The Feasibility of Shortening the Pretreatment Time for Improvement of the Biogas Production Rate from Rice Straw with Three Chemical Agents

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Solid-state chemical pretreatment at room temperature was adopted to evaluate the methane production potential of rice straw. Dried rice straw was homogenized with distilled water at a 1:3 ratio and pretreated for 6 h, 24 h, 72 h, 120 h, and 168 h. The effects of the pretreatment time with three chemicals (H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$) on the methane yield were investigated. The results showed that the anaerobic digestion performance was improved for solid-state chemical pretreatment of room temperature-treated rice straw. The biogas yields from the treatments with H_2O_2 for 168 h, $Ca(OH)_2$ for 72 h, and $NH_3 \cdot H_2O$ for 120 h were 386.6 mL/g VS, 413.5 mL/g VS, and 390.5 mL/g VS, which were 35.8%, 45.2%, and 37.2% higher than that from the untreated rice straw, respectively. Therefore, it is feasible to shorten the pretreatment time of rice straw for these three chemical solutions.

Keywords: Room temperature; Solid state; Chemical pretreatment; Straw; Methane production

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INTRODUCTION

Pretreatment of rice straw can be achieved through a variety of physical, chemical, and biological methods to remove some lignin and hemicellulose, which can increase the contact degree of enzymes for the hydrolysis of rice straw (Liu *et al.* 2005). The common pretreatment methods include physical, chemical, and biochemistry methods, as well as the combination of these methods. Among them, the physical method has the advantages of less pollution and simple operation, but its cost is high. The biological method is conducted under mild conditions, which leads to negligible pollution and a low cost, but this method needs a long pretreatment time and has a low efficiency. The chemical method has the advantages of convenience, rapidity, a low cost, simple operation, remarkable treatment effect, and short treatment time (Zheng *et al.* 2014; Li *et al.* 2015; Liu *et al.* 2015).

The majority of chemical pretreatment methods are known as liquid chemical treatments (He *et al.* 2008). This kind of treatment can lead to corrosion issues in equipment and needs a high volume of water to clean the raw materials after pretreatment or a lot of acid or chemicals for neutralization. The soluble organic matter in straw decreases through cleaning with water, which affects the subsequent biogas production. Additionally, when neutralizing with acid or chemicals, a large number of inhibitors, such as sodium and

chloride ions, are produced and this affects the growth of microorganisms during biogas production through anaerobic fermentation (Song *et al.* 2014; Li *et al.* 2016).

Many studies have shown that chemical pretreatment can cause the dissolution of hemicellulose and partial lignin, and it has a positive impact on cellulose degradation (Xu *et al.* 2010; Karuna *et al.* 2014; Sawatdeenarunat *et al.* 2015). Although chemical pretreatment has some disadvantages such as corrosion equipment, secondary pollution and so on, it has the advantages of convenience, a simple operation, low energy consumption, low investment, a short treatment time, low cost, and it can be performed at room temperature without the restriction of environmental conditions. Therefore, it is more suitable for the actual production of straw biogas (Khor *et al.* 2015). There are numerous chemical treatment solutions, which can be divided into acid and alkali treatments. Alkali pretreatment can be advantageous because of its low price and easy operation. Gao (2004) illustrated that the highest biogas yield can be achieved with sodium hydroxide pretreatment for 3 d. The biogas yield from rice straw was the highest after 30 d of treatment with NaOH (Qin *et al.* 2011), while treatment with calcium hydroxide ($Ca(OH)_2$) for 7 d is considered more effective (Song *et al.* 2014). Pang *et al.* (2005) showed that ammonia can be used to treat corn straw for 30 d and increases the biogas yield by 15%. Hydrogen peroxide (H_2O_2) is also known for its de-lignifying ability and oxidative effect (Silverstein *et al.* 2007; Zhao *et al.* 2014). However, the pretreatment time ranges from 3 d to 30 d, and an extended treatment period is unfavorable for the application of pretreatment technology. Additionally, most alkali pretreatments currently use a large amount of chemical solution and water to soak the substrates, which necessitates the recycling of chemicals, disposal of waste solution, and sometimes high temperatures, which could result in a high facility investment, high treatment cost, and environmental pollution (Zhang and Zhang 1999).

In this study, H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$ were applied as chemical reagents for rice straw pretreatment in the solid state at ambient temperature. A limited amount of water was used to keep the rice straw saturated, but no extra water was present inside of the material. This is why the process is called solid-state chemical pretreatment. It has a number of advantages over conventional chemical pretreatments, such as less water used, no waste solution generated, no waste solution treatment or disposal needed, and no energy input required (He *et al.* 2008). The effects of the solid-state chemical pretreatment at room temperature on the physicochemical properties of the rice straw and biogas production were systematically investigated to determine the optimum pretreatment times for solid-state chemical pretreatment at room temperature. The experimental conditions for the three chemical agents and their optimum concentration levels were selected based on the literature.

EXPERIMENTAL

Materials and Methods
Substrates

The air-dried rice straw was collected from an experimental field in Nanjing, China. The straw 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 further use. The total solid (TS) content, volatile solid (VS) content, and

carbon/nitrogen (C/N) ratio of the rice straw in this study were 91.56%±0.07%, 84.45%±0.50% (related to the dry mass), and 68.40%, respectively.

Inoculums of anaerobically digested sewage sludge were 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 day 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 thoroughly mixed and filtered through a screen with a pore size of 833 μm (20 mesh). This was performed to ensure the removal of easily degradable organic matter still present in the inoculums and remove 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.

Pretreatment method

To enhance the anaerobic digestibility of the straw, a solid-state chemical pretreatment methodology was adopted. The three chemicals H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$ were selected and had concentration levels of 3%, 8%, and 5%, respectively, based on the quality. The dried rice straw was homogenized with distilled water at a 1:3 ratio with selected pretreatment times of 6 h, 24 h, 72 h, 120 h, and 168 h, which were recommended by previous research (Song *et al.* 2012; Song *et al.* 2014; Li *et al.* 2015; Liu *et al.* 2015). Additionally, an un-pretreated straw (CK) group was set up with three parallel treatments and analyzed.

The typical procedure of the pretreatment test conducted at room temperature was as follows. The loading was 80 g of raw rice straw and the moisture content was adjusted up to 75% by adding chemical pretreatment agents to a plastic cup. The material was stirred to evenly mix it. After treatment, the rice straw was removed and cooled naturally. The moisture content of the straw was measured for each group after mixing.

Digestion tests

The anaerobic digesters used were a series of 1000-mL wide-mouthed bottles with 800 mL of working volume (Fig. 1).
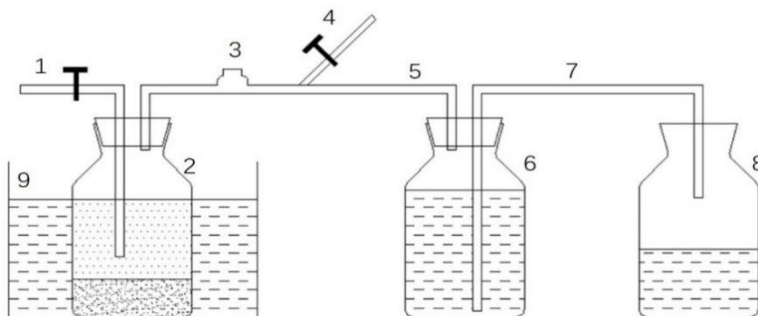

Fig. 1. Anaerobic fermentation devices: 1 – sampling orifice; 2 – fermentation flask; 3 – gas-sampling flask; 4 – vent; 5 – airway; 6 – biogas-collecting flask; 7 – aqueduct; 8 – water-collecting flask; and 9 – constant temperature trough

For each test, the digestion solution, which consisted of 48 g of pretreated straw materials (based on dry matter), 500 g of inoculum, and a certain amount of deionized water as the balance, was subjected to anaerobic digestion. Before that, the pH value of the digestion solution was adjusted to 7.5 and the remaining air in the digestion solution was

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removed by nitrogen purging to provide a suitable environment for anaerobic digestion. An untreated sample of rice straw was simultaneously tested as the CK group. All of the digesters were placed in a constant-temperature incubator at 37 °C ± 1 °C. Anaerobic digestion was terminated when the increase in the cumulative biogas yield was less than 2% for the next sampling within the following 3 d to 5 d, and the cumulative biogas yield was treated as the final biogas yield. The produced biogas was collected *via* a water displacement method and sampled with gas bags by gas chromatography (GC) analysis. Detailed explanations of the anaerobic digesters and digestion testing procedures can be found elsewhere (Li *et al.* 2015).

Analytical Methods

The daily biogas production was directly determined by the volume of displaced water in the graduated cylinder after the mixture was stirred manually. The methane concentration of the biogas was analyzed using GC (GC 9890A, Renhua, Nanjing, China) with a TDC-01column (ϕ 4mm×1 m)(Shimadzu, Kyoto, Japan) and using hydrogen as the carrier gas. The injector, oven, and detector temperatures were 100 °C, 150 °C, and 120 °C, respectively. The carrier gas flow rate was 50 mL/min, and the injection volume of the samples was 0.5 mL (Qian *et al.* 2016). The detection of TS and VS was done in accordance with the standard methods of Eaton *et al*. (2005). The total carbon and total nitrogen were analyzed by a CHN Vario EL analyzer (Perkin Elmer, Foster, USA). The pH value was precisely measured from the liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Greifensee, Switzerland) (Laser *et al.* 2002). The cellulose, hemicellulose, and lignin contents were determined with sequential fiber analysis using the method of Goehring and Van Soest with a FIWE Cellulose Analyzer (Velp Scientifica Company, Rome, Italy) (Van Soest *et al.* 1991). The cold-water, hot-water, 1% NaOH and benzene-alcohol extractives contents were determined were determined with NREL of the U.S. Department of Energy (Sluiter *et al*. 2005)

Statistical Analysis

All of the analytical results were conducted in triplicate. 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 2016 for Windows (Redmond, WA, USA) and Origin 9.0 for Windows (Northampton, MA, USA), respectively. Stepwise regression was used to determine the critical factors impacting the residual statistical analysis (SAs) in the liquid digestates. Multiple means were compared with a least significant difference test at a P value of 0.05 and 0.01. These statistical analysis were performed using the software SPSS (v. 21.0, IBM, Chicago, IL, USA).

RESULTS AND DISCUSSION

Characteristics of the Biogas Production
Volumetric biogas production rate

The volumetric biogas production rate is an important parameter in biogas fermentation. With the same reactor volume, more methane can be produced if the volumetric gas production rate is high, which means that a high biogas production can be achieved by adopting a smaller fermentation system and it saves on the construction area and investment cost for a biogas plant (Han *et al.* 2011).

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Figure 2 shows the volume biogas production rates for the different chemical treatment solutions. The volumetric biogas production rates of the rice straw pretreated by the three chemical treatment solutions with different pretreatment times were similar, except for the CK group. The results showed that the volumetric biogas production reached a maximum value on the third day, but the peak value was different among the different pretreatment times for each treatment agent. The peak pretreatment times for H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$ were 168 h, 72 h, and 72 h, and the peak values were 1.26 L/(L·d), 1.26 L/(L·d), and 1.29 L/(L·d), respectively. After maintaining a short period of high-volume biogas production, the rates decreased gradually and then gradually increased to a second peak biogas production on the eighth day. After that, all of the production rates of the treatment groups decreased slowly, and the only peak biogas production value of 0.98 L/(L·d) was reached on the fourth day of fermentation with the CK group. According to the results for the volume biogas production, the average volumetric biogas production rates from straw fermentation were the highest with H_2O_2 at 168 h, $Ca(OH)_2$ at 72 h, and $NH_3 \cdot H_2O$ at 120 h, and the values were 0.65 L/(L·d), 0.70 L/(L·d), and 0.66 L/(L·d), respectively. The results showed that pretreatment with the three chemical treatments could improve the volumetric biogas production rate from anaerobic fermentation of rice straw, and the average volumetric biogas production rates with H_2O_2 for 168 h, $Ca(OH)_2$ for 72 h, and $NH_3 \cdot H_2O$ for 120 h were 35.4%, 45.8%, and 37.5% higher than that of the CK group, respectively. Also, the rate of the group treated with $Ca(OH)_2$ for 72 h was the best among the three chemical treatments.
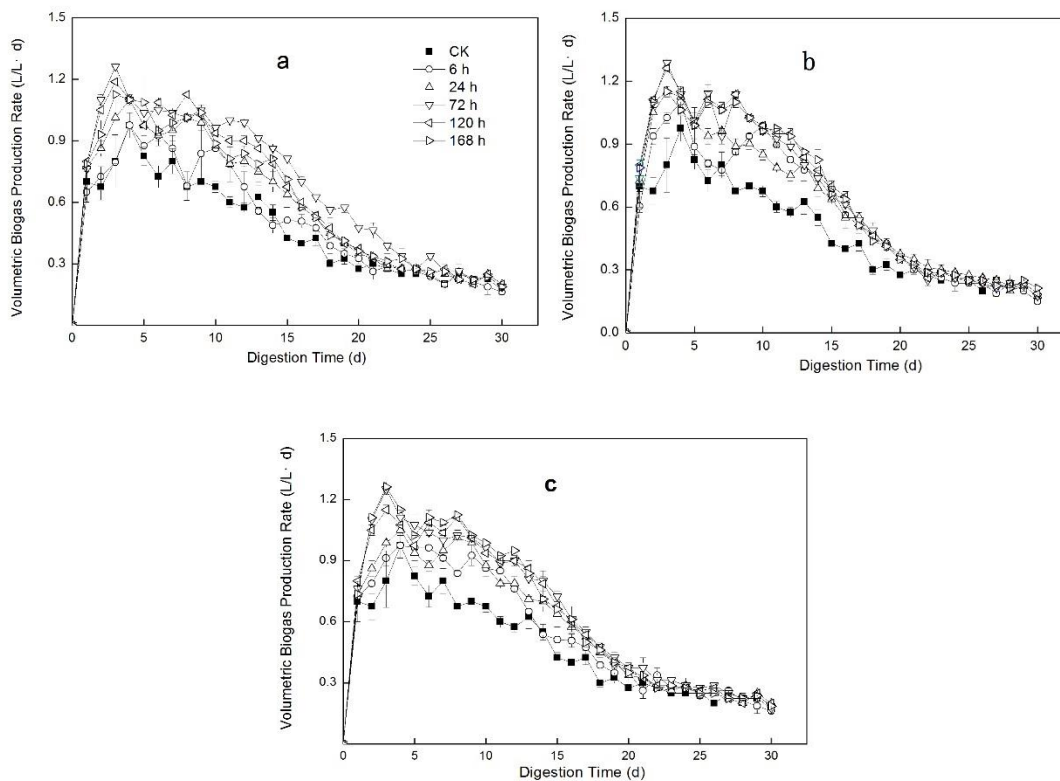

Fig. 2. Volumetric biogas production rates for the treatments with (a) $Ca(OH)_2$, (b) $NH_3 \cdot H_2O$, and (c) H_2O_2

Biogas yield

The biogas yield of the raw material is the biogas produced in the fermentation process from the unit weight of the TS, VS, or organic matter of the fermentation material (Feng 2008). The variation in the biogas produced from the VS with the different chemical treatment solutions is shown in Fig. 3.
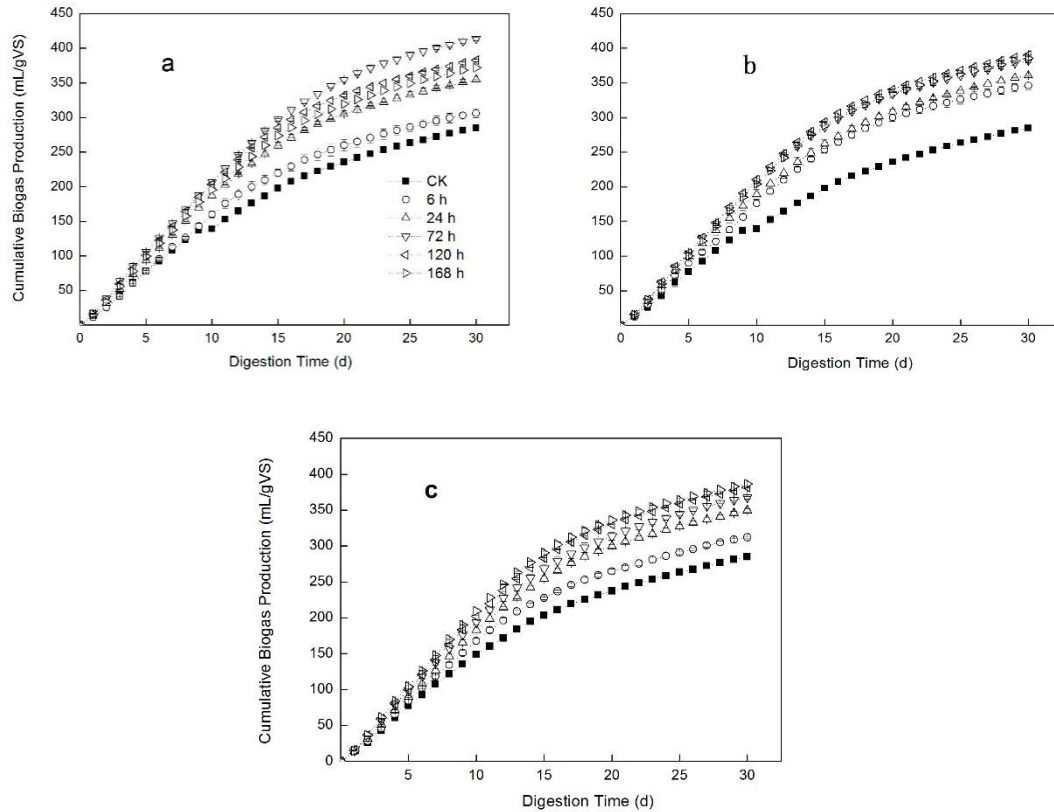

Fig. 3. Changes in the cumulative biogas yield during anaerobic digestion production rate and methane production rate for the treatments with (a) $Ca(OH)_2$, (b) $NH_3 \cdot H_2O$, and (c) H_2O_2; the $Ca(OH)_2$ treatment for 72 h was the best of the three chemical treatments.

The overall trend of biogas produced from the VS was the rapid growth in the early stage, which slowed afterwards. Then, the biogas yield gradually reached a stable value. Compared with the CK group, the biogas yield from VS in the straw increased obviously after pretreatment with the three chemical treatment solutions. However, the variation trend in the biogas yield from VS was different from the chemical treatment solutions following the increased pretreatment time, among which the treatments with H_2O_2 for 168 h, $Ca(OH)_2$ for 72 h, and $NH_3 \cdot H_2O$ for 120 h were the highest. The values were 386.6 mL/g VS, 413.5 mL/g VS, and 390.5 mL/g VS, which were increases of 35.8%, 45.2%, and 37.2% compared with the CK group, respectively. There was no significant difference between the treatments with H_2O_2 for 168 h and $NH_3 \cdot H_2O$ for 120 h ($P > 0.05$), and there was a significant difference between those two treatments and the treatment with $Ca(OH)_2$ for 72 h ($P < 0.01$). The methanogenic rate of the three optimum chemical treatments was 182.6 mL/g VS to 198.0 mL/g VS, which was 31.7% to 42.8% higher than that from the CK group at the end of the experiment (Table 1). These results were similar to those of Song

et al. (2012). Rice straw pretreated with these chemical solutions can improve biogas production.

Table 1. Biogas Production and Methane Content from the Different Treatments during Anaerobic Digestion

Pretreatment	Biogas Yield (mL/gVS)	Average Methane Content (%)	Methane Yield (mL/gVS)
CK	284.70±3.20 C	46.05±1.91 a	138.65±1.44 C
H_2O_2–168h	386.57±2.75 Ba	46.14±1.24 a	182.57±1.57 Ba
$Ca(OH)_2$–72h	413.46±2.52 A	46.63±2.62 a	198.03±1.18 A
$NH_3 \cdot H_2O$–120h	390.52±3.25 Ba	46.54±1.38 a	185.2±2.31 Ba
Note: The same column data marked with the same letters within the same factors indicate no significant difference ($P> 0.05$), and data marked with different letters have a significant difference ($P< 0.05$)			

Methane content

The change in the methane content in biogas can reflect the quality of biogas production from the anaerobic fermentation of rice straw after pretreatment. Figure 4 shows that the change trend of the treated groups was similar.
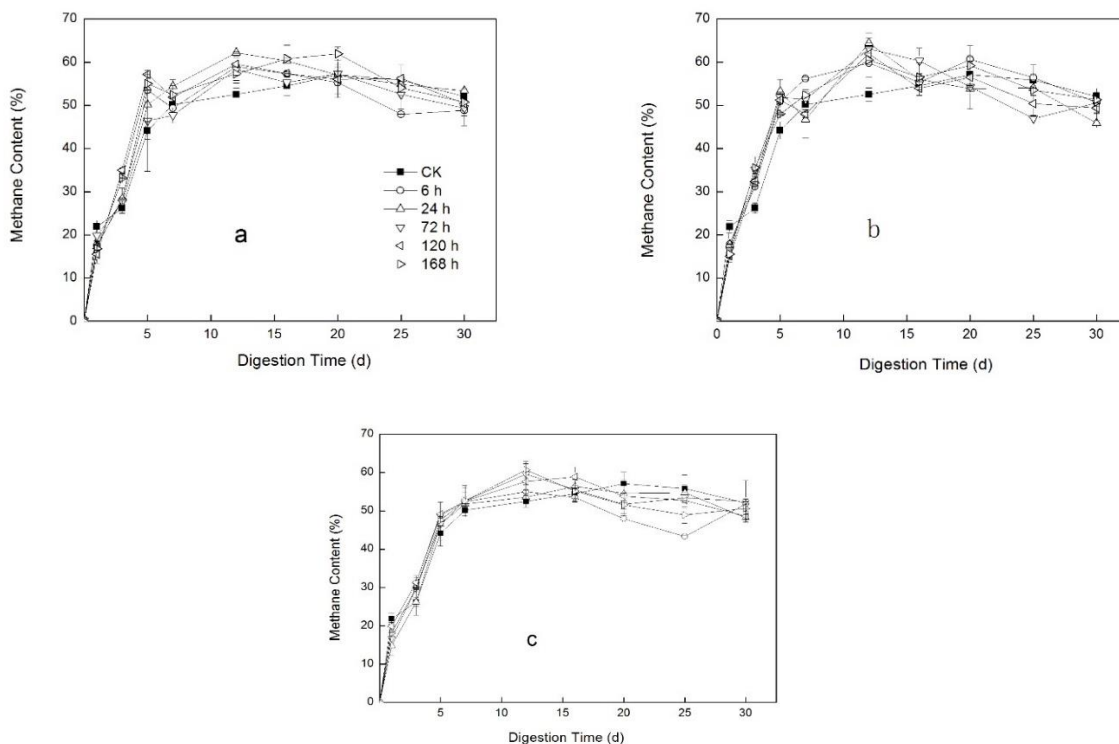

Fig. 4. Change in the methane content in the biogas from batch fermentation with (a) $Ca(OH)_2$, (b) $NH_3 \cdot H_2O$, and (c) H_2O_2

It is worth mentioning that the significant difference in the average methane content was investigated between the three chemical treatment groups and CK group during 30 d of fermentation. There was no significant difference between the different treatments ($P > 0.05$), which indicated that the three chemical treatments used had little effect on the biogas content produced through anaerobic fermentation of rice straw.

The methane content increased rapidly at the beginning of the fermentation process, continued to increase after a small decrease, then gradually decreased, and finally stabilized at approximately 50%. There was no significant difference in the methane content for the different pretreatment times, but the time to reach the peak methane content was different compared with the CK group. The methane contents of the three chemical treatment solutions peaked after 12 d, among which the treatments with H_2O_2 for 168 h, $Ca(OH)_2$ for 72 h, and $NH_3 \cdot H_2O$ for 120 h resulted in the highest contents at 60.7%, 62.2%, and 64.4%, respectively. The peak value the CK group reached after 20 d of fermentation was 57.1%.

Changes in the Physical-chemical Properties of the Straw

Changes in the lignocellulose composition

The straw structure contains a large amount of lignin, cellulose, and hemicellulose, which are crosslinked with each other and makes it difficult to degrade the straw. This special and complex structure restricts the efficiency of anaerobic fermentation of straw to produce biogas (Zhao 2011). Therefore, it is of great practical use to study various methods to pretreat raw materials to improve the biogas yield effectively (Zhao *et al.* 2017).

The effects of the three chemical treatments on the straw fiber components were different (Table 2). This was caused by the difference in the sensitivity of cellulose, hemicellulose, and lignin to pretreatment (Chen *et al.* 2009). With the prolongation of the pretreatment time, the hemicellulose and lignin contents decreased after treatment with H_2O_2 and $Ca(OH)_2$, while the cellulose content increased. Also, the hemicellulose content decreased. The lignin content remained unchanged, and the cellulose content increased slightly after treatment with $NH_3 \cdot H_2O$.

The hemicellulose and lignin contents decreased by 14.7% to 41.8% and 9.2% to 26.8% compared with the CK group, respectively. The hemicellulose and lignin contents in the $Ca(OH)_2$ treatment group decreased by 31.8% to 47.0% and 18.0% to 41.8%, respectively. The hemicellulose content in the $NH_3 \cdot H_2O$-pretreated groups decreased by 0.32% to 33.9%. This result was different from that of immersion in 4% $NH_3 \cdot H_2O$ for 30 d (Yang *et al.* 2003), which was similar to the results of Zhao *et al.* (2015) and Song (2013). This was probably because the impregnation and osmotic effect of the reagent is better than that of the soaking pretreatment.

In short, the lignocellulose components were partially removed with the chemical treatments, and the encapsulated cellulose was released so that the anaerobic microorganisms could gain access to more cellulose. As a result, the anaerobic digestibility of the rice straw was improved.

Changes in the straw extractives

By analyzing the contents of the extractives with different solvents, it could become clear whether pretreatment can produce more substances that are beneficial to anaerobic digestion, and the extractives contents from different solvents can be analyzed (He *et al.* 2008). The substances that dissolve in water from fiber raw materials are tannins, pigments, carbohydrates, plant tools, cyclic polyols, and some inorganic salts.

Table 2. Changes in the Lignocellulose Components Before and After Pretreatment with Straw

Treatment		Hemicellulose (%)	Cellulose (%)	Lignin (%)
3% H_2O_2	CK	41.23±0.33 A	22.58±0.18 F	15.44±0.26 A
	6 h	35.17±0.19 B	30.40±0.17 D	14.02±0.09 B
	24 h	28.72±0.36 Cc	32.23±0.11 C	13.10±0.18 CDb
	72 h	28.89±0.13 Cc	34.93±0.19 B	12.75±0.11 Db
	120 h	23.98±0.15 D	38.45±0.15 A	11.31±0.10 E
	168 h	29.25±0.31 Cc	29.48±0.13 E	13.50±0.05 Ca
8% $Ca(OH)_2$	CK	41.23±0.33 A	22.58±0.18 F	15.44±0.26 A
	6 h	28.10±0.13 B	29.36±0.03 E	12.66±0.06 B
	24 h	24.54±0.07 Cb	32.77±0.06 D	10.86±0.12 C
	72 h	21.86±0.07 E	34.45±0.17 C	10.02±0.09 D
	120 h	23.24±0.03 D	35.94±0.03 A	9.32±0.07 Ea
	168 h	25.07±0.15 Ca	35.55±0.07 B	8.99±0.11 Eb
5% $NH_3 \cdot H_2O$	CK	41.23±0.33 Aa	22.58±0.18 Dc	15.44±0.26 BCbc
	6 h	41.10±0.09 Aa	22.55±0.04 Dc	15.19±0.20 Cc
	24 h	37.12±0.10 B	23.21±0.08 C	15.34±0.07 BCc
	72 h	32.17±0.10 C	24.27±0.06 Bb	15.90±0.18 ABba
	120 h	30.41±0.05 D	24.63±0.12 Ba	15.79±0.12 ABCab
	168 h	27.27±0.12 E	26.07±0.10 A	16.15±0.12 Aa

Note: The same column data marked with the same letters within the same factors indicate no significant difference ($P> 0.05$), and data marked with different letters have a significant difference ($P< 0.05$)

Most of these water-soluble substances can be exploited by microorganisms, and hot solutions can dissolve furfural acid and decayed raw material components because of action from light, heat, oxidation, and bacterial spoilage, in addition to extracting water extract and some lignin, pentose, poly-hexose, and resin acid from the raw materials. A solution of benzene-alcohol can dissolve resins, waxes, fats, tannins, pigments, some carbohydrates, and trace amounts of lignin. The cell wall components in rice straw, such as resins, waxes, and fats, are difficult to deconstruct by microorganisms (Yang 2001).

The contents of the cold-water, hot-water, and 1% NaOH extractives increased by various degrees with the prolongation of the pretreatment time compared with the CK group (Table 3). Among them, the contents of the cold-water, hot-water, and 1% NaOH extractives increased by 43.1% to 92.1%, 25.2% to 59.9%, and 44.4% to 55.4% in the H_2O_2 treatment group, respectively, increased by 116.5% to 243.7%, 88.9% to 159.2%, and 21.8% to 34.6% in the $Ca(OH)_2$ treatment group, respectively, and increased by 12.5% to 56.9%, 43.1% to 70.6%, and 24.1% to 36.9% in the $NH_3 \cdot H_2O$ treatment group, respectively. Generally speaking, the chemical structure of the water extract was simple and the molecular weight was small compared with the cellulose, hemicellulose, and lignin components. Therefore, it is easily used by anaerobic microorganisms, while the 1% NaOH extractives can dissolve some substances that are not conducive to anaerobic fermentation, and the lignocellulose component was fully exposed and degraded by fermentation microorganisms. Therefore, the increase in the cold-water, hot-water, and 1% NaOH extractives was beneficial to improving the biodegradability of the rice straw.

At the same time, the benzene-alcohol extractives content in the rice straw after pretreatment with H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$ decreased by 36.2% to 57.5%, 20.7% to 58.4%, and 39.6% to 54.6%, respectively, compared with the CK group. The benzene-alcohol extracts content decreased, which indicated that the cell wall structure of the rice straw could not only be destroyed by the three chemical pretreatments and lignocellulose was degraded into small molecules, but also some of the straw resin, wax, fat, and other components that are not conducive to the biological utilization of straw can be effectively removed. This is beneficial to the subsequent anaerobic digestion process. This result was similar to that of He *et al.* (2008).

Table 3. Changes in the Extractives Before and After Pretreatment of the Straw

Treatment		Cold-water extractives (%)	Hot-water extractives (%)	1% NaOH extractives (%)	Benzene-alcohol extractives (%)
3% H_2O_2	CK	6.47±0.09 E	10.06±0.15 D	27.38±0.08 D	10.87±0.51 A
	6h	9.26±0.11 D	12.59±0.17 C	39.54±0.14 C	6.94±0.15 Ba
	24h	9.67±0.06 C	13.17±0.16 B	40.96±0.10 B	6.80±0.18 Ba
	72h	10.41±0.13 B	15.60±0.23 Ab	42.51±0.12 Aa	6.00±0.03 Cb
	120h	12.43±0.14 Aa	15.55±0.14 Ab	42.55±0.14 Aa	6.24±0.08 Cb
	168h	12.19±0.14 Aa	16.09±0.15 Aa	42.32±0.03 Aa	4.62±0.13 D
8% $Ca(OH)_2$	CK	6.47±0.09 F	10.06±0.15 F	27.38±0.08 E	10.87±0.51 A
	6h	14.01±0.06 E	19.00±0.17 E	33.35±0.02 D	5.18±0.11 Db
	24h	16.76±0.18 D	19.49±0.05 D	34.56±0.19 C	8.62±0.14 B
	72h	18.51±0.05 C	19.89±0.05 C	36.17±0.11 Ba	4.52±0.10 D
	120h	19.09±0.07 B	21.92±0.06 B	36.86±0.05 A	5.54±0.14 Da
	168h	22.24±0.09 A	26.07±0.14 A	36.12±0.07 Ba	6.04±0.20 C
5% $NH_3 \cdot H_2O$	CK	6.47±0.09 E	10.06±0.15 E	27.38±0.08 E	10.87±0.51 A
	6h	7.28±0.06 Da	14.65±0.11 Da	37.48±0.11 Aa	6.48±0.03 Ba
	24h	7.46±0.08 Da	15.89±0.11 B	35.78±0.05 C	6.56±0.07 Ba
	72h	9.30±0.12 B	15.30±0.05 C	36.49±0.09 B	5.50±0.09 Cb
	120h	10.15±0.07 A	17.16±0.12 A	37.22±0.05 Ab	5.24±0.14 CDc
	168h	8.60±0.10 C	14.40±0.08 Da	33.97±0.07 D	4.94±0.16 Dd

Note: The same column data marked with the same letters within the same factors indicate no significant difference ($P > 0.05$), and data marked with different letters have a significant difference ($P < 0.05$)

Discussion

McInerney *et al.* (1979) proposed the theory of three stages of anaerobic fermentation. First, the complex insoluble macromolecules are hydrolyzed into soluble small molecule organics by the hydrolytic bacteria, then it is permeated through the cells to decompose into volatile organic acids, alcohols, and aldehydes. Finally, hydrogen and oxygen are produced. Volatile acids are mainly produced in the acid production stage, including acetic acid, propionic acid, and butyric acid, which account for 80% and the largest proportion is acetic acid (Isci and Demirer 2007; Song 2007). Finally, the methanogenic stage is decomposed by methanogens to form methane and carbon dioxide, and hydrogen can also be used to reduce carbon dioxide to form methane or transform formic acid to methane.

Table 4. Correlation Analysis of the Physical-chemical Indicators of the Rice Straw Obtained Before and After Pretreatment with Different Reagents

Physical-chemical Indices	Cold-water extractives	Hot-water extractives	1% NaOH extractives	Benzene-alcohol extractives	Hemicellulose	Cellulose	Lignin
Cold-water extractives	1.000						
Hot-water extractives	0.917**	1.000					
1% NaOH extractives	0.042	0.021	1.000				
Benzene-alcohol extractives	-0.282	-0.410	-0.492	1.000			
Hemicellulose	-0.787**	-0.666**	-0.298	0.492	1.000		
Cellulose	0.727**	0.525*	0.495	-0.218	-0.811**	1.000	
Lignin	-0.919**	-0.760**	-0.193	0.124	0.740**	-0.879**	1.000
Note: * significant correlation ($P<0.05$); ** highly significant correlation ($P< 0.01$)							

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For plant materials, such as crop stalk, their biodegradability depends on the extent to which cellulose and hemicellulose are wrapped in lignin. Cellulose and hemicellulose are biodegradable, while lignin is difficult to degrade. When lignin is wrapped on the surface of cellulose and hemicellulose, it is difficult for the enzymes to hydrolyze the cellulose and hemicellulose. Therefore, the hydrolysis stage is the rate limiting step of the process in anaerobic biological treatment of biomass with a high fiber content (Kübler *et al.* 2000; Pavlostathis *et al.* 2007). Therefore, the purpose of straw pretreatment is to remove lignin and hemicellulose, reduce the cellulose crystallinity, increase cellulose accessibility, and improve the enzymatic hydrolysis of rice straw (Mata-Alvarez *et al.* 2000; Hendriks and Zeeman 2009).

The contents of the cold-water and hot-water extractives negatively correlated with the hemicellulose and lignin ($P < 0.01$), which indicated that the increase in the water extractive was mainly because of the degradation of hemicellulose and lignin in the rice straw. The increase in the lignin content was mainly because of the concentration effect caused by the decrease in the cellulose and hemicellulose contents, which was related to a highly significant negative correlation between the cellulose content, and hemicellulose and lignin contents in Table 4.

A solid state NaOH pre-treatment method was developed (Pang *et al.* 2008), but it could not be commercialized because it needs 30 days just as pretreatment time which is not feasible on a commercial scale and the wet state NaOH pretreatment is better because of its lesser treatment time (3 days) and due to more water availability, solubilization and swelling of the lignin is very fast (Zheng *et al.* 2009). However, the methods that were less than the 7-day pretreatment cycle were screened out in this study. Although processing cycles than the wet state pretreatment is long, but it can avoid the occurrence of secondary pollution. Therefore, the solid-state pretreatment method in this study is more adaptable to the requirements of environmental protection and high processing cycle occasions with low requirements.

CONCLUSIONS

1. The chemicals H_2O_2, $Ca(OH)_2$, and $NH_3 \cdot H_2O$ were found to differently enhance the lignocellulosic digestibility and biogas production of rice straw. The biogas yields from the treatments with H_2O_2 for 168 h, $Ca(OH)_2$ for 72 h, and $NH_3 \cdot H_2O$ for 120 h were 386.6 mL/g VS, 413.5 mL/g VS, and 390.5 mL/g VS, which were 35.8%, 45.2%, and 37.2% higher than the CK group, respectively.

2. The cold-water, hot-water, and 1%NaOH extractive contents increased by various degrees with the prolongation of the pretreatment time compared with the CK group, but the benzene-alcohol extractives content gradually decreased.

3. To shorten pretreatment time for three kinds of chemical solutions is feasibility.

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