Effects of Ammoniation Pretreatment at Low Moisture Content on Anaerobic Digestion Performance of Rice Straw

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The effect of ammonia pretreatment on the anaerobic digestion performance of rice straw was investigated. The rice straw was pretreated with four different moisture contents (30%, 50%, 70%, and 90%) and three concentrations of ammonia (2%, 4%, and 6%). The results showed that the anaerobic digestion achieved best performance for the rice straw pretreated by ammonia concentration of 4% and moisture content of 70%, and biogas yield achieved the highest value (396.92 $mL \cdot g^{-1}$ total solid), which was 21.9% higher than that of the untreated rice straw. Higher moisture was recommended as it could increase system stability, shorten anaerobic digestion time, and increase biogas production. The composition analyses indicated that ammonia pretreatment could effectively destroy the chemical structure of lignocellulose, and partially decompose cellulose, hemicellulose, and lignin. Cellulose and hemicellulose were converted by 79.2% and 63.2%, respectively, at optimal ammonia amount and moisture content. The ammonia pretreatment proved to be a simple and effective methods to improve the anaerobic digestion performance of rice straw.

Keywords: Ammoniation pretreatment; Anaerobic digestion; Rice straw

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INTRODUCTION

China is one of the largest agricultural countries in the world. It was reported (Wang 2007; National Bureau of Statistics of China 2013) that 220 million tons of rice straw is generated annually in China, accounting for about 30% of the total amount of agricultural residues. At present, a large proportion of straw resources in China are not effectively utilized. Most of them were burned in the open or discarded in piles, causing serious environmental pollution and waste of resources. Using the straw for anaerobic digestion not only can solve the environmental pollution problem caused by straw burning, but also provide a new source of bulk raw material for biogas promotion on a larger scale and within a wider scope.

Rice straw is mainly composed of cellulose, hemicellulose, and lignin. These three ingredients are intertwined and not readily utilized by anaerobes, greatly reducing the biogas production efficiency of the straw. To improve the anaerobic digestion efficiency of the straw, much attention has been turned to straw pretreatment to destroy the complex structure of straw (especially lignin), thereby reducing the difficulty of anaerobic bacteria in utilizing the straw (Ojia *et al.* 2007; He *et al.* 2008).

Common pretreatment methods for straw include mechanical, biological, chemical, thermal, and thermo-chemical or various combinations of these (Emiliano *et al.* 2010; Zhong *et al.* 2011a,b; Monlau *et al*. 2013).

The mechanical method is easy to perform and of short duration time; however, mechanical size reduction may not be a cost-effective process because it requires a lot of energy (Barakat *et al.* 2013). Ghosh and Bhattacharyya (1999) used a biological method incorporating *Phanerochaete chrysosporium* (Pc) and *Polyporus ostreiformis* (Po) to pretreat the rice straw and found that the total biogas production of rice straw increased by 34.7% and 21.1%, respectively; however, the strain must be maintained and it presented difficult requirements for the operator and equipment conditions, making application impractical. Chemical pretreatment includes acidification, alkalization, and ammoniation (Chen *et al.* 2010), and it has been widely applied in engineering practice due to its fast effect and short pretreatment time.

The ammoniation pretreatment method has been widely studied, mainly focusing on ethanol production, ammoniated forage, and anaerobic digestion. Soaking in aqueous ammonia was adopted for corn stover and rapeseed straw for ethanol production at room temperature and 69 ˚C (Kim and Lee 2005; Kang *et al*. 2012). Cañeque and Velasco (1998) focused on the application of ammoniated straw as animal feed, and they found that straw pretreated at 30% relative humidity and at a relatively high temperature increased the digestion rate and N content of the straw. Oji *et al.* (2007) on the composition change of corn stalk after ammoniation pretreatment showed that ammoniation pretreatment could effectively increase the nitrogen content of corn stalk and reduce its cellulose and hemicellulose contents. Ammonia pretreatment significantly improved cumulative biogas yield, biogas production rate, and volatile solid (VS) reduction of straw (Song *et al.* 2012; Li *et al*. 2014).

It is important to adjust moisture content for pretreating straw. Barakat *et al*. (2014) reported one eco-friendly dry alkaline pretreatment method (30% moisture content) for wheat straw without production of waste and liquid fractions. The physico-chemical and -biological ‘‘solid state’’ pretreatment may be used directly in a downstream process without washing, drying, and pH adjustments (Barakat *et al*. 2013). Solid-to-liquid ratios of pretreatment ranged from 1:2 to 1:15 (66.67% to 93.75% moisture content) for ethanol production.

Zhang and Zhang (1999) showed that 2% ammoniation pretreatment on rice straw for anaerobic digestion could effectively increase total methane production by 17.5% at 6:l weight ratio of tap water and the straw (85.7% moisture content). An experiment by Ma *et al.* (2011) on the anaerobic digestion of rice straw after ammoniation pretreatment (30% moisture content) showed the biogas production per gram VS after 4% ammoniation pretreatment increased by 34.8%. Thus, moisture content of pretreatment was not a consistent conclusion in lignocellulosic pretreatment.

The objectives of this work were to: (1) investigate the effect of ammonia pretreatment on the anaerobic digestion performance of rice straw at different ammonia amounts and moisture contents; (2) analyze the changes of compositions and the effects on biogas production.

EXPERIMENTAL

Materials

In this experiment, rice straw from the suburbs of Ji County, Tianjin, was air-dried and chopped with a cutter to lengths of approximately 5 mm for further use. The anaerobic sludge was provided by a biogas station in the Shunyi District, Beijing. The amount of sludge inoculant was calculated in accordance with the mixed liquor suspended solids (MLSS) quantity. The physico-chemical properties of the rice straw and anaerobic sludge are shown in Table 1.

Table 1. Basic Characteristics of Rice Straw and Anaerobic Sludge (%)

	Total solid (TS)	Total volatile solid (VS)	Total carbon	Total nitrogen	Cellulose	Hemi-cellulose	Lignin
Rice straw	94.50	86.50	42.29	0.60	34.33	26.62	7.56
Anaerobic Sludge	8.45	5.29	30.13	3.27	Not applicable	Not applicable	Not applicable

This experiment adopted batch anaerobic digestion (Fig. 1). The reaction device was composed of a 1-L anaerobic digestion bottle, a 1-L gas-collecting bottle, and a 1-L beaker. The anaerobic digestion device was placed in the shaker at a constant temperature of 35 ± 2 °C with shaking speed of 120 rpm.

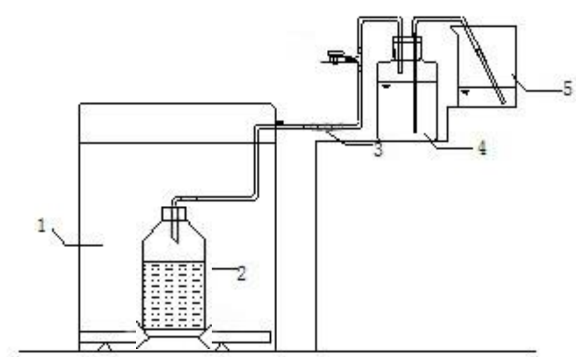

Fig. 1. Diagram of a batch anaerobic digester. (1) the shaker; (2) 1L anaerobic digestion bottle; (3) connection hose; (4) 1L gas-collecting bottle; (5) 1L beaker

Methods

The pretreatment reagent was ammonia water with a mass fraction of 25% (w/w). In the pretreatment process, the chopped rice straw was placed in a sealed bag, air was purged from the bag, and ammonia was added in the amount of 2% (w/w), 4% (w/w), and 6% (w/w) based on the dry weight of rice straw. The bag was rapidly sealed, and the contents in the bag kneaded to fully mix the straw with the ammonia. According to the results of Barakat *et al*. (2013, 2014), it is important to adjusted moisture contents for pretreatment straw. So, in this work, four water content levels of rice straw were designed (30%, 50%, 70%, and 90%). When the pH of this system became stabilized (pH$\pm$0.2), the pretreatment process was deemed complete. The pretreatment time was 7 days according to Zheng *et al.* (2009). The sealed bags with contents were stored at 30 ± 2 °C for later use.

The anaerobic sludge was added to each digester according to MLSS in the digester at 15 g·L^{-1}, based on the research from Zheng *et al.* (2009). Rice straw (52gTS) and anaerobic sludge (12gMLSS) were placed in a 1-L bottle, and tap water was added to bring the entire volume to 0.8 L. The reactor vessel was connected to a gas collection and water drainage device and shaken at 120 rpm at a constant temperature of 35 ± 2 °C for 65 d. A daily record of the volume of biogas production and methane percentage was maintained to evaluate the pretreatment. The controls for comparison were also set with the untreated rice straw. The digestion experiment for different pretreatments was repeated 3 times.

Analytical Methods

The untreated and ammonia-pretreated rice straw samples were analyzed for total solid (TS) and volatile solid (VS) according to the APHA standard methods (American Public Health Association 1998). The total carbon (TC) was analyzed with a TC analyzer (PrimacsSLC; The Netherlands). The total nitrogen (TN) was analyzed with a Kjeldah nitrogen analyzer (FOSS SCINO KT260; Shanghai, China) (EPA 1993). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents of samples were determined with an A2000I cellulose instrument (ANKOM Technology; Macedon, NY) according to the procedure of van Soest *et al.* (1991). All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Determination of Ammoniation Pretreatment Time

Pretreatment time is a key factor affecting the pretreatment (Garba 1996), as it affects the efficiency of actual production. The change in pH with different pretreatments of straw with 30%, 50% (Fig. 2), 70%, and 90% moisture content showed the same trend.

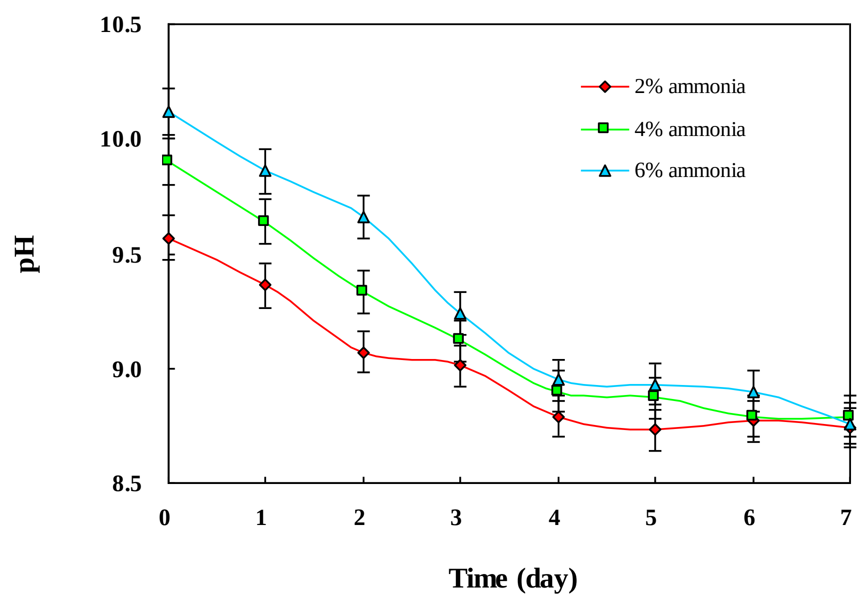

Fig. 2. Change in pH during ammoniation in straw with 50% moisture

After adding ammonia water at different concentrations, all pH values decreased for 3 d before leveling off. At the beginning of the pretreatment, the pH values ranged from 9.6 to 10.1 and generally declined with prolonged days. The pH values decreased to a range of 9.0 to 9.3 on the 3rd day, and 8.7 to 8.9 on the 4th day for different ammonia concentrations, and started to stabilize afterwards. Therefore, 5 days was determined and used for pretreatment.

Daily Methane Production

During the anaerobic digestion process, 3 to 4 peaks were found for the daily methane production under different pretreatment conditions (Fig. 3). The first peak value of ammoniation pretreatment appeared at the 4th day. The daily methane production from rice straw with 4% ammonia at 70% moisture content showed a peak on the first day at 469 mL, 123% higher than that of untreated rice straw. Later, the 2^{nd} daily methane production peak appeared between the 6th and 10th. Therein, the 2^{nd} daily methane production peak value of rice straw with 4% ammonia at 90% moisture content was the highest of 1500 mL, and the 2^{nd} daily methane production peak value was 166% higher than that of untreated rice straw. For the straw with 70% to 90% pretreatment moisture content, the 2^{nd} daily methane production peak value appeared about 20 days earlier than that of the untreated straw. So, for ammoniation pretreatment, increasing moisture content not only could make the peak value appear earlier, but also increase methane production.

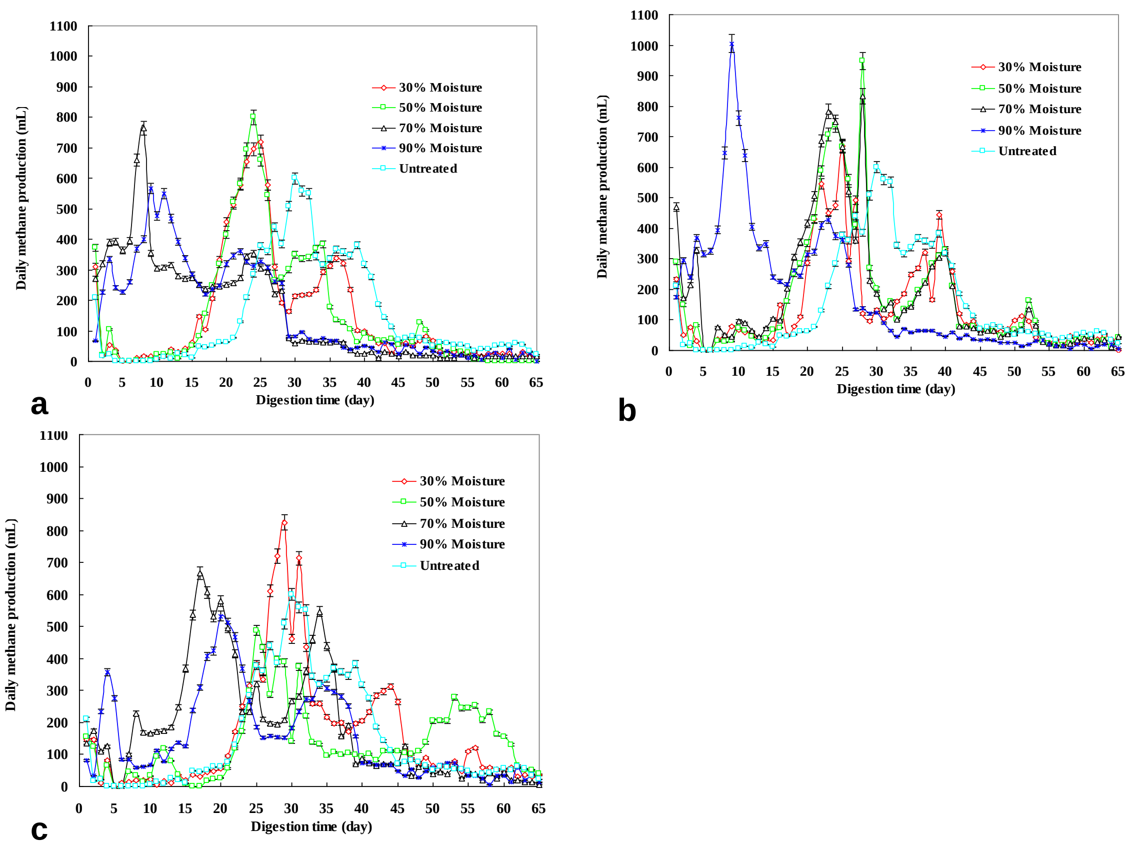

Fig. 3. Daily methane production of pretreated rice straw with (a) 2%, (b) 4%, and (c) 6% ammonia pretreatments

Total Biogas Production and Technical Digestion Time

Total biogas production was not significantly different ($p > 0.05$) for 30% moisture content at four ammonia amounts, but significantly different for 4% ammoniation pretreatment at different moisture contents ($p < 0.05$) (Fig. 4). For the moisture contents of 30%, 50%, 70%, and 90%, the total biogas production reached 18,310 mL, 20,280 mL, 20,640 mL, and 19,640 mL, which was 8.20%, 19.79%, 21.91%, and 16.00% more than that of the controls, respectively. The rice straw with moisture content of 70% obtained the highest biogas production. The rice straw pretreated with 2%, 4%, and 6% ammonia achieved total biogas production of 18,880 mL, 20,640 mL, and 19,341 mL, respectively, which was 11.52%, 21.91%, and 14.24% more than that of the controls, respectively. The biogas production was also higher than that reported by Zhang and Zhang (1999).

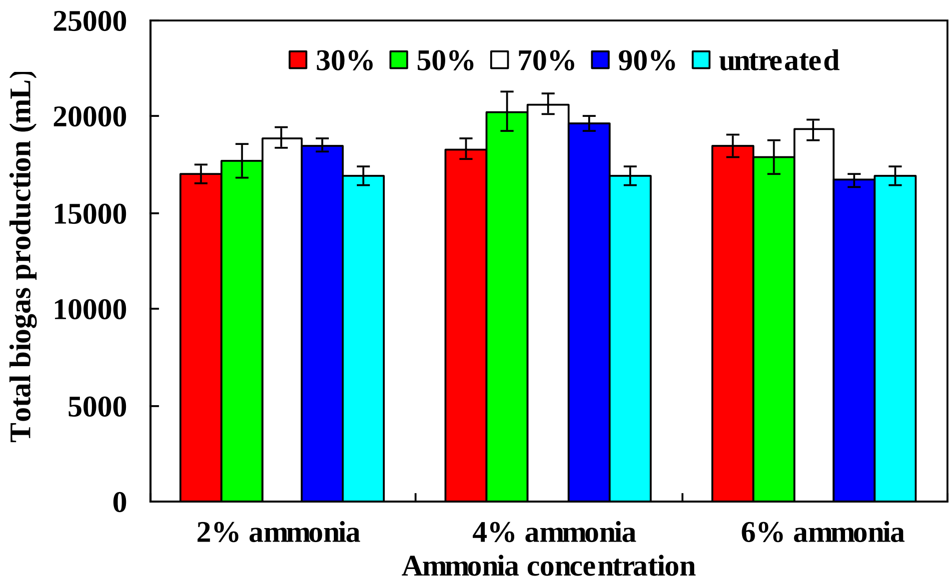

Fig. 4. Total biogas production with different pretreatments

Digestion time is another indicator of substrate biodegradability and was thus investigated in this study. Technical digestion time (T_{90}) is defined as the time needed to produce 90% of the maximal digester gas production (Zhou *et al.* 2005). Shortening T_{90} can effectively reduce production cost and improve production efficiency. Technical digestion time generally decreases with increasing moisture content. When the moisture content was 30% to 50%, T_{90} was 38 to 56 days, and when the moisture content was 70% to 90%, T_{90} was 28 to 39 days, 10 to 28 days shorter than that of the rice straw at 30% to 50% moisture content. In the case of 4% ammonia content and 70% to 90% moisture, T_{90} was the least (30 days), which was 13 days (30.23%) and 11 days (26.83%) shorter than that of rice straw at 30% and 50% moisture content, respectively, and 16 days (34.78%) less than that of the controls. T_{90} was shortened by 12 to 28 days compared with Ma *et al.* (2011). This may be because higher moisture content increases the contact probability between ammonia and straw cells. Such comprehensive pretreatment not only provides an effective N source but also neutralizes the organic acid produced at the earlier acidification stage of anaerobic digestion, alleviating the inhibiting effect of excessive ammonia on anaerobic digestion (Mason *et al.* 1988).

Biogas Yield per TS

Biogas yield based on TS is another important parameter for measuring the biodegradable property of straw in the anaerobic digestion process, and it can adequately represent the effectiveness of straw pretreatment. Under different ammoniation pretreatment conditions, biogas yield based on TS increased with the increased moisture content in the pretreatment (Table 2). When the moisture content was 70%, biogas yield based on of TS was 363.08 mL to 396.92 mL. The biogas yield based on TS reached the maximum value of 396.92 mL with 4% ammonia at 70% moisture content, 21.91% more than that from the untreated straw. This showed that increasing moisture can increase the biodegradable ingredients of straw and increase biogas production, effectively increasing the use ratio of straw.

Table 2. Comparison of Biogas Yield with Different Pretreated Rice Straw

Ammonia content (%)	Moisture content (%)	T_{90} (d)	Biogas yield of per gram of TS (mL)
Untreated	Not applicable	46 ± 3	325.58
2	30	39 ± 2	327.69
	50	38 ± 3	340.96
	70	28 ± 3	363.08
	90	31 ± 2	356.15
4	30	43 ± 4	352.12
	50	41 ± 2	390.00
	70	30 ± 3	396.92
	90	30 ± 3	377.69
6	30	48 ± 3	355.19
	50	56 ± 5	343.85
	70	39 ± 4	371.94
	90	39 ± 3	321.15

System Stability

The factors affecting the stability of the anaerobic digestion system include pH, ammonia nitrogen, and alkalinity. Figure 5 shows that after anaerobic digestion, the pH of the system remained between 7.2 and 7.5, which was within the suitable pH range of 6.5 to 7.8 for the growth of methanogens (Ren and Wang 2004). Lay *et al.* (1997) found that NH_4^+ concentration was the key factor affecting the activity of methanogens. When the pH value ranged from 6.5 to 8.5, the activity of methanogens decreased with increasing ammonia nitrogen concentration. Therefore, ammonia nitrogen and alkalinity of the anaerobic sludge were important parameters to measure system stability. Figure 5 shows that anaerobic sludge of the untreated rice straw had the lowest ammonia nitrogen, which was 603.4 $mg{\cdot}L^{-1}$. For the sludge with different ammonia concentrations (2%, 4%, and 6%), it had the highest ammonia nitrogen in the case of 90% moisture, which was 1188 $mg{\cdot}L^{-1}$ to 2565 $mg{\cdot}L^{-1}$, and it was the second ammonia nitrogen in the case of 70% moisture. Ammonia content (≤1500 $mg{\cdot}L^{-1}$) in biogas sludge increased the anaerobic digestion efficiency (Ren and Wang 2004), but when the ammonia content reached 2000 $mg{\cdot}L^{-1}$, the ammoniacal nitrogen obviously suppressed the activity of methanogens (Koster and Lettinga 1988). The ammonia nitrogen concentration was 1249.9 $mg{\cdot}L^{-1}$ to 1352.4 $mg{\cdot}L^{-1}$ with 2% to 6% ammonia content at 70% moisture content, which did not exceed the inhibition range of anaerobic digestion. For 6% ammonia content at 90%

moisture, the ammonia nitrogen concentration was 2565 mg·L^{-1}, which was already in the moderate inhibition range of anaerobic digestion (Ren and Wang 2004). This precisely explains why its biogas production based on TS was the lowest. The alkalinity of straw increased with increasing moisture in the pretreatment, and alkalinity increased accordingly while ammonia nitrogen increased. Figure 5 shows that the alkalinity of different ammonia-pretreated rice straw anaerobic sludge ranged from 5970 mg·L^{-1} to 11595 mg·L^{-1}; at 90% moisture, anaerobic sludge had higher alkalinity, and the highest value of 11595 mg·L^{-1} shows a consistent trend with ammonia nitrogen.

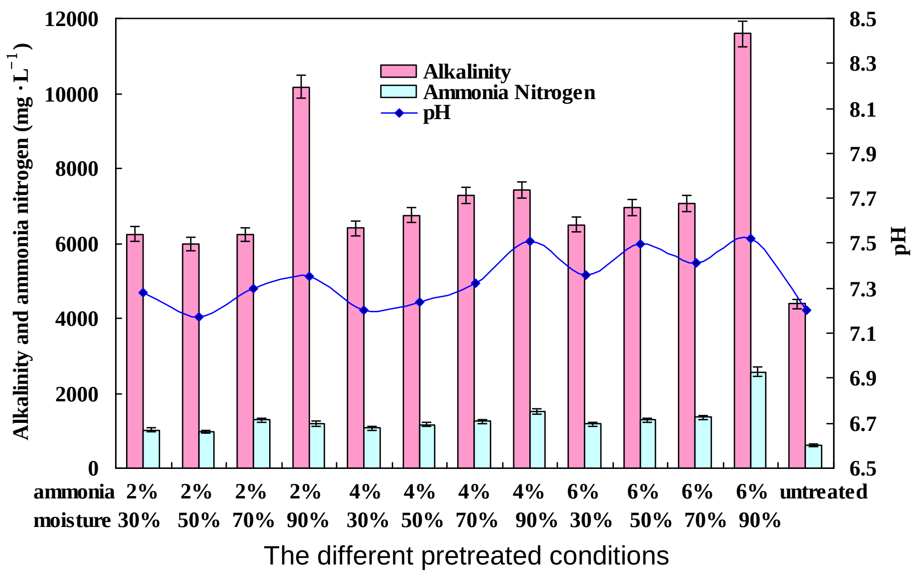

Fig. 5. Changes in pH, ammonia nitrogen, and alkalinity of different ammonia-pretreated anaerobic sludge after anaerobic digestion

Changes in Compositions

Cellulose and hemicellulose are the main components of rice straw, and also the major carbon source for anaerobic bacteria growth. Lignin, cellulose, and hemicellulose contents before and after straw ammoniation pretreatment and after anaerobic digestion were analyzed, and the changes were examined (Table 3). The results showed that cellulose and hemicellulose contents of untreated straw were 34.31% and 26.61%, respectively, and the lignin content was relatively small (7.62%). After ammoniation pretreatment, the hemicellulose content in straw was less, from the original 26.61% (untreated straw) to 21.16%, a decrease of 20.48%. Compared to the untreated straw, the lignin content was also reduced to some extent, indicating that lignin was removed partially after ammoniation pretreatment. This could cause more cellulose and hemicellulose to be released and increase the microbial accessibility. The lignocellulose data after anaerobic digestion showed that, under different ammoniation pretreatment conditions, the contents of cellulose, hemicellulose, and lignin were reduced to varying degrees, from 30.96%, 24.99%, and 7.29% after pretreatment to 11.60%, 17.51%, and 5.05% after anaerobic digestion, respectively, down 26.87%, 29.93%, and 30.73%, respectively. When the ammonia content was 4% and the moisture content was 70%, the conversion ratios for cellulose and hemicellulose were 79.16% and 63.18%, respectively;

therein, compared to the conversion ratio under other moisture conditions, the hemicellulose conversion ratio had the largest degree of increase, at 17.57% and 24.84%, respectively, more than that at 30% and 50% moisture contents.

Table 3. Changes in Compositions after Ammonia Pretreatment and After Anaerobic Digestion (%)*

Pretreatment condition		After ammonia-pretreatment			After anaerobic digestion		
ammonia content	moisture content	Cellulose	Hemi-cellulose	Lignin	Cellulose	Hemi-cellulose	Lignin
2	30	32.68 ± 0.41	26.10 ± 0.50	7.24 ± 0.20	15.82 ± 0.92	23.34 ± 1.80	6.48 ± 0.17
	50	31.17 ± 3.62	26.61 ± 0.03	7.09 ± 0.04	14.97 ± 0.02	20.83 ± 2.94	6.32 ± 0.10
	70	30.35 ± 0.74	26.27 ± 0.01	5.97 ± 0.66	13.97 ± 0.26	21.11 ± 1.63	5.17 ± 0.05
	90	29.41 ± 0.18	24.99 ± 0.30	4.65 ± 0.05	12.39 ± 0.33	17.51 ± 1.02	4.57 ± 0.24
4	30	34.10 ± 0.42	25.70 ± 0.03	7.29 ± 1.08	16.93 ± 1.27	24.04 ± 1.26	5.05 ± 0.10
	50	32.34 ± 5.65	24.67 ± 0.81	5.20 ± 0.13	13.99 ± 1.04	18.80 ± 1.14	4.63 ± 0.10
	70	30.90 ± 0.61	24.17 ± 0.11	4.49 ± 0.54	13.93 ± 0.68	18.12 ± 1.27	3.76 ± 0.55
	90	30.96 ± 1.86	25.27 ± 0.14	4.96 ± 0.13	11.60 ± 1.36	16.95 ± 0.28	4.10 ± 1.11
6	30	30.30 ± 3.38	25.11 ± 0.17	6.70 ± 0.07	12.65 ± 0.26	19.23 ± 3.08	5.72 ± 1.36
	50	30.53 ± 1.67	22.37 ± 0.13	6.00 ± 0.15	11.28 ± 0.41	17.31 ± 1.75	5.06 ± 0.91
	70	30.61 ± 0.01	21.65 ± 0.16	5.42 ± 1.22	10.35 ± 0.50	16.22 ± 3.00	4.52 ± 1.13
	90	28.28 ± 2.82	21.16 ± 0.02	5.33 ± 0.16	10.12 ± 1.05	16.33 ± 0.70	4.65 ± 0.61
Untreated		34.31 ± 1.59	26.61 ± 0.53	7.62 ± 1.17	14.11 ± 0.12	24.52 ± 1.47	6.59 ± 1.69

* Values are means ± SD (n ≥ 3)

Water-soluble extractives were mainly generated from the decomposition of lignocellulose. For the pretreated rice straw, the contents of cold-water and hot-water extractives reached 20.32% to 25.31% and 26.71% to 30.91%, respectively. Both extractives increased with the increase of ammonia concentration and moisture content. The rice straw pretreated by 4% ammonia and 70% moisture content achieved the highest cold-water extractives and hot-water extractives contents of 24.6% and 15.7%, respectively, which was higher than those of the untreated rice straw (Table4).

Table 4. Changes of Extractives Contents of Differently Treated Rice Straw (%,DM) *

Extractives	Untreated	2%NH_3、30%H_2O	4%NH_3、70%H_2O
Cold-water,%	20.32±1.52	21.16±0.78	25.31±0.50
Hot-water,%	26.71±0.97	27.37±1.04	30.91±0.88

* Values are the means ± SD(n=3)

CONCLUSIONS

1. Under different ammoniation pretreatment conditions, the rice straw pretreated by 4% ammonia and 70% moisture content achieved the highest biogas production of 396.92 mL·g^{-1} TS, which was 21.91% higher than that of the untreated one.
2. Ammoniation pretreatment could effectively destroy the lignocellulose structure of straw and decompose lignocellulose composition. The contents of cellulose, hemicellulose, and lignin were reduced from 30.96%, 24.99%, and 7.29% after pretreatment to 11.60%, 17.51%, and 5.05% after anaerobic digestion, respectively. When the ammonia content was 4% and the moisture content was 70%, the conversion ratios of cellulose and hemicellulose reached 79.16% and 63.18%, respectively.
3. Ammoniation pretreatment proved to be effective method for improving rice straw biodegradation efficiency and increasing biogas production.

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