$ and 0.972) with the gas production of the two-stage wet-dry fermentation, as shown in Fig. 2. In contrast, the degradation rate of cellulose and hemi-cellulose in the dry fermentation process gradually decreased, which corresponded significantly ($P < 0.01$, $R^2 = 0.965$ and 0.987) with the gas production of the two-stage dry-wet fermentation, as shown in Fig. 4. This showed that the degradation of the components of the wood fiber contributed greatly to the degradation of organic matter and the production of methane from straw.

In addition, the degradation rate of hemicellulose and cellulose for the 20-d experimental group was the highest in the two-stage wet-dry fermentation process. This result suggested that increasing or decreasing of the wet fermentation period from 20-d for the two-stage wet-dry fermentation would not improve the degradation rate of lignocellulose with straw. In comparison, the 5-d experimental group for the two-stage dry-wet fermentation process had the highest degradation rates, which indicated that increasing the dry fermentation period and decreasing the wet fermentation time would have an obvious inhibitory effect on the degradation rate of lignocellulose in straw.

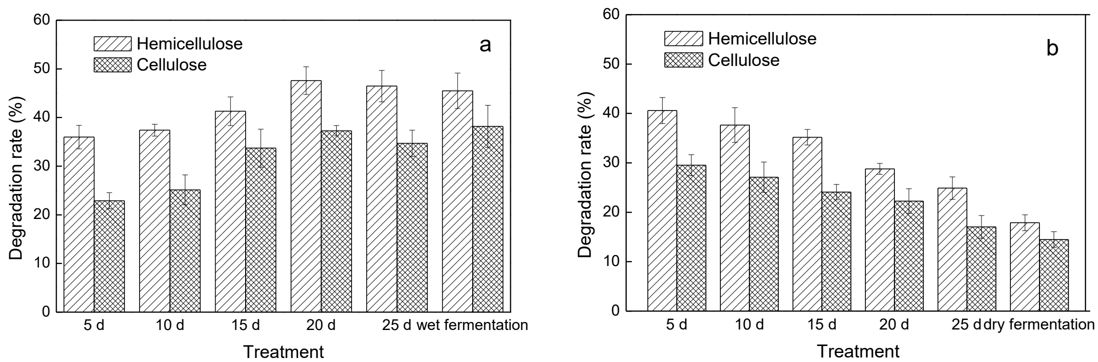

Fig. 8. Degradation rate of the cellulose and hemicellulose in the single-stage and two-stage fermentation system: (a) wet-dry fermentation; (b) dry-wet fermentation

Discussion

The determination of the most suitable type of fermentation process with straw by comparing the effects on the biogas production from the single-stage wet and dry fermentation and two-stage wet-dry and dry-wet fermentation has important practical value. When compared to the single-stage wet fermentation (295.7 mL/gVS), the cumulative biogas production of the 15-d (289.3 mL/gVS), 20-d (313.8 mL/gVS), and 25-d (298 mL/gVS) experimental groups was not affected by the conversion from the wet stage to the dry stage, which was consistent with the research results from Lian *et al.* (2014). The 20-d experimental group compared to the single-stage wet fermentation exhibited a 5.77% increase in the biogas production. The cumulative gas yield of the two-stage dry-wet fermentation improved to a different degree when compared to the single-stage dry fermentation (161.3 mL/gVS), and the growth rate decreased gradually as the stage conversion time increased. The 5-d experimental group had the highest biogas production yield, and the cumulative gas production was higher by 70.50% when compared to the single-stage dry fermentation. As a result, the two-stage wet-dry fermentation (15-d, 20-d, and 25-d experimental groups) and dry-wet fermentation (5-d experimental group) obtained better fermentation efficiency when compared to their respective control groups. The two-stage wet-dry fermentation was more successful in improving the cumulative gas production, and among the 15-d, 20-d, and 25-d experimental groups, the gas production was higher by 79.33%, 94.51%, and 84.72% when compared to the two-stage dry-wet fermentation (5-d experimental group), respectively. It was found that the two-stage wet-dry fermentation process was more suitable when straw was used as the raw material, and that 20 d was the best conversion time for the two-stage wet-dry fermentation process.

In addition, the biogas production rate of straw has characteristics of having relatively high values in the early stage and lower values later. It was found, through the analysis to the advantages of the engineering applications for the two-stage wet-dry fermentation process, that the problem of acidification that occurs in dry fermentation can be avoided directly when using the two-step approach. Also, the problems of feeding difficulty for semi-continuous fermentation and poor mass transfer of the fermentation material in the dry fermentation process are solved by using two-stage wet-dry fermentation. The average volume of gas production and straw treatment were significantly improved with a slight decrease in the wet fermentation period. The volume of the anaerobic reactor can be greatly reduced in engineering applications, which reduces the construction costs greatly. The application prospects are very good for the two-stage wet-dry fermentation.

CONCLUSIONS

1. The results of batch fermentation experiments showed that the effect on the biogas production from single-stage wet fermentation and two-stage wet-dry fermentation was superior to the effects from the single-stage dry fermentation and two-stage dry-wet fermentation.
2. The cumulative gas production increased gradually when the wet stage length for the two-stage wet-dry fermentation increased, and was similar to the gas production from the single-stage wet fermentation. The 20-d experimental group had the highest gas production. In comparison, the gas production decreased gradually when the dry stage length increased for the two-stage dry-wet fermentation, and the 5-d experimental group had the highest gas production among the dry-wet fermentation experimental groups.
3. It was determined that the two-stage wet-dry fermentation was the most beneficial to the improvement of the gas production by analysis characteristics of the biogas production from the two-stage wet-dry and dry-wet fermentation. For the two-stage wet-dry fermentation, the 15-d, 20-d, and 25-d experimental groups were 79.33%, 94.51%, and 84.72% higher when compared to the best two-stage dry-wet fermentation (5-d experimental group), respectively. It was found that the two-stage wet-dry fermentation process was the most suitable when straw was used as the raw material.
4. The changes in the gas production from the anaerobic fermentation process correlated significantly with the degradation rates of VS, cellulose, and hemicellulose ($P < 0.01$), for both single-stage dry and wet fermentation and two-stage fermentation. The RC and GM models were applied for the analysis of the kinetics for the two-stage wet-dry and dry-wet fermentation processes, respectively, after comparison of the accumulated methane yield fitting with the LM, RM, and RC models.

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