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ORIGINAL PAPER

Comparison of Different Pretreatments of Rice Straw Substrate to Improve Biogas Production

Ben-lin Dai[1,2] · **Xu-jing Guo**[3] · **Dong-hai Yuan**[4] · **Ji-ming Xu**[1,2]

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Abstract Chemical and biological pretreatments (with NaOH, HCl, $CO(NH_2)_2$ and cellulase) were used to pretreat rice straw at ambient temperature (about 20 °C) to improve its biodegradability and increase anaerobic biogas production. The NaOH and $CO(NH_2)_2$ pretreatments reduced the percentage contents of hemicellulose and lignin. The HCl pretreatment mainly dissolved the hemicellulose and resulted in decreases of 12.5–7.1% of the hemicellulose. The percentage content of cellulose showed a dramatic decrease, from 38.3 to 10.9%, after the cellulase pretreatment. Compared with untreated rice straw substrate, the total biogas yield ratios were 3.38–5.91, 1.63–2.99, 1.93–5.22 and 3.62–6.45, with a hydraulic retention time of 30 days, under NaOH, HCl, $CO(NH_2)_2$ and cellulose pretreatments, respectively. The highest yields of biogas and methane were obtained from 40 U/g total solids (TS) cellulase-pretreated rice straw (20.433 and 9.918 L respectively). Biogas production yields of volatile solids (VS) were 123.7, 273.8, 318.5, 353.5 mL/g for control, 6% $CO(NH_2)_2$-, NaOH- and 40 U/g TS cellulase-pretreated rice straw substrate, respectively. Compared to untreated rice straw substreates, cumulative biogas production yields increased 16–103, 25–122% for NaoH- and cellulase-pretreated rice straw substrate, respectively. The results suggested that the highest removal efficiencies of TS and VS were obtained from 6% NaOH-pretreated (53.80 and 36.80%), 6% $CO(NH_2)_2$-pretreated (54.90 and 36.10%) and 40 U/g TS cellulase-pretreated (51.30 and 37.30%) rice straw substrate. In short, NaOH, HCl, $CO(NH_2)_2$ and cellulase pretreatment was suitable to enhance the biogas production. However, to choose the optimal treatment, the energy requirements relative to the energy gain as extra biogas production have to be taken into account, as well as the costs of chemicals or enzymes.

Keywords Rice straw · Pretreatment · Biogas · Methane

Introduction

It is indisputable that future energy security has become one of the most critical of global problems [1]. Combustion of fossil fuels—i.e., petrol, natural gas and coal—has led to unacceptably high levels of emissions of CO_2, NO_x and SO_x, all of which cause increased air pollution and have adverse effects on human health [2]. In order to meet rising energy demands and to solve the problems caused by environmental pollution and climate change, while maintaining rapid economic growth, there is an urgent need to develop alternative low-emission energy technologies [3]. Anaerobic digestion is regarded as one means of producing renewable energy-rich biogas and simultaneously reducing the problems associated with the disposal of organic waste [4–7].

✉ Xu-jing Guo
gxj530520@126.com

[1] Jiangsu Key Laboratory for Biomass-based Energy and Enzyme Technology, Huaiyin Normal University, Huaian 223300, China

[2] Jiangsu Collaborative Innovation Center of Regional Modern Agriculture & Environmental Protection, Huaiyin Normal University, Huaian 223300, Jiangsu, China

[3] College of Resources and Environment, Chengdu University of Information Technology, No.24 Block 1, Xuefu Road, Chengdu 610225, China

[4] Key Laboratory of Urban Stormwater System and Water Environment, Ministry of Education, Beijing University of Civil Engineering and Architecture, Beijing 100044, China

Agriculture is very important not only for human sustenance and for sustaining economic development, but agricultural waste such as wheat or rice straw can also play an important role in meeting the growing demand for energy. In particular, lignocellulosic crop waste has a huge unutilized energy generation potential [1, 8]. China is one of the largest agricultural production countries in the world, generating more than 800 million metric tons of residual straw every year [9]; the annual amount of rice straw ranges from 180 to 270 million tons [10]. More than 60% of this straw is burned in the open, creating atmospheric environmental pollution [11, 12]. Clearly, developing a use for this discarded straw could improve the air quality in China. Furthermore, this straw could be used to produce biogas through anaerobic digestion, creating a new fuel source while reducing environmental pollution.

Lignocellulosic agricultural crops have a major drawback, however: a rigid crystalline structure that makes them resistant to microbial degradation [13–15]. Although the cellulose, hemicellulose and polysaccharides can be easily degraded by microorganisms or enzymes, the cellulose is tightly wrapped by lignin and hemicellulose, preventing contact between microbes and enzymes [16], and a pretreatment step is required to open up the structure and reduce the crystallinity of the lignocelluloses [17]. Pretreatment of lignocellulosic biomass can improve the biodegradation rate and the overall product yield in anaerobic digestion processes [18–20].

Various pretreatment methods have been employed for improving the biodegradation rate of lignocellulosic biomass and biogas production [21–24]. For example, NaOH and enzymatic treatments have been successfully applied to treat digested biofibers to improve biogas production [18]. Steam treatment can hydrolyze hemicellulose and improve the accessibility of cellulose for enzymatic hydrolysis [25]. In this study, four pretreatment methods were used to pretreat rice straw: alkaline (NaOH), acid (HCl), carbamide ($CO(NH_2)_2$) and enzymes. The pretreatments considered have been selected due to their low input energy requirements. Changes in the composition of the rice straw as a result of the pretreatments were also investigated. The objective of this study was to determine the biomethane potential of rice straw and how it could be improved with these pretreatment methods.

Materials and Methods

Experimental Materials

Naturally dried rice straw and cattle dung was collected from Wangying town in Huaiyin city of China. All straws were cut into 2- to 3-cm sizes and then crushed using a pulverizer (DFY-1000C, Wenling city, China). Inoculum was obtained from a household biogas digester in Wangzhuang, Dingji town, Huaian city, China. The inoculum in this study was a kind of compound bacterium agent, screened from high temperature compost and cow dung, then cultivate by high temperature domestication. It included deodorizing, cellulose and lignin decomposition composite microorganisms [26]. The treatment of inoculum stored before being used in experiments for 8 days at 4 °C. Before use, the inoculum was sieved through a 1-mm screen to remove large particles. The total solid (TS) and volatile solid (VS) values were, respectively, 92.9 and 81.4% for the rice straw, 87.4 and 56.8% for cattle manure, 7.3 and 4.1% for the incoculum. The C/N ratio, TS, VS, cellulose, hemicellulose and lignin proportions of raw materials were outlined in Table 1.

Pretreatment of Rice Straw

Powdered 2, 4, 6 and 8% (w/w) NaOH, HCl and $CO(NH_2)_2$ (96.0% purity) was added to 60.0 g of oven dried rice straw followed by addition of water to maintain 10.0% total solids concentration. The prepared substrates were kept in a temperature controlled incubator for, maintained at 35 °C .Cellulase was also used in the pretreatment of the rice straw. Enzyme doses were 10, 20, 30 and 40 U/g TS and hydrolysis was terminated by the addition of 3 mL DNS solution, followed by boiling the mixture for 5 min at 35 °C. For the destruction of the straw complex crystal structure, the pretreated processes were conducted for 192 h (8 days).

Anaerobic Digestion

The methane potentials of raw and washed rice straw after pretreatment were determined by anaerobic digestion in 2 L glass bottles. Batch anaerobic digestion processes were conducted under a mesophilic condition at 38 °C [27]. Each bottle was supplemented with 300 mL inoculum, 60 g dried cattle dung, and 60 g untreated or pretreated rice straw samples. Untreated rice straw is defined as control group. Furthermore, 300 mL inoculum, and 60 g dried cattle dung

Table 1 The compositions of raw material and inoculum

Materials	Rice straw	Cattle manure	Inoculum
C/N	42.5 ± 0.3	57.2 ± 0.4	20.00 ± 0.24
TS (%)	85.6 ± 2.1	87.4 ± 0.9	7.3 ± 0.2
VS (%)	74.9 ± 1.4	56.8 ± 1.1	4.1 ± 0.4
Ash content (%)	10.1 ± 0.6	–	–
Cellulose (%)	38.3 ± 0.8	–	–
Hemicellulose (%)	21.3 ± 0.5	–	–
Lignin (%)	12.5 ± 0.2	–	–

were used as a blank so that the gas production of the rice straw alone could be determined. Then, deionized water was added to bring the total volume up to 2 L. The initial pH value was adjusted to 7. Bottles were closed with butyl rubber seals and aluminum caps. The headspace of each bottle was flushed with nitrogen to obtain anaerobic conditions.

In order to maintain a constant temperature, fermentation devices were fixed in a constant-temperature water bath. A temperature controller, a sensor, and a heating wire were used to control the temperature (Fig. 1).

Analytical Methods

TS and VS were measured according to the Standard Methods for the Examination of Water and Wastewater [28]. Briefly, samples were dried at 103 °C for 2 h, cooled once, and then weighed to determine the amount of TS; samples were dried at 550 °C for 30 min, cooled once, and then weighed to determine the amount of fixed solid (FS), and VS was then calculated: VS = TS−FS. Biogas volume was monitored every day using the water displacement method, and the corresponding cumulative biogas volume was calculated. The measured volume was then converted to a volume of gas at standard temperature and pressure using the ideal gas law. Gas composition was determined using a gas chromatograph (GC-2010ATF, TDX-02B, Japan) equipped with a Φ4 mm × 0.5 mm deactivation column and a thermal conductivity detector (TCD). Two different analytical columns, Rtx-WAX (30 m × 0.25 mm × 0.25 µm) and Molecular sieve-13 × 60/80 mesh (2.0 m × 3.2 mm × 2.1 mm), were used for CH_4. A standard gas was used to calibrate the system and this had the following composition: 30% CO_2; 30% N_2; 40% CH_4. Biogas production was calculated using a airtight plastic cylinder with a number of dials (mL). The contents of lignin, hemicellulose, and cellulose were determined by measuring lignin, acid detergent fiber (ADF), and neutral detergent fiber (NDF) [29]. Lignin, cellulose and hemicellulose degradations were defined as the percentages of lignin, cellulose and hemicellulose reduction respectively. The cellulase activity was measured as filter paper activity (FPA) based on the method of Eveleigh [30]. All values were measured twice and average will be used in this study.

Results and Discussion

Changes in Cellulose, Hemicellulose, and Lignin

The mass percentages of cellulose, hemicellulose and lignin in the untreated and pretreated rice straw are shown in Fig. 2; the percentages in the untreated rice straw were 38.3, 21.3 and 12.5% respectively. The 2% NaOH pretreatment showed the minimum percentage of cellulose; this increased slightly in the 6% NaOH. The percentages of hemicellulose and lignin, however, decreased with increasing NaOH content, indicating that the effect of increasing alkalinity was to boost the percentage, especially of lignin. The OH- in NaOH can weaken the hydrogen bonding between cellulose and hemicellulose, and break the bonding of ester and ether between lignin and polysaccharides, resulting in the separation and partial decomposition of cellulose, hemicellulose and lignin [31, 32]. Dissolved hemicellulose was further hydrolyzed into a monomer structure, furfural and other products (volatiles). Moreover, insoluble lignin transformed into soluble hydroxyl lignin was conducive to microbial biodegradation [32, 33].

The percentage of cellulose showed a slight decrease in the hydrochloric acid pretreatment. The 4% HCl pretreatment had the minimum percentage of cellulose. However, the hemicellulose content showed a dramatic decrease with increasing hydrochloric acid content. Compared with the raw rice straw, the mass percent of hemicellulose dropped, from 21.3 to 7.1%. The content of lignin, though, increased in the HCl pretreatment.

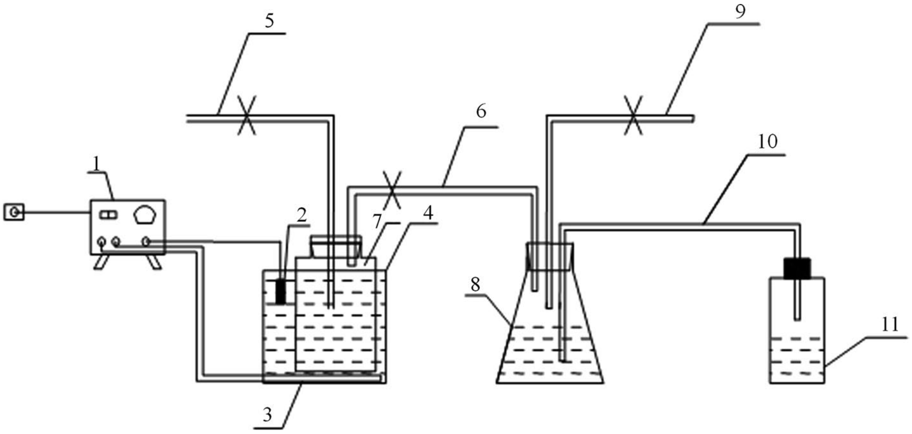
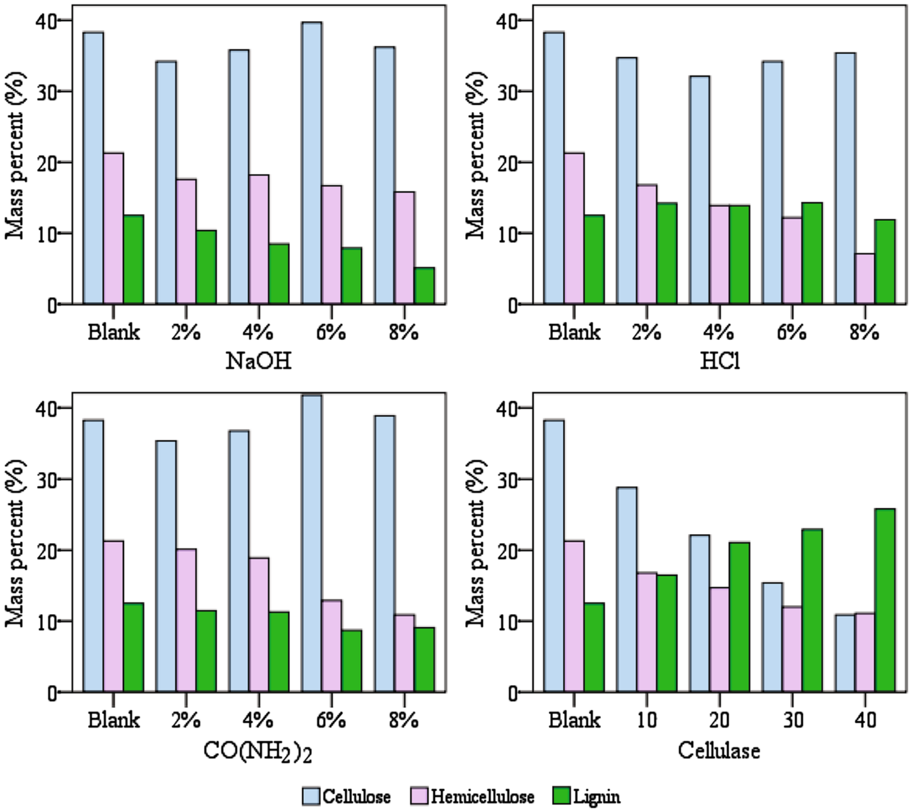


Fig. 1 Schematic of biogas fermentation experimental device: (*1*) Temperature controller, (*2*) sensor, (*3*) land hotline, (*4*) constant-temperature water tank, (*5*) sampling location, (*6*) gas-guide tube, (*7*) bottle of anaerobic fermentation, (*8*) gas-collecting bottle, (*9*) sampling location, (*10*) water pipe, (*11*) water bottle

Fig. 2 Mass percentages of cellulose, hemicellulose, and lignin under the different pretreatment methods. (Color figure online)



Hydrochloric acid mainly dissolved the hemicellulose and broke the crystal structure of the lignin. Thus, cellulose surrounded by lignin was exposed and could then be more easily degraded by microbes [34]. However, dissolved lignin is not conducive to further degradation, due to rapid coagulation and precipitation under acidic conditions [35]. Therefore, dilute acid was more advantageous to the lignocelluloses pretreatment.

The same trend appeared for the changes in cellulose, hemicellulose and lignin in the $CO(NH_2)_2$ pretreatment as in the NaOH treatment. The effects of the pretreatment for hemicellulose in the 6 and 8% $CO(NH_2)_2$ were obviously higher than those in the 6 and 8% NaOH. However, the effects of the pretreatment for lignin were relatively low in the $CO(NH_2)_2$ pretreatment, compared to the NaOH pretreatment. Through the ammoniation pretreatment, the straw fiber structure was broken and ammonium salt was formed [36]. Ammonium salt can promote microbial growth and improve the degradation of rice straw. Carbamide pretreatment can destroy the fiber ester bonds and expose the cellulose, accelerating the dissolution of both cellulose and hemicellulose [37].

As shown in Fig. 2, there was an obvious trend in the cellulase pretreatments: the percentages of cellulose and hemicellulose decreased with increasing amounts of the biological reagents, and conversely, the percentage of lignin increased. The results showed that cellulase pretreatment was advantageous to the degradation of cellulose and hemicellulose.

Methane Fermentation Parameters

Changes of pH

Appropriate pH is necessary for biogas microorganism growth. The suitable pH ranges from 5.0 to 6.5 for hydrolysis bacteria, fermentation bacteria, and hydrogen-producing acetogen, and from 6.6 to 7.8 for methane bacteria (Fig. 3). A marginal decrease in pH was observed between 1 and 6 days of retention time, as the value of pH ranged from 7.12 on the second day to a minimum value of 5.13 on the sixth day for 2% NaOH, from 7.14 to 5.43 for 4% NaOH, from 7.22 to 5.96 for 6% NaOH, and from 7.31 to 6.12 for 8% NaOH. Further, from the sixth day onward, an obvious increase in the value of pH was observed. The pH values ranged from 6.83 to 7.78 after 10 days of anaerobic fermentation. Compared with the NaOH pretreatment, the values of pH showed dramatic fluctuation in the HCl pretreatment. In the fermentation process, the values of pH were relatively lower in the HCl pretreatment than in the NaOH one. The 4 and 6% HCl-pretreated samples showed relatively stable pH values, from 6.35 to 7.05, after 12 days of anaerobic fermentation. The ammoniation pretreatment also showed relatively stable pH values, from 6.78 to 7.41,

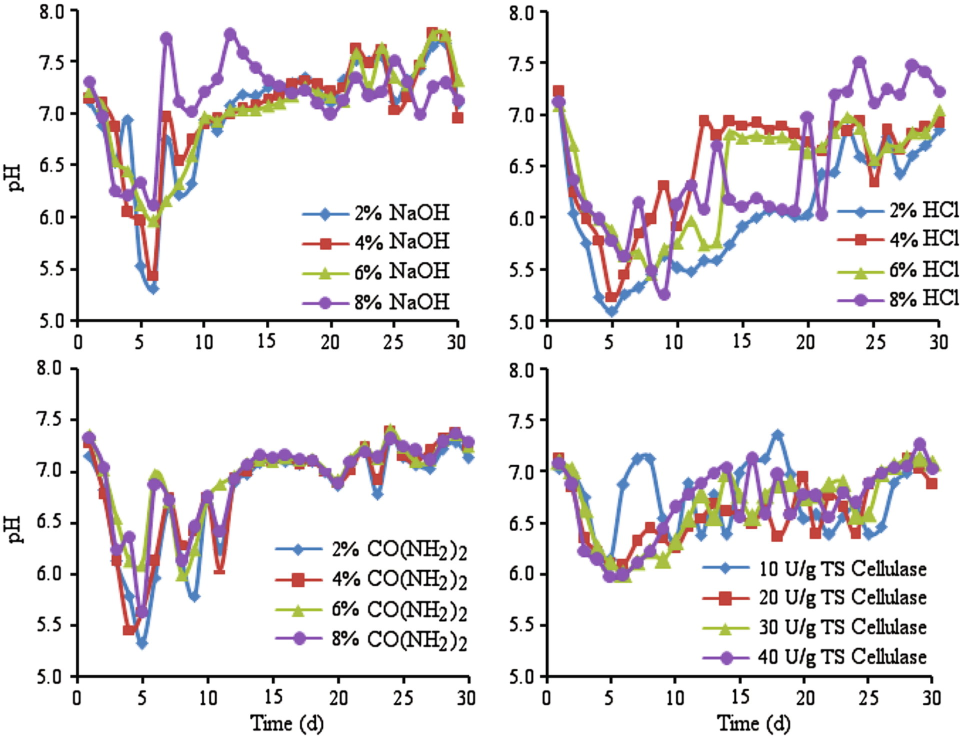


Fig. 3 Changes in pH values in the fermentation processes

after 11 days of anaerobic fermentation. The cellulase pretreatment showed a minimum value on the fifth day, but it fluctuated around 6.50 in the cellulase pretreatment after 10 days of anaerobic fermentation. As a whole, alkali and ammoniation treatments showed relatively high pH values.

Cumulative Biogas Production

Figure 4 shows cumulative biogas production yields produced from untreated and pretreated rice straw substrates. A sharp decrease in biogas production was observed after 26 days of hydraulic retention time (HRT). For both blank, untreated and pretreated substrate, biogas production essentially ceased after 30 days of HRT. Blank group and untreated rice straw substrate resulted in a total of 3.167 and 9.209 L of biogas by the 30th day of HRT. Furthermore, it was observed that about 97.0% of the biogas yield occurred in the first 26 days of HRT.

NaOH-pretreated rice straw substrate yielded totals of 10.704, 12.053, 18.720, and 14.957 L of biogas for 2, 4, 6, and 8%, respectively, under an HRT of 30 days. More than 99.0% of biogas production yield occurred in the first 26 days of HRT, while during the following 4 days of HRT only about 1.0% of the biogas was produced. The 6% NaOH pretreated substrate obtained the maximum total biogas production yield. However, the HCl-pretreated rice straw substrate resulted in total biogas production yields of 9.468, 6.057, 6.826, and 5.148 L for 2, 4, 6, and 8% HCl, respectively, after 30 days of HRT. It was observed that about 95.0% of the biogas production yields occurred in the first 26 days of HRT. Biogas production showed a dramatic increase after 20 days of HRT in the 2% HCl pretreated substrate. As shown in Fig. 4, the 6% $CO(NH_2)_2$-pretreated rice straw substrate yielded the maximum total biogas production 16.540 L under an HRT of 30 days. More than 99.0% of biogas production yield occurred in the first 26 days of HRT, and during the following 4 days of HRT only about 1.0% of the biogas was produced in the 6% $CO(NH_2)_2$-pretreated rice straw substrate. The cumulative biogas yields of each of the $CO(NH_2)_2$-pretreated rice straw substrate samples ranked in the decreasing order of 6% (16.540 L)—2% (12.823 L)—8% (9.823 L)—4% (5.668 L). The total biogas yield increased with increasing cellulase enzyme activity from 10 to 40 U/g TS. Total biogas yields were 11.471, 13.920, 18.374, and 20.433 L for 10, 20, 30 and 40 U/g TS cellulase-pretreated rice straw substrate, respectively.

In this study, biogas production yields of VS were 123.7, 154.3–318.5, 40.6–129.1, 51.2–273.8, and 170.0–353.5 mL/g for control, NaOH, HCl, $CO(NH_2)_2$ and cellulase enzyme pretreated rice straw substrate respectively. The 6% $CO(NH_2)_2$-, NaOH- and 40 U/g TS cellulase-pretreated net rice straw substrate had resulted into biogas production yields of 273.8 mL/g VS, 318.5 mL/g VS and 353.5 mL/g VS, respectively. Compared to untreated rice straw substreates, cumulative biogas production yields increased 16–103, 25–122% for NaoH- and cellulase-pretreated rice straw substrate,

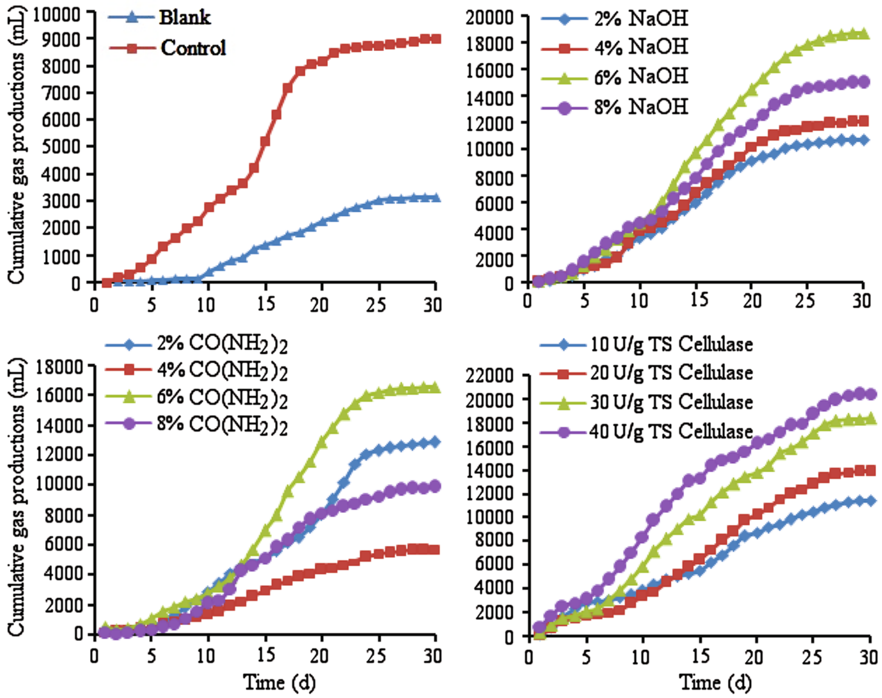


Fig. 4 Total biogas production yields observed, for untreated and pretreated rice straw substrates

respectively. The 2% HCl pretreated substrate only produced 3% higher biogas production compared to that of untreated rice straw substrate. The 4, 6 and 8% HCl pretreated substrate decreased 34, 26 and 44% respectively. Acidification is a potential factor that can be seen from Fig. 3. Cumulative biogas production yields increased 39, 87, and 3% for 2, 6 and 8% NaoH-pretreated rice straw substrate compared to that of untreated rice straw substrate. However, the 4% NaoH-pretreated rice straw substrate decreased 38% in biogas production.

Table 2 shows the ratios of total biogas yields in the 26th and 30th days, between untreated and pretreated rice straw substrates. The ratios more directly reflect the effect of pretreatment on the increase in biogas yield, in the anaerobic fermentation process. The ratio of the 6% NaOH-pretreated rice straw substrate was observed at its highest—5.90—on the 26th day and showed no obvious change on the 30th day. The ratios showed a slight decrease in the 2, 4 and 8% NaOH-pretreated rice straw substrates. The ratios for the HCl-pretreated rice straw substrates were relatively low. The ratio of the 6% $CO(NH_2)_2$-pretreated rice straw substrate showed a dramatically increased biogas yield, compared to that for raw substrate. Obviously, the ratio increased gradually with increasing cellulase enzyme activity, with a maximum ratio of 6.31 on the 26th day. The results also showed that anaerobic fermentation reached a marginal stage of biogas production on the 26th day.

Methane Content and Cumulative Methane Production

Figure 5 presents the volumetric contents of methane from untreated and pretreated rice straw substrates. The range of methane content for blank group varied from 4.1 to 29.1%, over a 1- to 30-day period of HRT. The range of methane

Table 2 Ratios of total biogas yield on the 26th and 30th days, between untreated and pretreated rice straw substrates

Reagents	Content	26 days	30 days
NaOH	2%	3.40	3.38
	4%	3.82	3.81
	6%	5.90	5.91
	8%	4.76	4.72
HCl	2%	2.97	2.99
	4%	1.88	1.91
	6%	2.10	2.16
	8%	1.59	1.63
$CO(NH_2)_2$	2%	4.04	4.05
	4%	1.77	1.79
	6%	5.29	5.22
	8%	3.06	3.10
Cellulase	10 U/g	3.52	3.62
	20 U/g	4.30	4.40
	30 U/g	5.78	5.80
	40 U/g	6.31	6.45

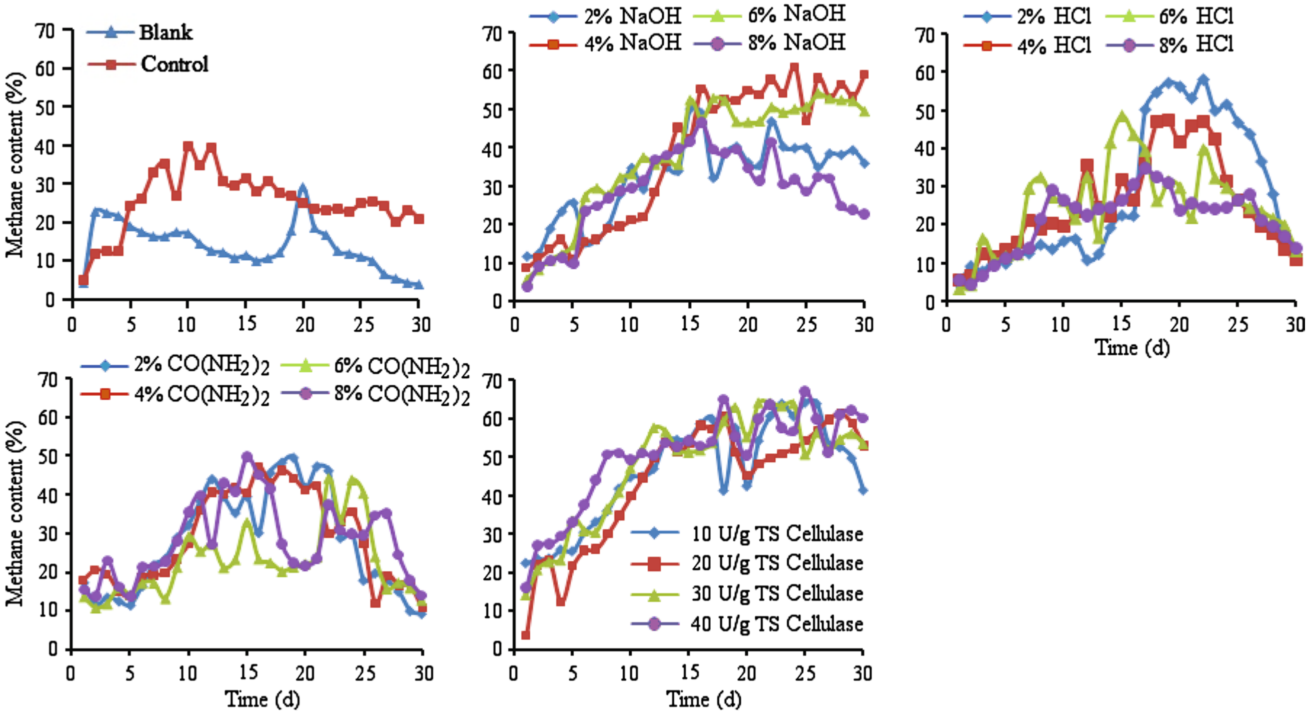


Fig. 5 Volumetric contents of methane from untreated and pretreated rice straw substrates

content for untreated rice straw substrate varied from 4.9 to 39.6%, over a 1- to 30-day period of HRT. The maximum methane content observed was 29.1% on the 20th day of HRT and 39.6% on the 10th day of HRT. Relatively low methane content resulted in low methane production. This may be because of low activity of methanogens.

The range of methane content for 2% NaOH was found to vary from 11.8 to 49.8% over a 1- to 35-day period of HRT. The maximum methane content observed was 49.8% on the 15th day of HRT; a sharp and continuous drop in methane was observed from the 15th day onward. Methane content for 8% NaOH showed the same trend as 2% NaOH. Methane contents for 4 and 6% NaOH-pretreated rice straw substrate ranged from 42.2 to 60.8%, and from 47.2 to 54.0%, respectively. The maximum methane contents observed were 60.8% on the 24th day of HRT for 4, and 54.0% on the 26th day of HRT for 6%, for NaOH. More than 45% of the methane content lasted for 14 and 15 days, respectively, for 4 and 6% NaOH-pretreated rice straw substrate. The methane content was consistent with other literature [20]. Most of the methane content was below 30% for HCl-pretreated rice straw substrate. The maximum methane content observed for 6% HCl-pretreated rice straw substrate was 48.6%, on the 15th day of HRT. The range of methane content for 2% HCl-pretreated rice straw substrate was found to vary from 43.7 to 58.1% during days 17 to 26 of HRT. More than 40% of the methane content lasted for 7, 10, 3 and 5 days, for 2, 4, 6, and 8% $CO(NH_2)_2$-pretreated rice straw substrate, respectively. Methane contents for cellulase-pretreated rice straw substrate showed a dramatic increase after 10 days of anaerobic fermentation. More than 40% of the methane content lasted for 20 days, for all the cellulase-pretreated rice straw substrates. Specifically, methane content for 40 U/g TS cellulase-pretreated rice straw substrate was found to vary from 50.6 to 64.0% during days 11–30 of HRT.

Figure 6 shows the variation in cumulative methane yields for untreated and pretreated substrates of rice straw. The overall methane yield was 0.452 L after 30 days of HRT. It was observed that about 99.0% of the methane yield occurred in the first 26 days of HRT. The specific methane yields of NaOH-pretreated rice straw substrate were 3.634, 4.651, 7.652 and 4.892 L for 2, 4, 6 and 8% NaOH, respectively. The maximum methane yield observed for 6% NaOH-pretreated rice straw substrate was 7.652 L However, 96.0% of the methane yield was observed to occur in the first 26 days of HRT. Methane yields were relatively low for HCl-pretreated rice straw substrate: less than 1.8 L for 4, 6 and 8% HCl pretreatments. The maximum methane yield observed for 6% HCl-pretreated rice straw substrate was 3.515 L More than 99.1% of methane yield occurred in the first 26 days of HRT, and during the following 4 days of HRT only about 0.9% of the methane was obtained for the 6% $CO(NH_2)_2$-pretreated rice straw substrate. The cumulative methane yields for each of the $CO(NH_2)_2$-pretreated rice straw substrates ranked in the

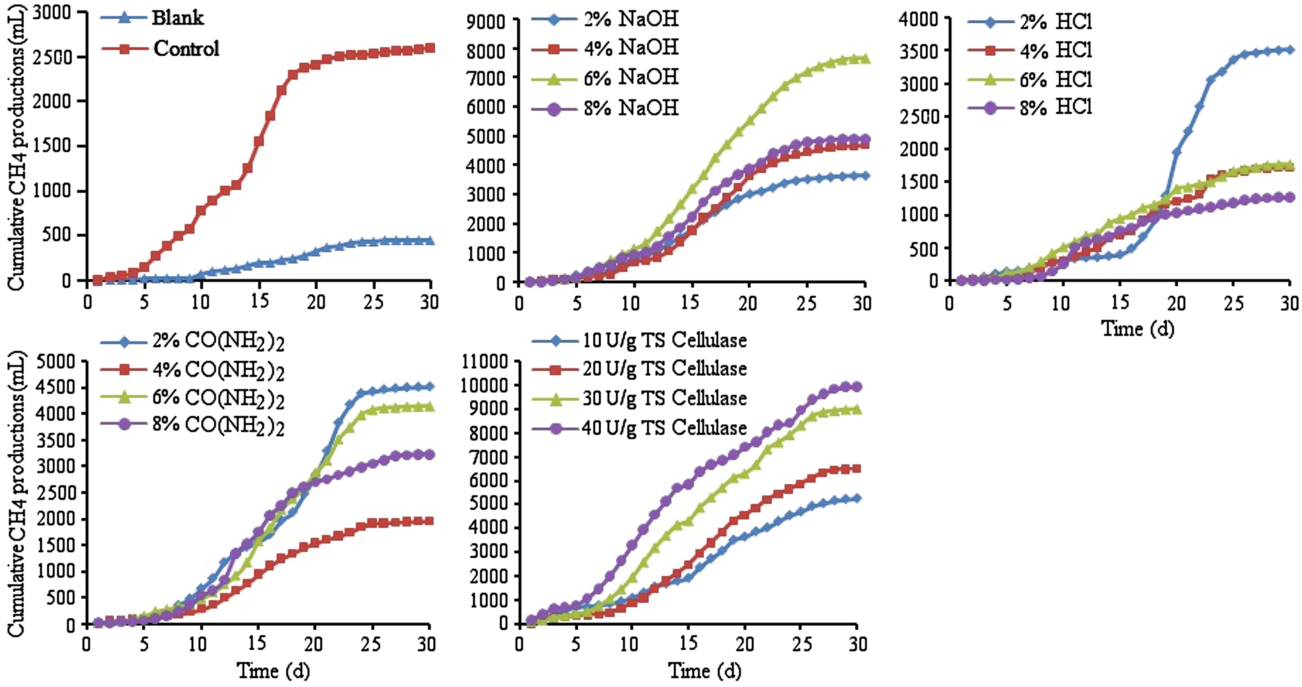


Fig. 6 Cumulative methane production from untreated and pretreated rice straw substrates

decreasing order of 2% (4.504 L)—6% (4.143 L)—8% (3.220 L)—4% (1.943 L). Although the biogas yield for the 2% $CO(NH_2)_2$-pretreated rice straw substrate was less than that for the 6% $CO(NH_2)_2$-pretreated rice straw substrate, the methane yield was relatively high. The same trend appeared for biogas production yield as for methane yield: it increased with increasing cellulase enzyme activity from 10 to 40 U/g TS. The maximum methane yield was 9.918 L for 8% cellulase-pretreated rice substrate. However, the methane yield for 30 U/g TS cellulase enzyme activity was relatively high (8.994 L), making up 90.7% of the methane yield for 40 U/g TS cellulase enzyme activity. Compared to untreated rice straw substreates, cumulative methane production yields increased 0.84–2.56 times for NaoH, $CO(NH_2)_2$ and cellulase-pretreated rice straw substrate, respectively. Only 2% HCl-pretreated rice straw substreates increased 0.18 time than that in the control group.

From an economic point of view, 30 U/g TS cellulase enzyme activity might be more appropriate for the pretreatment of rice straw. The results suggested that 6% NaOH-, 2% HCl-, 2% $CO(NH_2)_2$- and 30 U/g TS cellulase-pretreated rice straw obtained relatively high methane yields, in this study.

TS and VS Mass Removal Efficiencies

In this study, rice straw was investigated for biodegradability improvement under different pretreatment methods, and increases in biogas production and energy gain were attributed to the improved biodegradability of the variously treated rice straw substrates. It appears, though, that the biodegradability was improved through the synergistic effect of complex microbial agents [38, 39].

Biogas is generated from the biological conversion of substrates; thus, biogas production may be indicated by reductions in the amount of dry matter of the substrate, represented by TS and VS [1]. The TS and VS reductions in the straw using the four pretreatment methods were calculated, and the results are shown in Table 3. It was observed that more rice straw became available to anaerobic microorganisms after all the pretreatments, representing significant improvements in biodegradability. A variety of pretreatment methods have been used to convert the lignocellulosic rice straw into degradable components, such as fermentable sugars or more digestible starches [17, 40]. Given that more biogas generation requires the digestion of more substrates, increased TS and VS reductions could explain why the biogas production of biologically treated rice straw was greatly increased after pretreatment, as discussed above. The TS and VS mass removal efficiencies of 6% NaOH-pretreated substrate were observed to be highest—53.8 and 36.8%, respectively—on the 30th day, as were the TS and VS mass removal efficiencies of 2% HCl-pretreated rice straw substrate: 34.5% and 23.6%, respectively. Although 6% $CO(NH_2)_2$-pretreated rice straw substrate showed relatively high TS and VS removal efficiencies, methane yield was slightly lower than that for 2% $CO(NH_2)_2$-pretreated rice straw substrate. The TS and VS removal efficiencies of cellulose-pretreated rice straw substrate were also highest on the 30th day: 51.3 and 35.3%, respectively. The highest conversions of TS and VS mass into methane gas were observed for the 6% NaOH-pretreated substrate—34.3 and 23%, respectively—compared to that of untreated rice straw substrate.

Table 3 Removal efficiency of TS and VS using different pretreatment methods

Reagents	Content	TS (%)	Increase (%)	VS (%)	Increase (%)
Untreated		19.5		13.8	
NaOH	2%	41.6	22.1	27.4	13.6
	4%	45.6	26.1	31.6	17.8
	6%	53.8	34.3	36.8	23.0
	8%	44.6	25.1	30.6	16.8
HCl	2%	34.5	15.0	23.6	9.8
	4%	29.4	9.9	18.1	4.3
	6%	27.6	8.1	17.2	3.4
	8%	24.3	4.8	15.5	1.7
$CO(NH_2)_2$	2%	38.8	19.3	24.9	11.1
	4%	36.7	17.2	22.6	8.8
	6%	51.9	32.4	36.1	22.3
	8%	34.2	14.7	21.9	8.1
Cellulase	10 U/g	33.2	13.7	22.2	8.4
	20 U/g	40.8	21.3	28.8	15.0
	30 U/g	45.6	26.1	31.6	17.8
	40 U/g	51.3	31.8	35.3	21.5

Conclusions

This experimental study conducted on untreated and pretreated rice straw substrates showed that chemical and biological pretreatment could be an effective method for improving biodegradability and enhancing the efficiency of the biological conversion of rice straw into bioenergy. The NaOH and $CO(NH_2)_2$ pretreatments reduced the percentage contents of hemicellulose and lignin; the HCl pretreatment decreased the content of hemicellulose; and the cellulase pretreatment dramatically decreased the contents of cellulose and hemicellulose. The NaOH- and cellulase-pretreated rice straw substrates showed relatively high methane content. More than 40% of the methane content lasted for 20 days, for all the cellulase-pretreated rice straw substrates, during an HRT of 30 days.

The results of this methane fermentation study on chemically and biologically pretreated substrate of rice straw resulted in highly significant increases in biogas and methane production yields. The removal rates of TS and VS in all the pretreatment methods showed obvious increases compared with the untreated rice straw substrate. The results suggest that 6% NaOH-, 2% HCl-, 2% $CO(NH_2)_2$- and 30 U/g TS cellulase-pretreated rice straw were the most appropriate for producing relatively high methane yields, in this study. This study can provide a guidance for our next experiment to choose the combination of pretreatment methods for improving biogas production and reducing the costs of chemicals or enzymes.

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