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Improvement of methane production from rice straw with rumen fluid pretreatment: A feasibility study

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ABSTRACT

To overcome the inherent recalcitrance of rice straw during anaerobic digestion process, effective pretreatment is required for promoting methane production. In the present study, a biological pretreatment using rumen fluid was proposed. The rice straw was pretreated with the rumen fluid at 39 °C for 120 h under anaerobic conditions. Various volatile fatty acids, especially acetic acid and propionic acid, were produced by the rumen fluid pretreatment. The methanogenic process was carried out over a 30-day anaerobic digestion. The results indicated that the optimal pretreatment time for anaerobic digestion was 24 h, resulting in a biogas production increase of 66.5%, a methane yield increase of 82.6% and a technical digestion time decrease of 40.0%, compared with the control. At the end of anaerobic digestion, degradation efficiency of total solid and volatile solid was respectively improved by 16.4–33.3% and 14.8–31.7% for rumen fluid pretreatment. The promoted methane production and organic matter degradation could be mainly attributed to the effective hydrolysis of rice straw by the mixed microorganisms in rumen fluid. Methane production could be well explained by modified Gompertz model rather than the first order model, and a higher methane production rate of 29.31 ml/(g_{VS}·d), a rapider hydrolysis rate of 0.09 1/d, and a shorter lag phase of 1.62 d were obtained after 24 h pretreatment. Therefore, the rumen fluid pretreatment is promising for effective production of methane from rice straw and reduction of rumen fluid discharge from slaughterhouse.

1. Introduction

Rice is one of the main esculent cereals in central and southern China, and rice straw is a major by-product of rice production. As one of the biggest agricultural countries, China produces the rice straw of 203 million tons every year (National Bureau of Statistics of China, 2009). However, large quantities of rice straw are often dumped or burned in open environment, which is not a recommended practice in term of environmental and ecological aspects of sustainable development (Chandra et al., 2012a, b). The rice straw is composed mainly of cellulose, hemicellulose and lignin, and can be transformed into renewable energy (biomethane or ethanol) by anaerobic fermentation (Gu et al., 2014), which may alleviate many problems for environment and energy to a certain extent (Song et al., 2013). However, the cellulose, hemicellulose and lignin in lignocellulosic biomass are strongly linked to each other and form complex three-dimensional structures, which resist the accessibility of microorganisms (Malherbe and Cloete, 2002; Monlau et al., 2013). Therefore, suitable pretreatment methods are needed to destroy the structural and compositional barrier of lignocellulosic biomass (Yu et al., 2014). Various pretreatment technologies have been investigated for lignocellulosic biomass to enhance methane yield, such as chemical (Song et al., 2013), mechanical (Chen et al., 2014), thermal (wet oxidation) (Ferreira et al., 2013), biological (Yan et al., 2012) or combinations of them (Bruni et al., 2010). High energy consumption associated with mechanical pretreatment and strong corrosiveness to reactors for chemical pretreatment limit their large-scale application. Compared with these pretreatments, biological pretreatment is environmentally friendly because of its lower energy requirement and milder reaction conditions. Various

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microbial agents, such as white-rot fungi (Zhao et al., 2014), mesophilic lignocellulolytic microbial consortium (BYND-5) (Yan et al., 2012) and thermophilic microbial consortium (MC1) (Yuan et al., 2014) have been applied for the pretreatment of lignocellulosic biomass. These microbial communities can effectively improve the biomass biodegradability and methane yield. Considering the constant supply of these microbial agents and their screening cost, the direct utility of microbial agents might not be economically feasible. Therefore, the microbial agents with high lignocellulose degradation efficiency, low cost and environmentally friendly properties are needed to be explored.

Rumen fluid, including complex microbial population of bacteria, protozoa, fungi and archaea, is formed in the fore-stomach (reticulorumen) of cows and exhibits higher ability and activity to degrade lignocellulosic biomass than other normal anaerobic microorganisms (Yue et al., 2013; Creevey et al., 2014). It has been reported that the cellulose solubilization by rumen microorganisms are significantly faster than that by microbial communities from landfills or anaerobic digesters (O'Sullivan et al., 2006; Song et al., 2005). Hu and Yu (2005) studied anaerobic fermentation of corn stovers with rumen microorganisms as inocula, and found that the volatile solids (VS) was rapidly degraded and a higher production of volatile fatty acids (VFAs) was observed, compared with the conventional acidogenic bacteria derived from sewage (Hu and Yu, 2005). The high lignocellulose degradation rate and effective hydrolytic conversion to VFAs by the rumen fluid make it possible to improve the methane production. Besides, blood and rumen contents are major slaughterhouse wastes, causing high investment and operating costs when they are discharged to sewage treatment plants (Makinde and Sonaiya, 2010; Roy et al., 2013). The use of these natural microbial consortia should be cost-effective, and using the rumen fluid for the lignocellulosic pretreatment is a promising option.

However, this biological agent has been seldom tested for lignocellulosic pretreatment to increase methane production. Baba et al. (2013) reported that the waste paper was pretreated by rumen fluid for 6 h and 24 h, and daily methane yield respectively increased by 2.6 and 2.1 times, compared with that of control. The waste paper has been treated both chemically and thermally to remove lignin during paper-making process, and should be easier to be degraded than other lignocellulosic biomass. However, few researches on rice straw pretreatment by rumen fluid for methane production have been reported so far.

The objective of this work is to investigate the feasibility of biological pretreatment of rice straw by rumen fluid to improve the methane production, and determine the optimal pretreatment time. Studying the kinetics of methane production from feedstocks is important when designing and evaluating anaerobic digesters. First-order kinetic (Zhen et al., 2014) and modified Gompertz models (Lu et al., 2014) are most applied to describe the methane production from lignocellulosic materials. The first-order kinetic model is commonly applied to simulate anaerobic digestion process when the hydrolysis is rate-limiting (Gavala et al., 2003). The Gompertz model is commonly used in the simulation of methane and hydrogen production, and is useful to explain lag time and sigmoidal growth curve (Syaichurrozi, 2013). Therefore, the first-order and Gompertz models were used to assist in the interpretation of conclusions.

2. Materials and methods

2.1. Materials

The rice straw was collected from rice fields around Changsha, Hunan. The rice straw was air-dried at room temperature and chopped to 2–3 cm using a paper knife before stored in a refrigerator at 4 °C. After oven-dried at 45 °C for 24 h, the rice straw was ground to a size of 30-mesh by a grinder (HC-700. Huangcheng, China).

The rumen fluid was taken from the fresh stomach of cattles from a local slaughterhouse in Changsha of China, brought to the laboratory in a sealed bottle. The rumen fluid sample was filtered through four layers of gauze with N_2 protection and stored at 39 °C, since this temperature is close to the body temperature of ruminant animals (ranged from 37.8 to 40 °C) (Feng, 2004). The samples were used in experiments within 5 h of being collected from the fresh stomach. The main characteristics of rice straw and rumen fluid are shown in Table 1. With a high C/N ratio of 64.1, the rice straw is not ideal as the sole feedstock for anaerobic digestion. The rumen fluid presented a high concentration of total nitrogen (TN) and NH_4^+-N, thus could serve as nitrogen source during methanogenic process without addition of extra nitrogen.

The seed sludge for methane production was collected from a continuous biogas plant (Changsha, China) with an organic loading rate at about 4.5 kg VS/(m^3·d), a hydraulic retention time of 25 d and a operating temperature of 32 ± 1 °C. The main raw materials of this biogas plant were swine manure and crop straws. After concentrated, the seed sludge was used as the inocula. The characteristics of seed sludge were: 56.2 g/L TS, 34.8 g/L VS. It was cultured in a thermostatic water bath (HH-8. JOYN, China) at 35 °C for a few days until no biogas production, then used as the seed sludge in subsequent anaerobic digestion.

2.2. Rice straw pretreatment with rumen fluid

The rice straw pretreatment was performed in 250 ml conical flasks. Firstly, the rice straw of 3 g, rumen fluid of 60 ml, and deionized water of 60 ml were thoroughly mixed in flasks without adding any nutrient media. The initial pH was maintained at 7.0 by NaOH and HCl. Then the flasks was purged with N_2 for 5 min to remove O_2 and sealed with a rubber stopper. All the flasks were incubated at 39 °C on a incubator shaker (ZHWY-2012C, Shanghai Zanyu Instrument Co. LTD., China) at 120 r/min for 120 h. The constant temperature of 39 °C and initial pH of 7.0 were used, as these conditions are close to the actual rumen environment. Inside of the actual rumen, the temperature maintained between 37.8 and 40 °C and the pH varied between approximately 6.5 and 7.2 (Feng, 2004). The biogas volume generated during the pretreatment was recorded at a certain time interval by water displacement, and the biogas composition was measured by gas chromatography (SP7820,

Table 1
Properties of rice straw and rumen fluid used in experiments.

Samples	Parameter	Value
Rice straw	Total solid (TS) (%)	90.0 ± 0.3
	Volatile solid (VS) (%)	77.8 ± 1.6
	Total organic carbon (TOC) (%TS)	43.6 ± 2.1
	Total kjeldahl nitrogen (TKN) (%TS)	0.7 ± 0.13
	C/N ratio	64.1 ± 3.7
	Cellulose (%TS)	37.2 ± 0.9
	Hemicellulose (%TS)	26.4 ± 1.7
	Lignin (%TS)	6.9 ± 1.0
Rumen fluid	pH	7.1 ± 0.0
	TS (g/L)	10.1 ± 0.8
	VS (g/L)	6.2 ± 1.3
	TN (mg/L)	412.9 ± 22.3
	NH^{4+}–N (mg/L)	288.1 ± 18.9
	Acetic acid (mg/L)	1131.4 ± 37.1
	Propionic acid (mg/L)	414.3 ± 11.3
	Butyric acid (mg/L)	425.6 ± 23.4
	Valeric acid (mg/L)	55.6 ± 13.6

Jinputech Co., Beijing, China). The pretreated rice straw and hydrolysate were used for composition determination, chemical analyses, and subsequent anaerobic digestion.

2.3. Rice straw anaerobic digestion

The 120 ml hydrolysate and residual rice straw pretreated by rumen fluid were digested in batch anaerobic digester without adjustment of C/N ratio. $NaHCO_3$ was used to adjust the initial pH to 7.2. The volume of anaerobic digester was 1000 ml, with a working volume of 500 ml. The anaerobic digester was purged with N_2 for 5 min to remove O_2 and immediately sealed using a rubber stopper with drilled holes for biogas collection. The anaerobic digestion was carried out at a mesophilic temperature of 35 °C for 30 d. The C/N of unpretreated rice straw was adjusted to 25 with urea ($CO(NH_2)_2$), which is optimal for anaerobic microorganism growth (Liu et al., 2015), to be used as the control. The seed sludge was mixed with substrate at a ratio of 1:1 (VS/VS) in all reactors. The blank (CK) anaerobic digester contained only rumen fluid and the seed sludge. The methane yield of anaerobic digestion was calculated as Eqs. (1)–(2):

$$V_{\text{Methane yield}}(\text{ml/g}_{\text{VS}}) = \frac{V_{\text{Methane}}(\text{Total}) - V_{\text{Methane}}(\text{CK})}{VS_{\text{Substrate}}} \quad (1)$$

$$V_{\text{Methane}}(\text{Total}) = V_{\text{Pretreatment}} + V_{\text{Methanogenic process}} \quad (2)$$

where $V_{\text{Methane yield}}$ is the final methane yield, which is normalized per substrate mass VS added (ml/gVS); V_{Methane} (Total) and V_{Methane} (CK) represents the final methane production (ml) from samples and the blank, respectively; $VS_{\text{Substrate}}$ refers to the mass of VS added. $V_{\text{Pretreatment}}$ and $V_{\text{Methanogenic process}}$ (ml) is the methane production during the pretreatment process and methanogenic process, respectively.

2.4. Analytical methods

The TS, VS, NH_4^+-N, TOC and TKN were analyzed according to APHA methods (APHA., 2005). The pH value was measured by a pH meter (PHSJ-4A, Shanghai Kangyi Instrument Co. Ltd., China). The content of lignin, hemicellulose and cellulose was determined by a Fibretherm Fibre Analyzer (Gerhardt, Bonn, Germany) according to the procedure proposed by Van Soest et al. (1991). The biogas composition was detected by a gas chromatograph (SP7820, Jinputech Co., Beijing, China) with a column carbon molecular sieve (TDX-01) and a thermal conductivity detector (TCD). The temperature of oven, injector port, and TCD was 140, 150 and 150 °C, respectively. Argon was used as the carrier gas at a 30 ml/min flow rate. The determination of VFAs was conducted according to the method described by http://www.sciencedirect.com/science/article/pii/S0957582012001619Luo et al. (2011). The filtrate was firstly acidified with 3% H_3PO_4 in a 1.5 ml gas chromatography (GC) vial. An Agilent 6890N GC (Santa Clara, CA, USA) with a capillary free fatty acid phase (polarity) column (DB-FFAP, 30 m × 0.25 mm × 0.25 mm) and a flame ionnization detector (FID) was employed to measure VFAs. The temperature of injection and detector was 250 and 300 °C, respectively. N_2 was the carrier gas with a flow rate of 2.6 ml/min. The GC oven was programmed to raise the temperature to 180 °C. The initial temperature of GC oven was 70 °C for 3 min, followed with a ramp of 20 °C/min for 5.5 min and with a final temperature of 180 °C for 3 min. The total VFA (TVFA) was recorded as the sum of measured acetic acid, pro-pionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and iso-valeric acid.

2.5. Degradation efficiency

The degradation efficiency of different chemical compositions of rice straw was calculated as Eq. (3) (Yue et al., 2007):

$$E_i = \frac{X_{i,ini} \times \text{TS}_{i,ini} - X_{i,fin} \times \text{TS}_{i,fin}}{X_{i,ini} \times \text{TS}_{i,ini}} \quad (3)$$

where E_i is the degradation efficiency of composition i of rice straw; $X_{i,ini}$ and $X_{i,fin}$ were the initial and final content of composition i, respectively (i represents cellulose, hemicellulose, lignin, TS, and VS).

2.6. Kinetic modeling

First-order model and modified Gompertz model were applied to fit the observed methane production (Li et al., 2013), which are shown as Eqs. (4) and (5), respectively:

$$B(\text{t}) = B_0[1 - \exp(-kt)] \quad (4)$$

$$B(\text{t}) = B_0 \exp\left\{-\exp\left[\frac{\mu_m e}{B_0}(\lambda - t) + 1\right]\right\} \quad (5)$$

where B is the cumulative methane production (ml/g_{VS}) at time t, B_0 is the final methane production (ml/g_{VS}), k represents the first-order rate constant (1/d), μ_m refers to the maximum methane production rate (ml/(g_{VS}·d)), λ is the lag phase time (d) and e is equal to 2.72, t stands for the anaerobic digestion time (d).

3. Results and discussion

3.1. Rice straw pretreatment with rumen fluid

3.1.1. Change of VFAs and pH during pretreatment

Acetic acid and propionic acid were the dominate VFAs in rumen fluid pretreatment products (as shown in Fig. 1a), which respectively increased from 565.7 to 4583.6 mg/L and from 207.2 to 2856.9 mg/L after 120 h pretreatment. N-butyric acid increased slightly from 103.24 to 398.14 mg/L. However, no significant change was observed for iso-butyric acid and valeric acid. Fig. 1b shows the opposite tendency of pH and TVFA concentration change, indicating that the decrease in pH was just caused by the VFA production. The pH can indirectly reflect the VFA production during rumen fluid pretreatment. As can be seen Fig. 1b, there were four phases of the rumen fluid pretreatment: first exponential phases (0–24 h), limiting-step phase (24–48 h), second exponential growth phase (48–72 h) and stationary phase (72–120 h). In the first exponential phases easily digestible organics were quickly consumed, and the organic conversion rate declined in the limiting-step phase; in the second exponential growth phase, part of refractory organics was converted, and the fermentation was almost stopped after all the biodegradable substrates were consumed in the stationary phase (Jin et al., 2014). The experimental VFA yield was 0.36 g/g_{VS}, which is quite consistent with that for *cattail* fermentation with rumen inocula (0.34–0.41 g/g_{VS}) (Hu et al., 2006), and significantly higher than that for rice straw fermentation with conventional anaerobic sludge inocula (0.13–0.29 g/g_{VS}) (Park et al., 2015). These results confirmed that the rice straw was effectively converted into VFAs by rumen microorganisms.

It is worth mentioning that the high VFA concentration is not benefit for subsequent methane production and the accumulation of propionic acid would result in failure of methanogenesis. The cellulose degradation could be inhibited at a VFA concentration

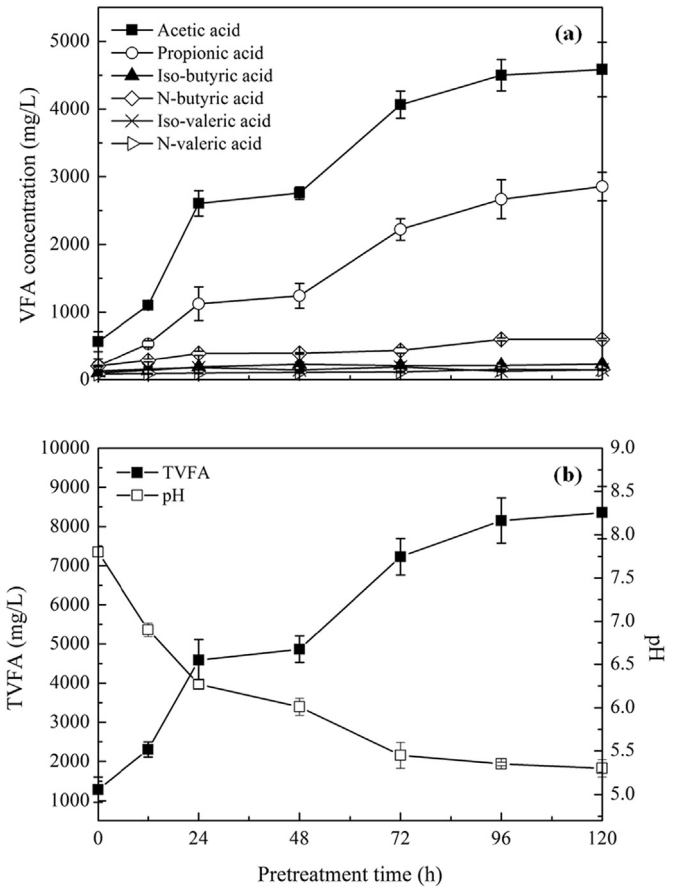

Fig. 1. Change of VFAs and pH during rumen fluid pretreatment, (a) individual VFA, and (b) TVFA and pH.

above 2000 mg/L (Siegert and Banks, 2005). The propionic acid could be difficultly and slowly used, because propionate assimilating microbes are one of the slowest growing microbes for its low free-energy gain and the complicated syntrophic relation to hydrogen-utilizing methanogens (Zhang et al., 2011). Wang et al. (2009) studied the effect of different VFA concentrations on methanogenic growth and methane yield, and found that the activity of methanogenic and acidogenic bacteria was not influenced when the initial propionic acid concentration was lower than 300 mg/L, while became significantly repressed when the initial propionic acid concentration reached 900 mg/L. These findings indicated that the influence of initial concentration of VFAs and propionic acid was important for further anaerobic digestion, and should be considered in the study of methane production by rumen fluid pretreatment.

3.1.2. Degradation of main rice straw components during pretreatment

The degradation of cellulose, hemicellulose and lignin during rumen fluid pretreatment is shown in Fig. 2. The degradation of cellulose and hemicellulose increased with the increase of pretreatment time. However, there was only a slight increase after 72 h pretreatment, indicating that the ruminal biodegradation mainly occurred in the first 72 h pretreatment. After 120-hour pretreatment, approximately 47.8% of cellulose and 58.9% of hemicellulose were degraded. These results were consistent with the change of VFAs over the 120 h pretreatment. On the other hand, 20.6% of lignin was degraded after rumen fluid pretreatment. The lignin is

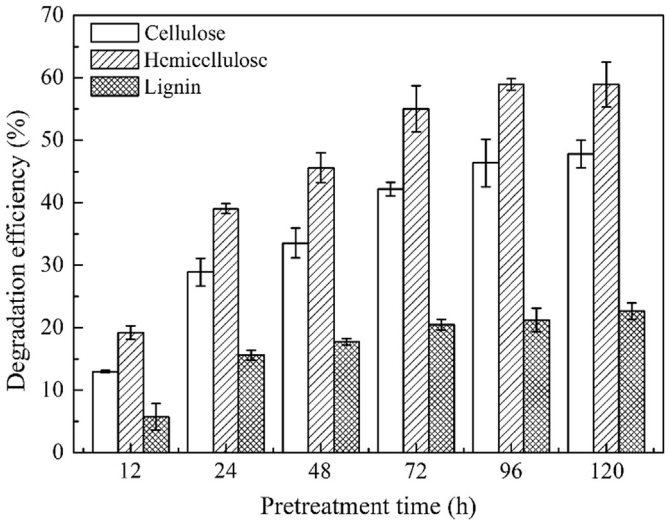

Fig. 2. Degradation of cellulose, hemicellulose and lignin during pretreatment.

more difficult to be attacked and degraded by microorganisms than the cellulose and hemicellulose due to its structural complexity and macromolecular properties. The lignin is usually biodegraded by an aerobic process using fungus (white and brown rot fungi) and a few special bacteria (*proteobacteria, Firmicutes and Actinomycetes*), most of which employ extracellular phenol oxidases and extracellular peroxidases (Bugg et al., 2011). But notable lignin degradation in this study was found in the rumen fluid pretreatment, suggesting that lignin could be degraded by rumen microorganisms. Hu et al. (2008) obtained the similar result that 25.5% of lignin in wheat straws was degraded by rumen microorganisms, while the lignin degradation reached approximately 30% when the corn stovers were fermented by rumen microorganisms (Hu and Yu, 2005). The rumen microbes degraded the lignin of lignocellulosic biomass, because the hydrolyzing enzymes could pass through the holes in fiber surface as a channeling (Yue et al., 2013). Cellulose, hemicellulose, and lignin are the main components of cellulosic biomass, and also the main carbon sources for anaerobic microorganisms. The availability and digestibility of cellulose and hemicellulose would significantly affect the subsequent anaerobic digestion.

3.1.3. Gas production during pretreatment

The cumulative gas production during rumen fluid pretreatment is shown in Fig. 3. The cumulative biogas production significantly increased with the increase of pretreatment time before 48 h, and did not obviously change after 48 h. The biogas produced during pretreatment was mainly composed of carbon dioxide (79.5%–90.6%), the methane content was only between 6.2% and 19.6%, and few hydrogen (0.3%–1.1%) was detected. The low methane content might be attributed to the inhibition of methanogenic bacteria activity due to VFA accumulation from the rumen fluid and the biomass acidification. This result was in agreement with that from corn stover fermentation with rumen microorganism (Hu and Yu, 2005). However, the higher carbon dioxide was produced as the pretreatment time prolonged, leaving less carbon for subsequent anaerobic digestion.

3.2. Anaerobic digestion of rice straw pretreated by rumen fluid

3.2.1. Biogas production during anaerobic digestion

Daily biogas production, cumulative methane yield and methane content are presented in Fig. 4. Fig. 4a shows the daily

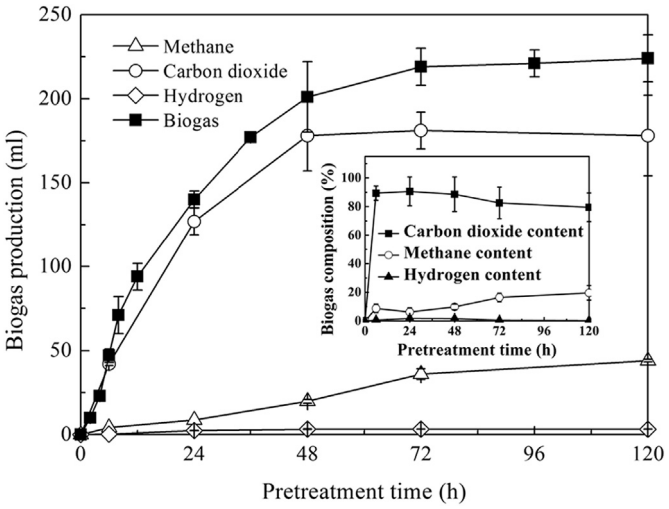

Fig. 3. Biogas production during pretreatment.

biogas production of rumen fluid pretreated rice straw during 30 d anaerobic digestion, Similar trend of daily biogas production was observed for rumen fluid pretreated straws and the control. Several biogas production peaks appeared as the digestion proceeded. However, the rumen fluid pretreated samples yielded higher daily biogas production over a shorter time, compared to the control, because the VFAs after pretreatment are readily available for methanogenic archea. The biogas production of control lasted longer digestion time. With the rumen fluid pretreatment time of 12, 24, 48, 72, 96 and 120 h, the total biogas yields were respectively 459.8, 495.9, 424.3, 389.1, 385.4, and 376.7 ml/g_{VS}, which were 27.0%–67.3% higher than that of the control (296.4 ml/g_{VS}). It was found that the total biogas yield reduced when the pretreatment time was higher than 48 h. The biogas production might be inhibited due to the high acetic and propionic acid concentration. Meanwhile, the propionic acid was slowly converted by propionate assimilating microbes. This result indicates that the rumen fluid pretreatment significantly enhanced the biogas yields.

Energy contained in the biogas was determined by both biogas volume and methane content. Fig. 4b shows cumulative methane yield of rumen fluid pretreated rice straw for 30 d anaerobic digestion. The methane yield was normalized per volatile solid added (ml/g_{VS}). It was observed that the pretreated rice straws achieved higher methane yield than the control. The cumulative methane yields were in a range of 218.5–285.1 ml/g_{VS} for rumen fluid pretreated samples, which was 40.5–82.6% higher than that of the control (156.1 ml/g_{VS}). The improvement of methane yield (82.6%) in this study was comparable with that for rice straw digestion with hydrogen peroxide pretreatment, lower that for rice straw digestion with hydrothermal-NaOH pretreatment, and higher than those for lignocellulosic biomass digestion with different biological pretreatments (as shown in Table 2). However, the hydrothermal-NaOH pretreatment required high energy and chemical consumption. The rumen fluid pretreatment need neither high energy nor chemical reagents, therefore, is one ideal method to accelerate the anaerobic digestion of lignocellulosic biomass.

Fig. 4a and b shows that the rumen fluid pretreated rice straw achieved higher biogas and methane yield. However, the total biogas and methane yield increased when the rumen fluid pretreatment time increased from 12 to 24 h, and decreased with the further pretreatment time increase. The lignocellulose might be ineffectively degraded by a short rumen fluid pretreatment (12 h). This trend was similar to the results observed by Hu et al. (2015).

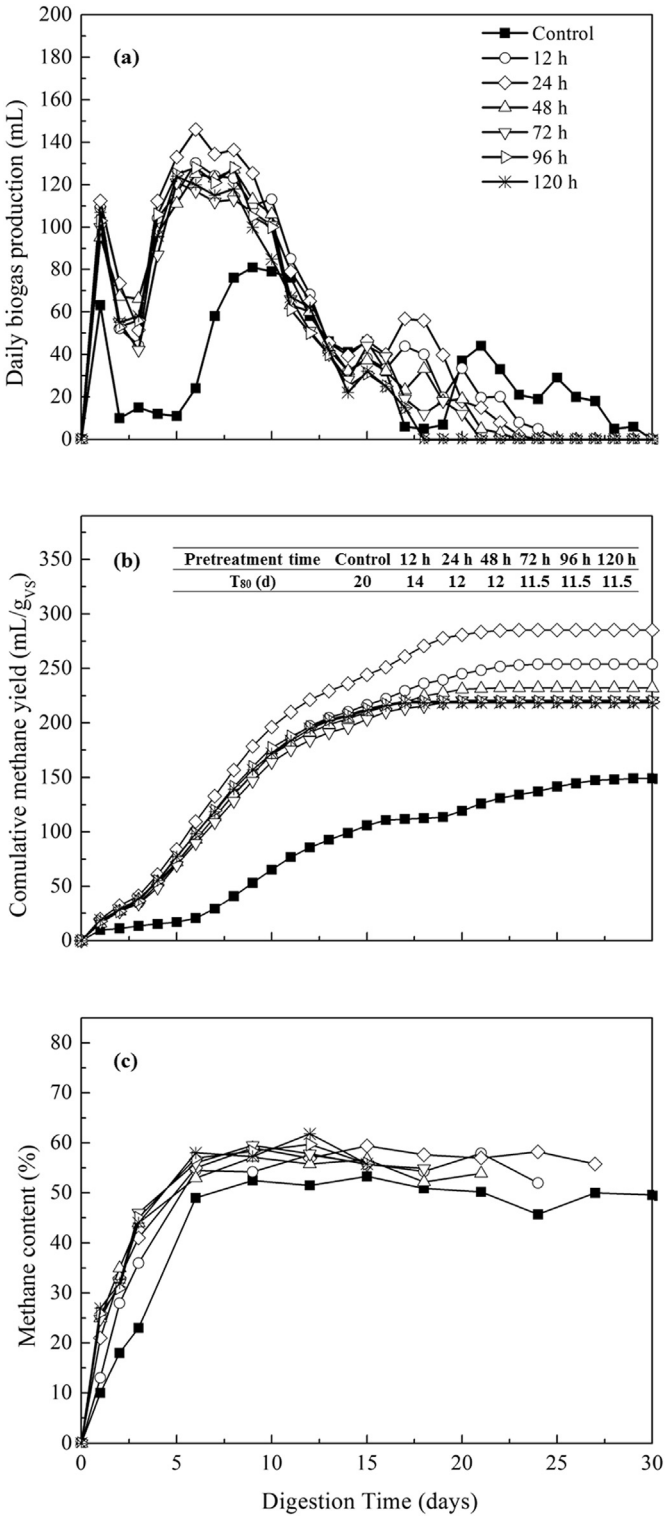

Fig. 4. Biogas and methane production of unpretreated and rumen fluid pretreated rice straw, (a) daily biogas production, (b) cumulative methane yield, and (c) methane content.

The biogas and methane yield decrease after the rumen fluid pretreatment over 24 h might be caused by two reasons. On the one hand, part of carbon was lost as carbon dioxide during rumen fluid pretreatment, leading to less carbon for subsequent anaerobic digestion. On the other hand, the propionic acid concentration in

Table 2
Comparisons of methane yield with different pretreatments.

Pretreatment	Substrate	Increase of methane yield %	References
Chemical pretreatment (3% H_2O_2, 20 °C, 6 d)	Rice straw	88	Song et al. (2013)
Hydrothermal pretreatment (5% NaOH, 200 °C, 10 min)	Rice straw	121.9	Chandra et al. (2012)
Biological pretreatment (White rot fungus, 38 °C, 3 weeks)	Rice straw	46.2	Ghosh and Bhattacharyya (1999)
Biological pretreatment (Liquid fraction of digestate, 20 °C, 3 d)	Corn stover	66.3	Hu et al. (2015)
Biological pretreatment (Rumen fluid, 39 °C, 24 h)	Rice straw	82.6	this study

the anaerobic system was relative higher (534.3–686.8 mg/L), when the rumen fluid pretreatment time was longer than 24 h. The higher propionic acid concentration might inhibit the methane production to a certain extent. Based on the results from Fig. 4a and b, it could be concluded that 24 h was the optimal rumen fluid pretreatment time in the present study.

Technical digestion time (T_{80}) can be defined as the digestion time to reach 80% of the total methane production (Zheng et al., 2009). It was observed that the T_{80} for the rice straw pretreated with rumen fluid was 30.0–42.5% shorter than that of the control. The significant reduction of digestion time might be attributed to that the rice straw become more readily biodegradable after rumen fluid pretreatment. Moreover, high methane production over a shorter time would bring economic benefit for improving the treatment capacity of biogas facility (Zhong et al., 2011).

From Fig. 4c, the average methane content of biogas produced from the rumen fluid pretreated rice straw was calculated, excluding the data in the first five days. The higher average methane content of 56.6–58.5% was observed for the rice straw pretreated for 48–120 h, while that for 24 h pretreatment was 56.1% and for the control was 50.3%. The methane content was in agreement with that of conventional anaerobic digestion of organic wastes (Samani et al., 2001). It was found that the methane content of the sample with 24 h pretreatment is slightly lower, but its methane yield was significantly higher than that with longer pretreatment (48–120 h). This is because of the higher biogas production for the sample with 24 h pretreatment.

3.2.2. TS and VS degradation during anaerobic digestion

Biogas and methane are generated from biological conversion of substrate, which can be represented by TS and VS change. The increases of biogas and methane production could be attributed to the improved TS and VS degradation due to rumen fluid pretreatment. The TS and VS degradation after anaerobic digestion is shown in Fig. 5. It can be seen that higher TS and VS degradation was achieved for all rumen fluid pretreated rice straws as compared to the control. The TS and VS degradation for rumen fluid pretreated rice straw was respectively between 57.4% and 65.7% and between 58.3% and 66.9%, while those for the control only 49.3% and 50.8%. After the rumen fluid pretreatment, the rice straw components became more available, and more rice straw was therefore used by anaerobic microorganisms. However, the TS and VS degradation for the rice straw above 48 h pretreatment was higher than that with 24 h pretreatment, but corresponding biogas and methane production was not improved. This may be attributed to the partial conversion of organic carbon to CO_2 during rumen fluid pretreatment and the inhibition of higher propionic acid concentration during methanogenic process (Barrera et al., 2015).

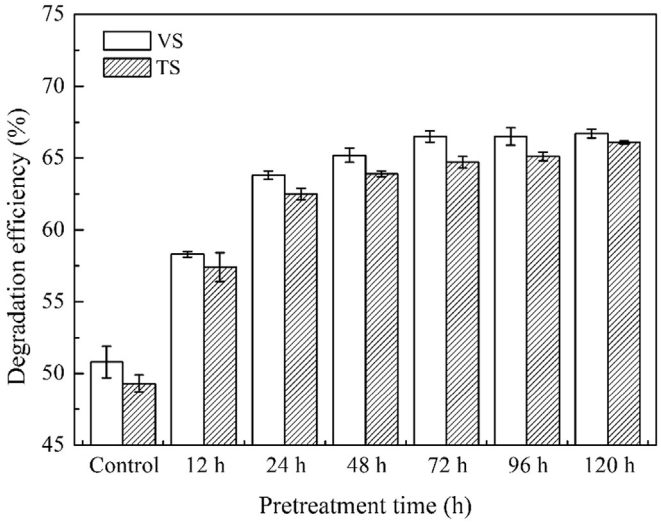

Fig. 5. TS and VS degradation after anaerobic digestion.

3.2.3. Methane production modeling

The modified Gompertz model and first-order kinetic model were used to evaluate methane production rate and hydrolysis rate of rice straw during anaerobic digestion after rumen fluid pretreatment. The kinetic parameters were summarized in Table 3. The modified Gompertz equation showed a better fit in describing methane production in terms of R^2 from 0.992 to 0.999, as compared to the first-order equation from 0.938 to 0.968. The maximum methane yield of 285.1 ml/g_{VS} was achieved for the rice straw with 24 h pretreatment, while the minimum methane yield of 149.0 ml/g_{VS} was for the control. The results of first-order model showed that the rumen fluid pretreatment significantly increased the k value as compared to the control (from 0.080 1/d to 0.099 1/d for rumen fluid pretreated samples and 0.024 1/d for the control). The k value represents the hydrolysis rate of anaerobic digestion, and a higher k value is helpful for improving the anaerobic digestion efficiency. According to the results of modified Gompertz model, the calculated lag-phase time (λ) declined from 3.21 d to 1.62 d, while the μ_m increased from 10.66 ml/(g_{VS}·d) to 29.31 ml/(g_{VS}·d) for the rice straw with 24 h pretreatment. A lower λ and a higher μ_m meant a faster startup and a higher efficiency of anaerobic digestion, respectively. The easily utilized compositions of rice straw increased after rumen fluid pretreatment. However, the predicated λ value for the rice straw with 96 h pretreatment increased to 2.06 d, which meant that the rumen fluid pretreatment did not benefit to the start-up of anaerobic digestion because of the higher propionic acid concentration, if the rumen fluid pretreatment time was too long. These modeling results also indicated that 24 h rumen pretreatment could be significantly enhanced the methane production rate and hydrolysis rate, shortened the lag phase time and increased the methane yield.

4. Conclusions

Biological pretreatment with rumen fluid was proved to be an effective method to improve rice straw biodegradability and methane production. The degradation efficiency of cellulose, hemicellulose and lignin were improved by rumen fluid pretreatment under anaerobic conditions. Rumen microbes mainly

Table 3
Experimental methane yields (EMY) and kinetics parameters for anaerobic rice straw digestion after different pretreatment time.

Pretreatment time	EMY	Modified Gompertz model			First-order model	
	(ml/g_{VS})	μ_m ($ml/(g_{VS}\cdot d)$)	λ (d)	R^2	K (1/d)	R^2
Control	156.1 ± 5.9	10.66 ± 0.44	3.21 ± 0.32	0.992	0.024 ± 0.006	0.938
12 h	253.8 ± 4.6	26.25 ± 0.68	1.95 ± 0.05	0.997	0.080 ± 0.007	0.966
24 h	285.1 ± 7.8	29.31 ± 0.76	1.62 ± 0.09	0.997	0.094 ± 0.002	0.968
48 h	232.1 ± 1.8	26.50 ± 0.39	1.81 ± 0.11	0.999	0.093 ± 0.003	0.963
72 h	220.6 ± 2.6	26.24 ± 0.41	1.96 ± 0.08	0.999	0.097 ± 0.005	0.957
96 h	219.7 ± 3.7	29.25 ± 0.55	2.06 ± 0.08	0.999	0.099 ± 0.011	0.941
120 h	217.5 ± 3.2	29.21 ± 0.60	1.95 ± 0.07	0.998	0.099 ± 0.010	0.950

produced acetic acid and propionate acid from rice straw, which could be used in the subsequent methanogenic process. 24 h rumen fluid pretreatment was considered the optimal, resulting in 66.5% more biogas production, 82.6% more methane yield, and 40.0% shorter T_{80} compared with the control. The improved methane yield was attributed to effectively degradability of rice straw during rumen fluid pretreatment, as indicated by increased TS and VS degradation. The modified Gompertz equation modeled better the anaerobic digestion of rice straw pretreated with rumen fluid with a maximum methane yield of 285.1 ml/g_{VS} for 24 h rumen pretreatment.

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