Mild Acid Hydrolysis of Rice Straw for Highly Efficient Methane Generation with Hydrolysates

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Glucose and xylose are the dominant hydrolysis products of lignocellulose, and they can be used as a potential substrate for biogas generation. To reveal the acid hydrolysis metabolism and enhance the methane production, mild acid hydrolysis of rice straw was conducted to obtain hydrolysates with different glucose to xylose ratios. The methane production and microbial metabolism process using mixed sugars and hydrolysates were investigated. The results showed that the cellulose and hemicellulose in the rice straw were converted to the desired sugars by controlling the acid hydrolysis conditions. The glucose to xylose ratios tested were 70:30, 60:40, 50:50, 40:60, and 30:70. The anaerobic digestion process had the highest methane yield with the 40:60 glucose to xylose ratio in the mixed sugars and hydrolysate, which were 334.1 mL/g and 318.2 mL/g based on the chemical oxygen demand (COD), respectively. The metabolic process with the 40:60 glucose to xylose ratio had good butyrate type fermentation and a high COD removal rate. The corresponding acid hydrolysis conditions were 1% HCl and a 7.5% solid loading at 60 °C for 48 h. Therefore, the mild acid hydrolysis conditions can adjust the glucose to xylose ratio in the hydrolysate for high methane production.

Keywords: Anaerobic digestion; Methane production; Acid hydrolysis; Hydrolysate; Glucose to xylose ratio

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INTRODUCTION

Biogas is a promising alternative to fossil fuel, as it is renewable and has a high energy conversion (Börjesson and Mattiasson 2008; Li *et al.* 2015). Lignocellulosic materials, such as crop straw and animal manure, are low-cost, renewable, and abundant sources for biogas production. However, because of its natural complex structure, one-step fermentation of lignocellulosic feedstock for biogas production is typically inefficient (Ren *et al.* 2008). Hence, a two-step fermentation process can be used, in which the biomass hydrolysis and fermentation of lignocellulosic hydrolysate are separately performed in two reactors. In the first step the lignocellulosic materials are hydrolyzed into reducing sugars (Cheng *et al.* 2008; Li *et al.* 2011), which primarily include xylose being converted from hemicellulose and glucose being converted from cellulose (Kim *et al.* 2012). These reducing sugars are easily degraded and utilized in the second step of anaerobic digestion (AD).

Anaerobic digestion that uses hydrolysate as the substrate has many advantages, such as a short hydraulic retention time, convenient feeding or discharging, and efficient degradation rate (Kongjan *et al.* 2010). There are many studies that have used hydrolysate

as the substrate in AD. These studies mainly have focused on using hydrolysis methods to enhance lignocellulose hydrolysis (Castro *et al.* 2011; Guerra-Rodríguez *et al.* 2012). Datar *et al.* (2007) used steam explosion with sulfuric acid (1.2%) to hydrolyze corn stover for sugars production and had a sugar yield of 21.8%. Cai *et al.* (2012) used a high temperature (123 °C) with dilute sulfuric acid (1% H_2SO_4) to hydrolyze corncob for 1.5 h, and 87% of the total xylan was recovered. Sun *et al.* (2007) used a formic acid solution to hydrolyze cotton fiber at 65 °C, which resulted in a 32% sugar reduction.

Although the reducing sugars production is important for AD, methane production is also influenced by various parameters, including the inoculum, temperature, pH, solid concentration, *etc.* (Yadvika *et al.* 2004; Yu *et al.* 2014). These fermentative factors affect the metabolic processes for the production of microbial and regulated products. In fact, some reports have found that the production of methane and volatile fatty acids (VFAs) is also affected by the carbon source (Czerkawski and Breckenridge 1969). Lignocellulosic hydrolysate contains different carbon sources, mainly xylose (pentose) and glucose (hexose). Prakasham *et al*. (2009) found that xylose is a preferred carbon source compared with glucose when used individually in AD. Kádár *et al.* (2004) investigated the hydrogen production of glucose and xylose, and found that the acetate yield from mixed carbon source fermentation was considerably lower than that from pure carbon source fermentation. This was consistent with the previous results obtained by the authors that found that AD can be acidified if solely using glucose or xylose as the carbon source. Lowe *et al.* (1987) studied the effect of glucose and xylose on the growth and fermentation of an anaerobic rumen fungus, and they found that glucose and xylose have organism doubling times of 5.56 h and 6.67 h, respecitvely. When using mixed sugars, the anaerobic fungus had a doubling time of 8.70 h. Therefore, the utilization pathways for glucose and xylose by anaerobic organisms had noticeable differences. A mixed sugars substrate, such as hydrolysate, may be a good choice for AD.

Based on previous research, the glucose to xylose ratio in the substrate has a remarkable effect on the degradation rate and mixed sugars production during AD (Ai 2013). Thus, to enhance the methane production, the glucose to xylose ratio in the hydrolysate needs to be optimized. Moreover, there are few reports on the effect of the glucose to xylose ratio on the anaerobic fermentation type. More importantly, the optimum glucose to xylose ratio for high methane production can be regulated by the hydrolysis parameters. However, to the knowledge of the authors, investigation regarding the effect of the hydrolysis parameters on the glucose to xylose ratio is limited. Among the hydrolysis methods, acid hydrolysis has a positive effect on the hemicellulose sugars recovery (Mosier *et al.* 2005), which is conducive to produce a mixed hydrolysate with a high xylose content.

Therefore, to enhance the methane production with hydrolysate from mild acid hydrolysis, this study first conducted acid hydrolysis at various conditions to obtain hydrolysates containing different glucose to xylose ratios. Then, the effects of the acid hydrolysis conditions on the glucose to xylose ratios and lignocellulose degradation were investigated. Moreover, the methane production and VFAs concentration from the batch AD experiments using mixed sugars and hydrolysate as the carbon sources were compared to reveal the metabolic processes of glucose and xylose. Finally, the chemical oxygen demand (COD) removal rate was calculated and compared with the mixed sugars and hydrolysate substrates.

EXPERIMENTAL

Materials

Rice straw was collected in the suburbs of Wuhan, China. After collection, the straw was air dried, ground using a hammer mill (9FQ-750, Billion Ming, Zhengzhou, China), and passed through a 10-mesh standard screen. The ground straw was then sealed in plastic bags and stored at room temperature until further use and analysis. The inoculum sludge was taken from a mesophilic anaerobic digester, which used swine manure as the substrate and had been operated for two years. The following feedstock characteristics were based on weight percentages. The total solids (TS) and volatile solids (VS) contents of the rice straw were 91.8% and 84.8%, respectively. The rice straw was composed of 34.4% cellulose, 28.8% hemicellulose, 4.11% lignin, and 1.21% ash. The TS and VS contents of the sludge were 8.8% and 95.6%, respectively.

Methods

Acid hydrolysis regulation design

Acid hydrolysis has been described in a previous study (Wang *et al.* 2015). Acid hydrolysis was performed using a single factor experiment to study the effects of the HCl concentration (0.5%, 1%, 2%, 3%, and 4% w/w), temperature (15 °C, 30 °C, 45 °C, and 60 °C), and solid loading (2.5%, 5.0%, 7.5%, and 10.0% w/w) on the glucose and xylose concentrations in the hydrolysate. While studying the effects of the HCl concentration and retention time (hydrolysis time), the temperature and solid loading were fixed at 60 °C and 7.5%, respectively. While studying the effects of the temperature, the HCl concentration and solid loading were fixed at 1.0% and 7.5%, respectively. While studying the effects of the solid loading, the HCl concentration and temperature were fixed at 1.0% and 60 °C, respectively. For each run, 37.5 g of dry matter were hydrolysed for 48 h. The treated mixture solutions were filtered with filter paper, the contents were diluted using 1 L of deionised water, and the treated rice straw was used for the dry matter and fibre composition analysis. A small amount of hydrolysate was used to analyse the sugar composition and COD. The remaining hydrolysate was stored at 4 °C for AD.

Anaerobic digestion and quantitative biogas measurement

By calculating the approximate glucose to xylose ratios in the hydrolysate, the appropriate hydrolysates with different glucose to xylose ratios from the acid hydrolysis were used as the carbon source for the batch AD experiment. Prior to feeding the reactors, the hydrolysates were neutralised to a pH value of 7.0 using 5 M sodium hydroxide and diluted to a fixed COD concentration of 8 g/L. To validate and compare the effect of the glucose to xylose ratios on the methane production, the mixed sugars solution was prepared using glucose and xylose (Sigma-Aldrich, Beijing, China), according to the glucose to xylose ratios and COD concentration in the hydrolysate. A 1.5-L distillation flask was used as the batch AD reactor. The working volume included 0.80 L of substrate and 0.35 L of sludge. To avoid the acidification of the bioreactor systems, 2.0 g/L $NaHCO_3$ was added in each run. All reactors were flushed with nitrogen to keep an anaerobic environment, and placed in a 37 °C water bath. The biogas production was measured by a drainage method, and the biogas components were determined at 24 h intervals. The hydrolysates were taken at 12-h intervals and put in a 4 °C refrigerator until further analysis.

Analytical methods

The TS, VS, and COD were determined according to standard methods (Clescerl *et al*. 1998). The glucose and xylose contents in the hydrolysate were analyzed using an Agilent 1220 liquid chromatography (Agilent Technologies, Palo Alto, USA), which was equipped with a Zorbax carbohydrate analytical column (4.6 mm × 150 mm; Agilent Technologies, CA, USA) and a refractive index detector (1200, Agilent Technologies, CA, USA). The fiber composition was determined using a laboratory analytical procedure (LAP) from the National Renewable Energy Laboratory (Sluiter *et al*. 2008). The concentrations of VFAs were determined with gas chromatography (GC; GC9790II, Fuli Analytical Instrument Co. Ltd., Taizhou, China) with a KB-Wax column (Kromat Corporation, New Jersey, USA) using a flame ionization detector (FID). Nitrogen was used as the carrier gas. The methane content in the biogas was determined by GC with a thermal conductivity detector (TCD; 9790II, Fuli Analytical Instrument Co. Ltd., Taizhou, China), 1.5-m stainless steel packed column with a 5A molecular sieve (Lanzhou Atech Technologies Co, Lanzhou, China), and Hayesep Q packed column (Lanzhou Atech Technologies Co. Ltd., Lanzhou, China). Argon was used as the carrier gas at a flow rate of 30 mL/min.

A modified Gompertz model was used to fit the cumulative methane production data from the batch experiments,

$$H(t) = P\exp(-\exp(\frac{R_m e}{P}(\lambda - t) + 1)) \quad (1)$$

where $H(t)$ is the cumulative methane volume (mL) at time t (h), P is the methane potential during the stationary phase (mL), R_m is the maximum methane production rate obtained during the exponential phase (mL/h), and λ is the lag time (h). These parameters were estimated by nonlinear curve fitting. The specific methane potential (P_s, mL/g of COD_{added}) and specific maximum methane production rate (R_s, mL/(g of COD_{added}·h)) were obtained by dividing P and R_m by the amount of COD added. The batch fermentation was compared using P_s, R_s, and λ.

RESULTS AND DISCUSSION

Mild Acid Hydrolysis and Properties of the Hydrolysates

During mild acid hydrolysis, the glucose and xylose concentrations in the hydrolysate were determined. The approximate glucose to xylose ratios were calculated and presented in Table 1. With an increase in the acid concentration, the glucose concentration first increased and then decreased. It reached a maximum of 8.17 g/L at a 2.0% acid concentration, 60 °C, and a 7.5% solid loading. The xylose concentration increased gradually as the acid concentration increased. This was because acid hydrolysis mainly degrades hemicellulose to produce xylose and arabinose (Jiang *et al*. 2015). Because the arabinose concentration was less than 0.59 g/L, the arabinose was not considered. The glucose to xylose ratios (40:60, 50:50, and 30:70) were determined according to the experimental data. To achieve a high reducing sugars concentration and reduced acid consumption, the hydrolysates at 1.0%, 2.0%, and 3.0% acid concentrations were chosen for subsequent AD.

Table 1. Acid Hydrolysis Conditions and Glucose to Xylose Ratios in the Hydrolysates

Acid Hydrolysis Conditions			Glucose Concentration (g/L)	Xylose Concentration (g/L)	Approximate Glucose to Xylose Ratio
Acid Concentration (%)	Temperature (°C)	Solid Loading (%)			
0.5	60	7.5	3.55 ± 0.08	3.32 ± 0.11	50:50
1.0			3.95 ± 0.30	6.29 ± 0.20	40:60
2.0			8.17 ± 0.15	7.81 ± 0.10	50:50
3.0			5.47 ± 0.10	11.99 ± 0.08	30:70
4.0			5.27 ± 0.12	12.18 ± 0.15	30:70
1.0	15	7.5	2.28 ± 0.08	0.56 ± 0.06	80:20
	30		2.95 ± 0.10	0.81 ± 0.04	80:20
	45		4.54 ± 0.10	1.13 ± 0.08	80:20
	60		3.95 ± 0.30	6.29 ± 0.20	40:60
1.0	60	2.5	3.61 ± 0.03	1.62 ± 0.04	70:30
		5.0	5.30 ± 0.12	3.37 ± 0.09	60:40
		7.5	3.95 ± 0.30	6.29 ± 0.20	40:60
		10.0	5.17 ± 0.10	5.68 ± 0.22	50:50

When observing the effect of the temperature, the effect of the glucose and xylose concentrations was similar to that of the acid concentration. It was observed that the glucose concentration was higher than the xylose concentration below 45 °C, which resulted in a high glucose to xylose ratio (80:20). The glucose and xylose concentrations were relatively low because the low temperature can lead to less degradation of the glucan and xylan. This was not beneficial to the biogas production from the hydrolysate during AD. At 60 °C, the xylose concentration was high, and there was a suitable glucose to xylose ratio of 40:60. Additionally, the effect of the solid loading on the glucose concentration was irregular. This was because a high solid loading was not conducive to the hydrolysis of the cellulose. With an increasing solid loading, the xylose concentration reached a maximum of 6.29 g/L. This indicated that the hemicellulose can achieve a better degradation at a 7.5% solid loading. For the 2.5% solid loading, there was a high glucose to xylose ratio (70:30). Moreover, by changing the solid loading, the 60:40, 40:60, and 50:50 glucose to xylose ratios were obtained. Therefore, the mild acid hydrolysis process can obtain hydrolysates with different glucose to xylose ratios by varying the acid concentration, temperature, and solid loading.

Changes in the Lignocellulose Content during Mild Acid Hydrolysis

After acid hydrolysis, the lignocellulose contents are given in Table 2 to investigate the formation mechanism of the hydrolysates with different glucose to xylose ratios. The data presented are the averages of two separate experiments. The acid hydrolysis control conditions resulted in different degradation intensities of the cellulose and hemicellulose. Compared with the raw rice straw, the cellulose content increased with an increase in the acid concentration, while the hemicellulose content noticeably decreased. As the temperature ranged from 15 °C to 45 °C, the cellulose and hemicellulose contents did not show remarkable changes. However, when the temperature reached 60 °C, the hemicellulose content was only 5.86%. This confirmed the previous results obtained with a high xylose concentration. Additionally, the 7.5% solid loading led to more cellulose and hemicellulose hydrolysis. Therefore, acid hydrolysis mainly degraded the hemicellulose,

which resulted in a high xylose content in the hydrolysate. Moreover, by altering the acid hydrolysis conditions, the cellulose can also be hydrolysed to obtain hydrolysates with different mono-sugar ratios.

Table 2. Changes in the Lignocellulose Content with Different Acid Hydrolysis Conditions

Acid Hydrolysis Conditions			Cellulose (%)	Hemicellulose (%)	Lignin (%)
Acid Concentration (%)	Temperature (°C)	Solid loading (%)			
0.5	60	7.5	43.99 ± 1.39	8.11 ± 0.44	8.56 ± 0.72
1.0			46.22 ± 0.91	5.86 ± 0.39	9.82 ± 0.59
2.0			47.76 ± 1.38	5.65 ± 0.38	10.13 ± 0.66
3.0			48.78 ± 1.23	4.48 ± 0.22	3.02 ± 0.35
4.0			47.47 ± 1.07	2.27 ± 0.10	5.01 ± 0.28
1.0	15	7.5	43.09 ± 0.85	15.50 ± 1.27	5.41 ± 0.22
	30		43.20 ± 1.17	11.15 ± 0.87	5.13 ± 0.15
	45		43.80 ± 1.08	11.53 ± 0.56	7.77 ± 0.29
	60		46.22 ± 0.91	5.86 ± 0.39	9.82 ± 0.59
1.0	60	2.5	49.00 ± 1.54	7.67 ± 0.48	9.18 ± 0.55
		5.0	46.34 ± 1.13	7.40 ± 0.36	9.71 ± 0.42
		7.5	46.22 ± 0.91	5.86 ± 0.39	9.82 ± 0.59
		10.0	46.82 ± 1.48	9.56 ± 0.58	8.59 ± 0.37

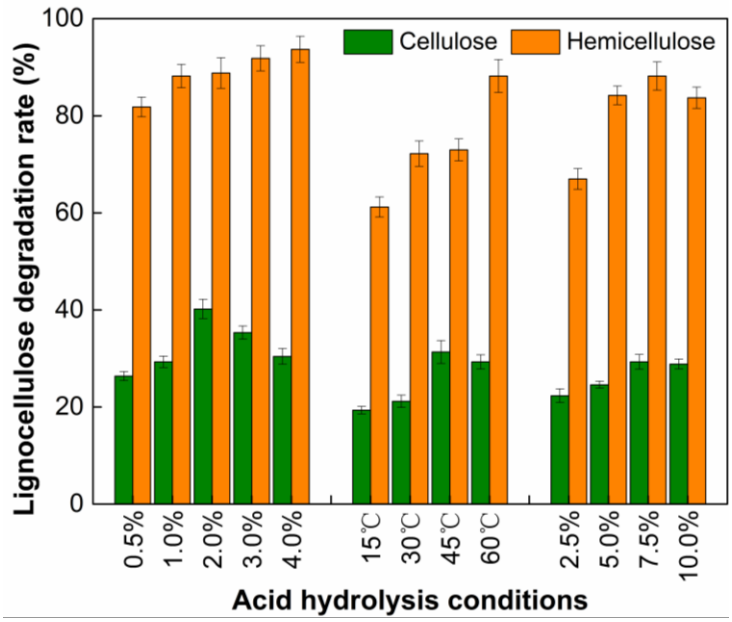

Fig. 1. Cellulose and hemicellulose degradation rates at the different acid hydrolysis conditions

The lignocellulose content can only reveal a relative variation. To distinguish the mild acid hydrolysis process for different glucose to xylose ratios, the degradation rates of the cellulose and hemicellulose are shown in Fig. 1. The degradation rate of cellulose or hemicellulose was calculated by dividing the mass of cellulose or hemicellulose with mass loss of cellulose or hemicellulose in acid hydrolysis. It was seen that the cellulose degradation rate increased at first. This resulted in a high glucose content in the hydrolysate

compared with that in the others. According to the results in Table 1, the 50:50 glucose to xylose ratio was obtained at a 2% acid concentration. When the acid concentration was above 2%, the hemicellulose degradation rate was strengthened, and the cellulose degradation rate was weakened. This resulted in a hydrolysate with a 30:70 glucose to xylose ratio. When the temperature was higher, the cellulose and hemicellulose degradation rates were higher. In particular, for hydrolysis at 60 °C, the hemicellulose degradation rate was nearly 90%. The solid loading had an important effect on the hemicellulose degradation rate and hydrolysis efficiency. With an increase in the solid loading, the hemicellulose degradation rate first increased and then decreased at solid loadings above 7.5%. The 2.5%, 5.0%, 7.5%, and 10.0% solid loadings had different glucose to xylose ratios, which revealed a complex acid hydrolysis process for degrading lignocellulose. In summary, the analysis of the lignocellulose degradation rate can explain the effect of the acid hydrolysis conditions on the formation of hydrolysates with different glucose to xylose ratios.

Effect of the Glucose to Xylose Ratios on the Methane Production from the Hydrolysates

The glucose to xylose ratios of 70:30, 60:40, 50:50, 40:60, and 30:70 in the hydrolysate were selected, and the corresponding acid hydrolysis parameters and COD concentrations are given in Table 3. The COD concentrations for all of the hydrolysates were above 8 g/L, which can be diluted to the same feeding COD concentration of 8 g/L for AD. These hydrolysates were used for AD to compare the methane production and microbial metabolism at different glucose to xylose ratios.

Table 3. Selected Glucose to Xylose Ratios and the Corresponding COD Concentrations after Acid Hydrolysis

Acid Hydrolysis Conditions			Glucose to Xylose Ratio	COD Concentration (g/L)
Acid Concentration (%)	Temperature (°C)	Solid Loading (%)		
1.0	60	2.5	70:30	8.9
1.0	60	7.5	60:40	13.4
2.0	60	7.5	50:50	15.4
1.0	60	5.0	40:60	9.1
3.0	60	7.5	30:70	16.3

The methane production yields from different carbon sources are shown in Fig. 2. It was seen that the methane yields varied with the glucose to xylose ratios. An increase in the methane yields was found with an increase in the xylose content in the substrates and reached a maximum at the glucose to xylose ratio of 40:60 for the mixed sugars and hydrolysate substrates. This may have been because the xylose uptake was better than the glucose uptake during AD (Prakasham *et al.* 2009), which resulted in suitable VFA components and a high methane yield. For the mixed sugars carbon source, a maximum yield of 334.1 mL/g of methane was observed with an AD substrate containing 40% glucose and 60% xylose. The AD of the hydrolysate also showed a similar trend. This indicated that at this glucose to xylose ratio (40:60), the growing microbial consortia effectively metabolized both carbon sources and produced VFAs and methane. The maximum methane yield from the hydrolysate was 318.2 mL/g at the 40:60 glucose to xylose ratio, which was slightly lower than that from the mixed sugars.

Some studies on AD for hydrogen production have shown that when glucose and xylose are available in the substrate, the xylose transport system is four times faster compared with that of the glucose (Temudo *et al.* 2009). Further analysis revealed that a glucose content of more than 40% in the substrate obviously decreased the methane production, which indicated that the glucose in the substrates had a regulatory role on the anaerobic microbial metabolism. This was probably because of the high xylose transport bioenergetics, which led to a decrease in the metabolic energy available for methane production. Thus, the utilization of carbon sources is limited. Another possible reason was that glucose and xylose AD systems are affected by the metabolites in the microbial population. To determine the effect of the glucose to xylose ratio on the metabolic processes, the changes in the sugars and VFAs were monitored in the AD process.

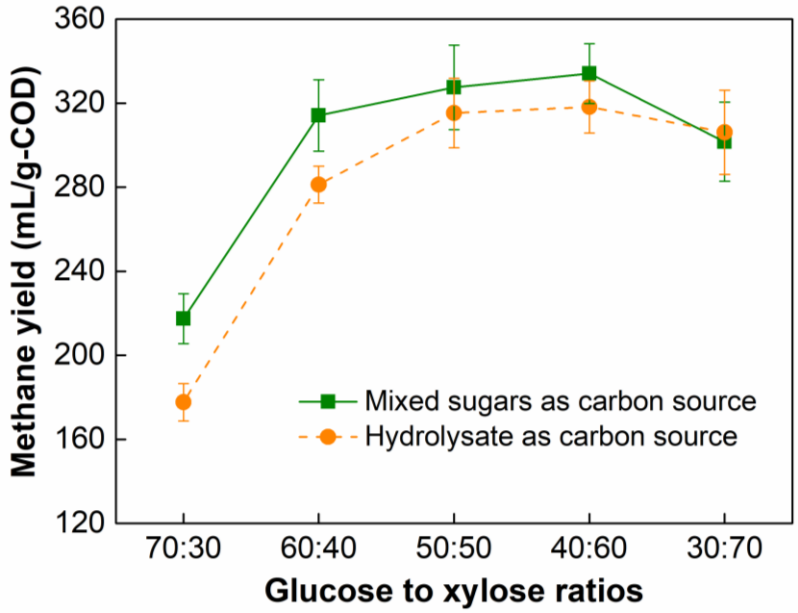

Fig. 2. Methane yield from the mixed sugars and hydrolysate substrates

Changes in the Glucose, Xylose, and VFAs Concentrations during AD

Because the hydrolysate composition was complex and to avoid the byproducts (such as acetic acid, *etc.*) in the hydrolysate affecting the anaerobic fermentation type, the glucose, xylose, and VFAs contents in the mixed sugars carbon source with different glucose to xylose ratios were determined and are presented in Fig. 3. The highest and lowest methane production yields (40:60 and 70:30) were chosen to compare the metabolic process in the AD. It was found that the decomposition of glucose and xylose was extremely fast. After 12 h of fermentation, 40% to 60% of the sugars in the substrate were broken down. After 24 h, the glucose and xylose were completely decomposed. Further analysis found that when xylose was a major component in the substrate (Fig. 3a), the xylose decomposition rate was faster than that of the glucose at 6 h to 12 h. Similarly, Fig. 3b shows that glucose was utilized quickly for the hydrolysate with a 70:30 ratio.

For the VFAs concentration, the 40:60 glucose to xylose ratio resulted in a butyrate type fermentation, which is characterized by the production of butyrate and acetate (Guo *et al.* 2008). The utilization of VFAs for methane production was relatively stable and efficient. This was also a good explanation for the high methane production. However, for the 70:30 glucose to xylose ratio, the fermentation type was not obvious and VFAs did not

have excellent methane conversion. Moreover, the propionate concentration was high compared with that of the 40:60 ratio. This may have indicated that with an increase in the glucose, the fermentation type was affected and gradually changed to propionate type fermentation. Propionate type fermentation can mainly produce propionate, acetate, and some valerate, without a remarkable gas production (Ren *et al.* 2007). Therefore, the optimum glucose and xylose ratio was 40:60, which had a positive effect on the VFAs and methane production.

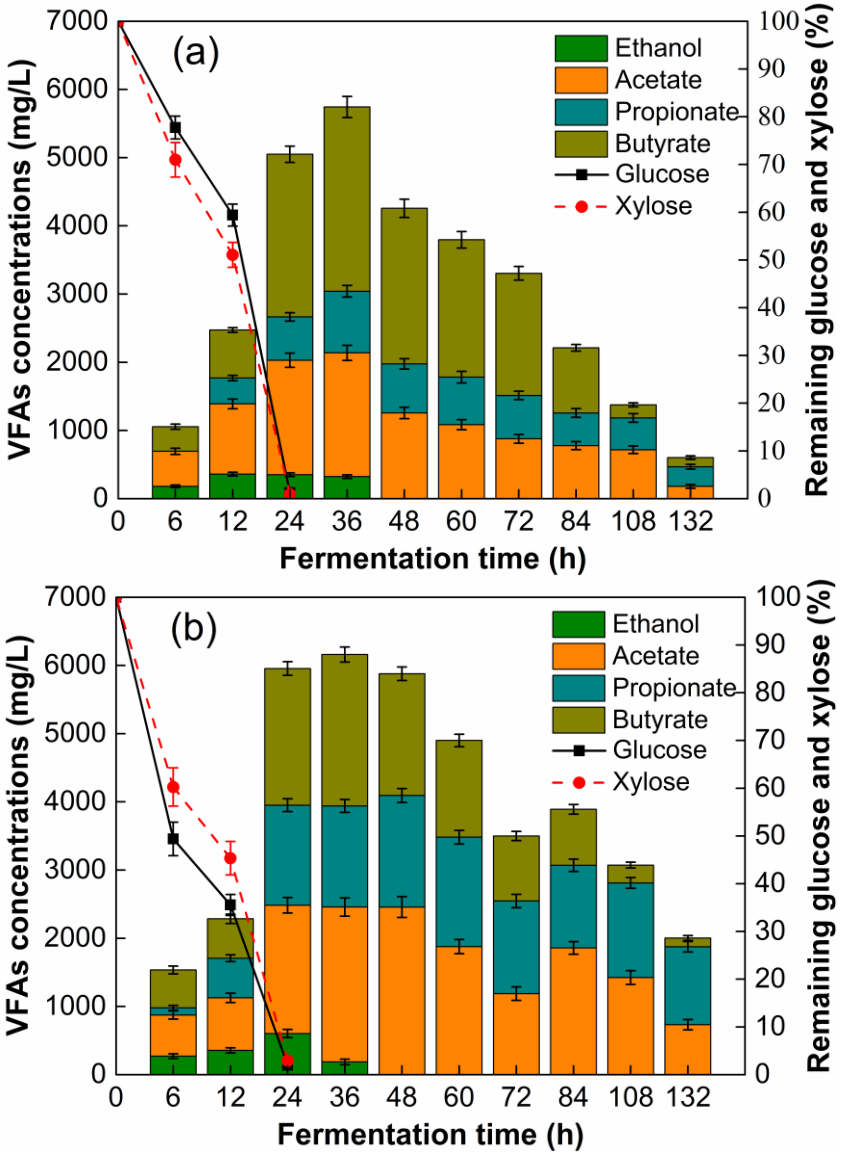

Fig. 3. Changes in the glucose, xylose, and VFAs concentrations during AD: (A) 40:60 ratio (highest methane production) and (B) 70:30 ratio (lowest methane production)

Gompertz Equation Kinetic Parameters and Comparative COD Removal Efficiency

The methane production yields from the mixed sugars and hydrolysate at different glucose to xylose ratios and the theoretical methane yield calculated using the Gompertz equation (Eq. 1) are shown in Fig. 4. The methane yields experimentally determined from

the mixed sugars at the 70:30 and 40:60 glucose to xylose ratios were 217.4 mL/g and 334.1 mL/g, respectively. The corresponding methane yields of the hydrolysates were 177.7 mL/g and 318.2 mL/g at the 70:30 and 40:60 glucose to xylose ratios, respectively. The specific methane yields (P_s) from the simulated results were slightly different from the experimental methane yields. Methane yield can be assessed based on the Gompertz model parameters and λ can be easily estimated. This study suggested that substrates with a 40:60 glucose to xylose ratio resulted in a high methane yield. For the mixed sugars and hydrolysate, the λ from the 40:60 glucose to xylose ratio was longer than that of the 70:30 ratio. This indicated that the hydrolysate with the 40:60 glucose to xylose ratio required a longer time to start fermentation.

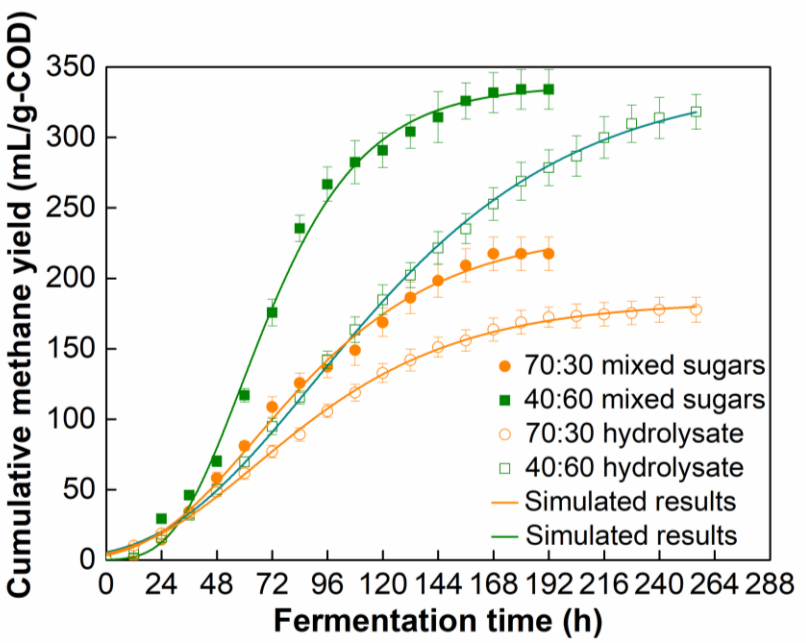

Fig. 4. Methane yield from the mixed sugars and hydrolysate at glucose to xylose ratios of 70:30 and 40:60 during batch fermentation

Table 4. Gompertz Equation Kinetic Parameters and COD Consumption during Batch Experiments with Different Substrates

Glucose to Xylose Ratio	P_s (mL/g)	R_s (mL/g·h)	λ (h)	CH_4 Concentration (%)	COD Consumption (%)	R^2
Mixed Sugars						
70:30	234.12	12.19	19.55	54.32	80.2	0.9976
40:60	336.98	27.22	29.50	57.93	88.9	0.9954
Hydrolysate						
70:30	183.75	8.68	15.13	57.31	71.8	0.9988
40:60	342.06	12.69	26.40	69.23	85.2	0.9990

Table 4 also shows the COD removal efficiency at different glucose to xylose ratios for the mixed sugars and hydrolysate substrates. The initial COD concentration was 8 g/L, and the COD concentrations in the AD effluents ranged from 0.88 g/L to 2.24 g/L for all of the reactors. The results showed that the COD removal efficiency increased with an increasing xylose proportion in the substrates. For the 40:60 glucose to xylose ratio, the

COD removal efficiency was the highest, at 88.9% (mixed sugars) and 85.2% (hydrolysate). This indicated that the COD removal was restricted to some extent by a high glucose content in the substrates (*e.g.*, 70:30). This was closely related to the metabolism of the glucose and xylose. These results were consistent with changes in the methane production and VFAs concentration. However, for the 30:70 glucose to xylose ratio, a slight decrease in the COD removal efficiency occurred. Similar trends were observed for the mixed sugars and hydrolysate. Moreover, the COD removal efficiency for the hydrolysate was lower than that for the mixed sugars carbon source. This was because the complex hydrolysate composition had a negative effect on the COD removal efficiency and methane production (Garde *et al.* 2002). Therefore, the hydrolysate at the 40:60 glucose to xylose ratio achieved the highest COD removal during AD.

CONCLUSIONS

1. The glucose to xylose ratio in the hydrolysate can affect the anaerobically metabolic process and methane production. By mild acid hydrolysis regulation, hydrolysates with 70:30, 60:40, 50:50, 40:60, and 30:70 glucose to xylose ratios were obtained.
2. The AD results indicated that the mixed sugars and hydrolysate with the 40:60 glucose to xylose ratio had a more efficient methane yield. The corresponding metabolic process was a good butyrate type fermentation, which had a high COD removal rate of 88.9%.
3. The acid hydrolysis conditions for the hydrolysate with a 40:60 glucose to xylose ratio were 1% HCl and a 7.5% solid loading at 60 °C for 48 h.

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