COMPARISON OF TWO CHEMICAL PRETREATMENTS OF RICE STRAW FOR BIOGAS PRODUCTION BY ANAEROBIC DIGESTION

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Lignocellulosic biomass is considered the most abundant renewable resource that has the potential to contribute remarkably in the supply of biofuel. Previous studies have shown that chemical pretreatment prior to anaerobic digestion (AD) can increase the digestibility of lignocellulosic biomass and methane yield. In the present study, the effect of rice straw pretreatment using ammonium hydroxide ($NH_3•H_2O$) and hydrogen peroxide (H_2O_2) on the biogasification performance through AD was investigated. A self-designed, laboratory-scale, and continuous anaerobic biogas digester was used for the evaluation. Results showed that the contents of the rice straw, *i.e.* the lignin, cellulose, and hemicellulose were degraded significantly after the $NH_3•H_2O$ and H_2O_2 treatments, and that biogas production from all pretreated rice straw increased. In addition, the optimal treatments for biogas production were the 4% and 3% H_2O_2 treatments (w/w), which yielded 327.5 and 319.7 mL/gVS, biogas, respectively, higher than the untreated sample. Biogas production from H_2O_2 pretreated rice straw was more favorable than rice straw pretreated with same concentration of ammonia, ranking in the order of 4% $\approx$ 3% > 2% > 1%. The optimal amount of H_2O_2 treatment for rice straw biogas digestion is 3% when economics and biogas yields are considered.

Keywords: Biogas production; $NH_3•H_2O$, H_2O_2; Pretreatment; Rice straw

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INTRODUCTION

Increasing energy demand and concerns related to non-renewable energy resources have led researchers to investigate alternative energy sources during the last two decades (Isci and Demirer 2007). The currently utilized renewable energy resources, including solar, wind, hydro, wave, and biomass account for approximately 14% of the primary energy consumption in the world. Biomass is a major contributor to renewable energy (representing approximately 10% of the total energy) (Antizar-Ladislao and Turrion-Gomez 2008). This resource is considered a global valuable energy alternative to fossil fuels because it can be converted to various available forms of energy, such as heat, steam, electricity, hydrogen, biogas, and liquid transportation biofuels (Converti *et al*. 2009; Zhong *et al*. 2011). China, one of the largest agricultural countries in the world, has abundant biomass resources including crop straw, firewood, agricultural residues, and organic wastes (Global Status Report 2007; Tu *et al*. 2011). It has been estimated that the total output of crop straw in China is 710 million tons per year, with rice straws comprising 203 million ton (National Bureau of Statistics of China 2009). Due to underdeveloped conversion technologies, rice straw has not yet become a practical option for energy production. Instead, quite a large amount of rice stalk is burnt or dumped into the field currently, causing serious problems such as air pollution, fire disaster, degraded soil conditions, and adverse impacts on air craft and traffic safety. Therefore, developing a cost-effective technology for rice straw utilization is imperative.

Anaerobic digestion (AD) of crop straw for energy production has emerged as a widely used technology because it provides both an option for utilizing waste and reducing greenhouse gas emissions by being a substitution for fossil fuels (Murphy and Power 2009). The AD process generally consists of four stages, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Biological hydrolysis is identified as the rate-limiting step because of the recalcitrant structure and composition of lignocellulosic biomass, such as lignin, which interlinks cellulose and hemicellulose layers (Noike *et al*. 1985; Wang *et al*. 1999; Tiehm *et al*. 2001). Pretreatments are often performed to facilitate the access of hydrolytic enzyme to degradable carbohydrates, including solubilization and biodegradation of the hemicellulosic and lignin parts of the substrates, to improve the AD conversion efficiency.

The application of several pretreatment methods, such as steam, chemical, and biological treatments, improves AD efficiency (Penaud *et al*. 1999; Frigon *et al*. 2011; Liew *et al*. 2011). Chemical pretreatment is considered to be a cost-effective method for enhancing the biodegradation of complex materials compared with steam and biological treatments (Lin *et al.* 2009). The methods of chemical pretreatment mainly include bicarbonate treatment (Liu *et al.* 1995), radiation (Xin and Kumakura 1993), alkaline peroxide treatment (Zhang and Cai. 2008; Zhu *et al.* 2010), and ammonia treatment (Zhong *et al.* 2011; Kim and Lee. 2005). Among them, ammonia treatment is one of the most effective methods for enhancing the biodegradation of complex materials. Kang *et al*. (2012) reported that the most optimal condition for ethanol production of rapeseed straw by immersion in aqueous ammonia was 19.8% ammonia water, 14.2 h pretreatment time, and a pretreatment temperature of 69.0°C. Alizadeh *et al*. (2005) found a sixfold increase in enzymatic hydrolysis yield and a 2.5-fold increase in ethanol yield on ammonia-treated switchgrass compared with that of the untreated sample. Zhong *et al*. (2011) showed that the biogas production of corn straw pretreated by ammonia was 120.5% and 196.6% higher compared with samples pretreated by urea and untreated samples, respectively.

Hydrogen peroxide (H_2O_2) is a well-known reagent used as bleaching agent in the paper and cellulose industry. Several researchers recently reported that H_2O_2 could be used as a pretreatment agent for AD because of its strong oxidizability (Dewil *et al*. 2007; Azabou *et al*. 2010; Teghammar *et al*. 2010; Dhar *et al*. 2011; Shahriari *et al*. 2012). However, these studies mainly focused on the pretreatment of waste sludge for AD. There has been a lack of studies focusing on the AD of agricultural straw. The objective of the present study is to compare the effect of $NH_3{\bullet}H_2O$ and H_2O_2 pretreatments on rice straw for the enhancement of biogas production. In addition, research on developing a better technique to improve the treatment efficiency of rice straw and increase biogas production is currently being conducted.

MATERIALS AND METHODS

Material Collection

The rice straw was obtained from a rice field near the Northwest A&F University (Yangling, Shaanxi, China). The rice straw was cut to a length of 20 to 30 mm using a grinder. The rice straw contained 95.8% dry matter. The inoculum was obtained from the 8 m^3 hydraulic biogas digester in a model village powered by household biogas (Yangling, Shaanxi, China.). The total solid (TS) content of the slurry was 4.9%.

Experimental Setup

Two chemicals, $NH_3 \cdot H_2O$ and H_2O_2, were used in this study. The concentrations of $NH_3 \cdot H_2O$ added were chosen based on previous research in the literature (Ma *et al*. 2011). In order to evaluate the effect of these two chemicals on biogasification, the same concentrations were used for H_2O_2. Certain amounts of $NH_3 \cdot H_2O$ and H_2O_2 were dissolved in distilled water to obtain concentrations of 1%, 2%, 3%, and 4% (w/w). The prepared $NH_3 \cdot H_2O$ and H_2O_2 solutions were added to eight beakers containing 500 g rice, and the ratio of solid to liquid was 1:3, respectively. Finally, all prepared beakers were covered with plastic films, secured with a plastic ring, and then stored in a chamber at ambient temperature (25±2) °C for 7 days. After pretreatment, the rice straw was directly used for anaerobic digestion (AD) without any treatment.

A laboratory-scale simulated experiment using a self-designed, constant-temperature anaerobic fermentation equipment was then conducted (Bai *et al*. 2009). The equipment consisted of an anaerobic digester (1 L Erlenmeyer flask), a biogas collector (1 L Erlenmeyer flask), and a 1 L measuring cylinder that measured the water discharged from the collector (Fig. 1).

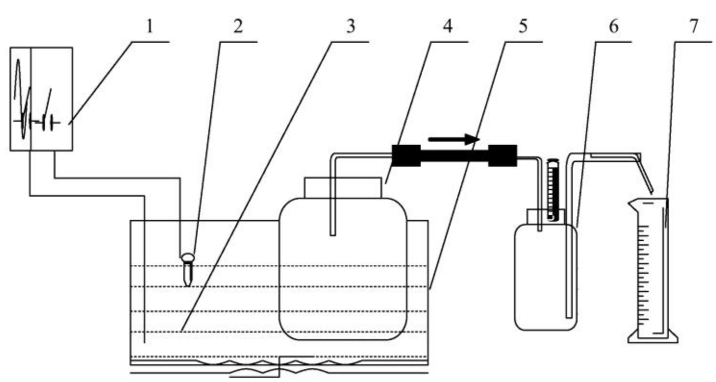

Fig. 1. Controllable and constant-temperature anaerobic fermentation device:
1. Relay; 2. Temperature controller; 3. Heater; 4. Anaerobic digester; 5. Trough at constant temperature; 6. Receiver; 7. Measuring cylinder

The digesters were placed in a constant-temperature trough (water-heated, 1°C fluctuation) using a temperature controller that relayed the display. The biogas generated in the digester was transported into the headspace of the bottle using a glass pipe. The water in the bottle was then pressed out and overflowed into the receiver through another glass pipe. The volume of the discharged water from the bottle represented the volume of the biogas generated in the digester.

Each pretreated rice straw was used as the digestion material, with the untreated rice straw as the control. Each treatment was performed in triplicate. The volume of each digester was 1 L, with 0.75 L working volume, and the fermentation process lasted for 30 days. The digesting material (500 g) and inocula (200 g) were added to each digester, and deionized water was added as necessary to obtain 8% TS content (Song *et al*. 2010). Moreover, the anaerobic fermentation was set at 37°C to eliminate the effect of the inocula, and 200 g of the inocula were digested and served as the blank. At the end of the experiment, the pretreated rice straw was dried in an electronic oven (HengFeng SFG-02.600, Huangshi, China) at 80°C for 48 h and then kept in a refrigerator for composition determination and AD experiments to investigate the effect of different chemical treatments on biogas production.

Analysis and Calculations

The daily biogas production for each anaerobic digester was recorded using the water displacement method. The corresponding cumulative biogas volume was also calculated. The TS, volatile solids (VS), and pH of the materials were determined using the standard water and wastewater examination methods (American Public Health Association 1995). Total carbon (TC) and nitrogen content (TN) were analyzed using the Walkley-Black (Nelson and Sommers 1982) and Kjeldahl (Bremner and Mulvaney 1982) methods, respectively. The volatile fatty acid (VFA) was analyzed through colorimetric method (Chinese Academy of Sciences, 1984), and the result was expressed in acetic acid. The cellulose, hemicellulose, and lignin contents were analyzed based on the method used by Wang and Xu (1987) as well as by Lin *et al*. (2009).

RESULTS AND DISCUSSION

Effect of the Pretreatments on the Main Compositions of Rice Straw

Table 1 shows the contents of hemicelluloses, cellulose, and lignin in the rice straw pretreated with $NH_3 \cdot H_2O$ and H_2O_2. The cellulose and hemicellulose contents in the ground rice straw decreased remarkably for all the chemical treatments, compared with the control. The 4% $NH_3 \cdot H_2O$ and 4% H_2O_2 treatments exhibited the highest reduction in lignin, cellulose, and hemicelluloses. TC contents behaved similarly to the cellulose and hemicelluloses, which decreased with increasing H_2O_2 and $NH_3 \cdot H_2O$ concentrations, in contrast to the behavior of the TN contents. These results were consistent with previous studies in which 30% to 60% of lignin, cellulose, and hemicellulose was decomposed in the anaerobic biogasification of alkaline-treated material (Luo *et al*. 2005; Lin *et al*. 2009; Zhu *et al*. 2010; Zhong *et al*. 2011). This result indicated that a higher pretreatment concentration is more effective in breaking down the lignocellulose matrix and changing chemical components. The higher $NH_3 \cdot H_2O$ and H_2O_2 greatly changed the microstructure of the cell wall and increased the accessibility of contents to the anaerobic microorganisms, facilitating usage of soluble compounds with low molecular weights by the microorganisms and thus increasing the biodegradability. The decrease in TC is probably due to the use of carbon source by hydrolytic microorganisms. Moreover, our study found an increased TN content compared with the control; this was in agreement with the results from Zhong *et al*. (2011) and Luo *et al*. (2005), wherein the TN content in the NaOH pretreated corn straw was 20% and 74% lower than that in the untreated one. The reasons to account for these findings still require further investigation.

Table 1. Main Composition of Rice Straw after Chemical Pretreatment

		$NH_3 \cdot H_2O$				H_2O_2			
Composition	Control	1%	2%	3%	4%	1%	2%	3%	4%
TN	0.49	0.74	0.98	1.17	1.39	0.53	0.68	0.76	0.84
TC	33.96	32.31	30.98	30.01	29.36	30.74	30.31	29.28	28.11
Cellulose	45.36	43.91	41.73	38.92	36.22	42.72	39.47	37.16	35.41
Hemicellulose	28.14	26.64	25.21	21.21	19.18	23.77	19.54	13.19	11.19
Lignin	6.78	6.72	6.57	6.51	6.47	6.69	6.47	6.39	5.96

TN: Total nitrogen; TC: Total carbon

Effect of the Pretreatments on Daily Biogas Production

The daily biogas production of the pretreated rice stalk is shown in Fig. 2. Similar trends of daily biogas production were found for all treatments. Biogas was generated after seeding, and it kept increasing until the peak value was reached. Biogas production experienced several small peaks before production finally ceased. However, the peak value of the biogas production and the time to reach the peak value were different for each treatment. The biogas production for the $NH_3 \cdot H_2O$ pretreatment reached its peak value (500 mL) on day 17 at 1%, whereas peak values of 548 (day 9), 700 (day 7), and 735 mL (day 5) were obtained for the 2%, 3%, and 4%, respectively. The days at which these peaks were reached were earlier than that of the control at 358 mL on day 18. A similar trend was also observed for the H_2O_2 pretreatment, which yielded peak values of 527 (day 12), 630 (day 13), 738 (day 6), and 803 mL (day 5) for the 1%, 2%, 3%, and 4% concentrations, respectively. The $NH_3 \cdot H_2O$- and H_2O_2-treated rice stalks took approximately 5 to 17 days and 5 to 12 days, respectively, to reach the peak value, compared with the 18 days required for the control (Fig. 2), which was 1 to 13 days later than that of the pretreatments. The result of this study implies that the two chemical pretreatments improved the biodigestibility of rice stalk, facilitating its use by anaerobic microorganisms, resulting in less time required for digestion. Furthermore, the peak value of the pretreatments was increased as the concentrations of the two treatments increased, indicating that a higher concentration of chemical pretreatment can provide more organic matter to anaerobic microorganism for biogas generation.

Meanwhile, the daily biogas production fluctuated considerably for all treatments, as indicated by the appearance of several small peaks. This phenomenon may be related to the dynamic balance between the metabolism of acidogen and methanogen in the AD (Qin *et al*. 2011). The highly concentrated substrate at the initial phase of AD supplied sufficient organic acid for methanogen, which hastened the growth of methanogen and resulted in an increased biogas yield. The shortage of organic acid as it gradually decreased in the substrate limited the activity of methanogen, but it stimulated acidogen, causing a drop in biogas yield. The activity of methanogen increased again when organic acid accumulated to an extent, resulting in the appearance of a peak in the biogas production. However, the peak value in the biogas production decreased because the concentration of the substrate was not as high as the initial concentration.

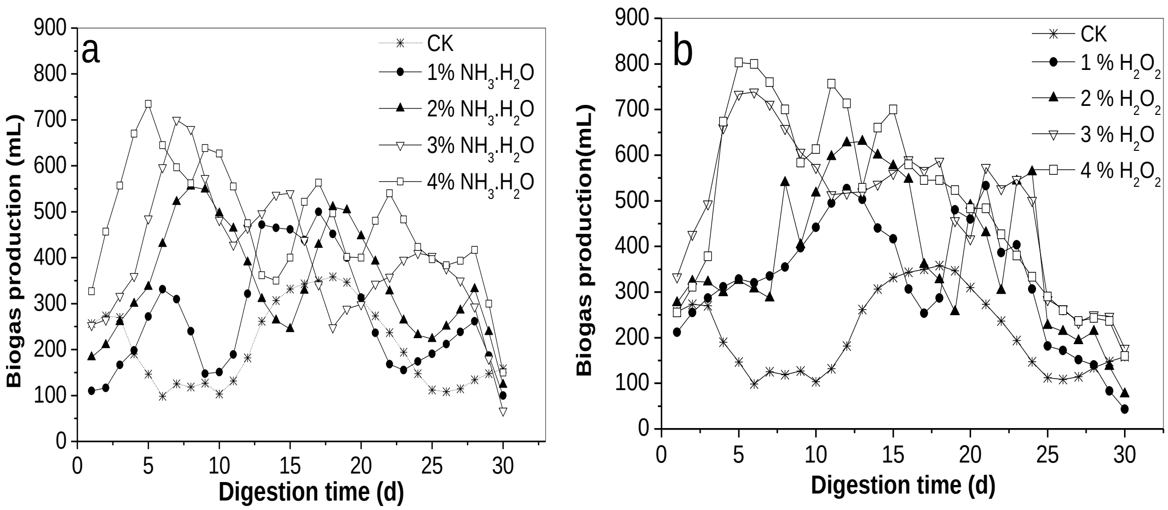

Fig. 2. Daily biogas production for each pretreatment. (a) $NH_3{\cdot}H_2O$ and (b) H_2O_2 pretreatments
CK: Rice straw without pretreatment

Effect of Pretreatments on Cumulative Biogas Production

The final cumulative biogas productions were 8291, 10395, 11970, and 14305 mL for 1%, 2%, 3%, and 4% $NH_3{\cdot}H_2O$, respectively, illustrating an increase of 31.0%, 64.2%, 89.1%, and 126.0% over the untreated ones (Fig. 3). The same trend was also observed for the H_2O_2 pretreatment (Fig. 3b), indicating that $NH_3{\cdot}H_2O$ and H_2O_2 pretreatments can significantly improve the biodegradability of rice stalk and increase biogas yield. This phenomenon was due to the fact that alkaline and acid pretreatments increase organic solubilization and the surface area available for enzymatic action (*Lin et al*. 2009). The rice straw pretreated with 4% $NH_3{\cdot}H_2O$ resulted in higher cumulative biogas production than the other $NH_3{\cdot}H_2O$ concentration, in agreement with the result from Ma *et al*. (2011), who reported that the cumulative biogas production of 4% $NH_3{\cdot}H_2O$ treatment was 19.6% higher than that of the 2% $NH_3{\cdot}H_2O$ treatment. However, this phenomenon was not observed for the H_2O_2 pretreatment. Only a slight difference of 2.3% was observed in the biogas yields of 3% and 4% H_2O_2. More hydroxyl ion in 4% hydrogen peroxide pretreatment could produce toxicity to methanogens, thus inhibiting the activity of the microorganisms and interfering with their metabolism (Chen *et al*. 2008). The biogas yield of each concentration during the initial 5 days in both pretreatments presented no significant difference (Fig. 3), whereas the biogas yield of pretreatment with a higher concentration was higher than that of the pretreatment with a lower concentration. This difference was caused by the instability of the digester at the beginning of AD, which was the environmental adaptation stage for methane bacteria. However, more biogas yield was attained with higher concentrated solution when the system was stable because of the greater ability to decompose the substance (Lin *et al*. 2009).

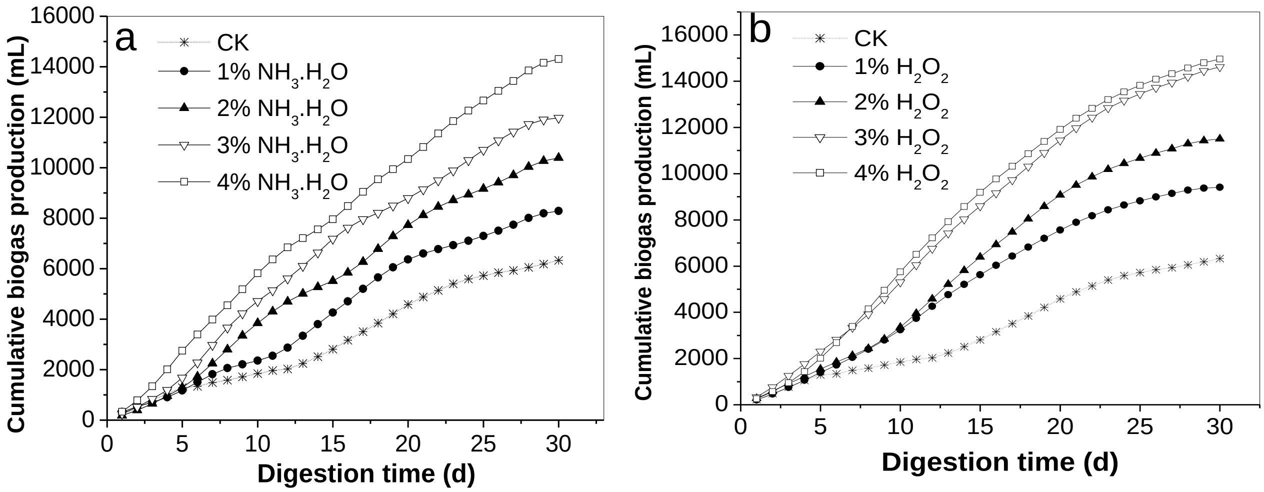

Fig. 3. Cumulative biogas production of each pretreatment. (a) $NH_3{\cdot}H_2O$ and (b) H_2O_2 pretreatments. CK: Rice straw without pretreatment

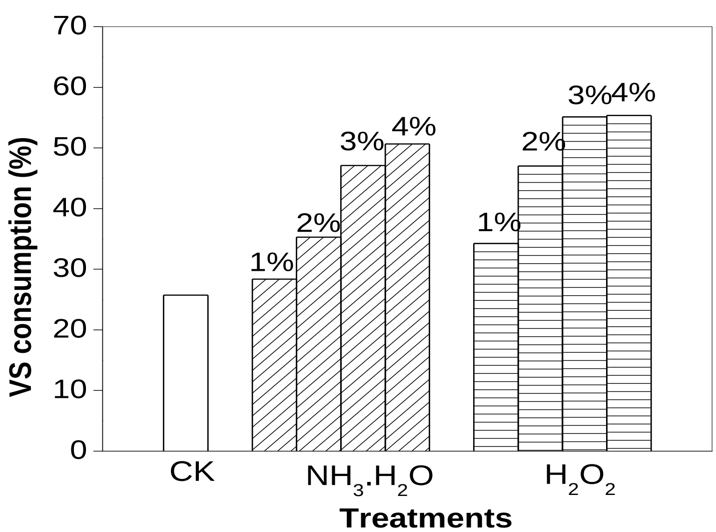

Fig. 4. Volatile solids (VS) consumption of each pretreatment. CK: Rice straw without pretreatment

Effect of Pretreatments on volatile solids (VS) Consumption

VS consumption in the $NH_3{\cdot}H_2O$ and H_2O_2 pretreatments was significantly greater than that of the control, probably because of the progressive hydrolysis of the complex organic matter present in the feeding (Fig. 4). This result was consistent with the finding of Ma *et al*. (2011), which indicates that the VS content of the rice straw pretreated with $NH_3{\cdot}H_2O$ decreased by 28.3% compared with the untreated sample. The highest amount of VS consumed in the $NH_3{\cdot}H_2O$ pretreatment was at 4%, followed by 3%, 2%, and 1%. A similar trend was also observed for the H_2O_2 pretreatment. However, no significant difference was observed between 3% and 4%. The main contents of VS are volatilizable inorganic salts and organic matter, which is the substrate of AD. Thus, VS consumption reflects the utilization of the material (Rowena *et al*. 2011; Chu *et al*. 2011). Rafique *et al*. (2010) showed that VS consumption corresponded directly to the biogas production for the chemical treatment in AD. The current study found similar results in that 4% $NH_3{\cdot}H_2O$ and 4% H_2O_2 exhibited the highest biogas production (Fig. 3), indicating that $NH_3{\cdot}H_2O$ and H_2O_2 pretreatments can improve digestibility and increase the biogas yield of rice stalk significantly.

Effect of Pretreatments on pH Value and Total VFA

The fermentative microorganisms can function in a wider range of pH values between 4.0 and 8.5 (Hwang *et al*. 2004). In the present study, the initial pH values of the $NH_3{\cdot}H_2O$ pretreatment were all above 7, whereas those in the H_2O_2 pretreatment were less than 7 (Fig. 5) because the original materials added to the bioreactor were alkaline and acid. The pH curves of the eight pretreatments were similar, showing a decreasing trend on the initial 10 to 12 days and increasing thereafter. This phenomenon was related to the variation in VFA concentration. VFA produced during AD reduced the pH level, in accordance with the result from Lin *et al*. (2009) who conducted a laboratory experiment on biogas production enhancement using NaOH pretreatment in AD. During the initial digestion phase, with the accumulation in VFA, the pH dropped and continued to do so until it reached its lowest value on the days 10 to 12; and then with VFA consumption, pH values increased steadily to over 7.0.

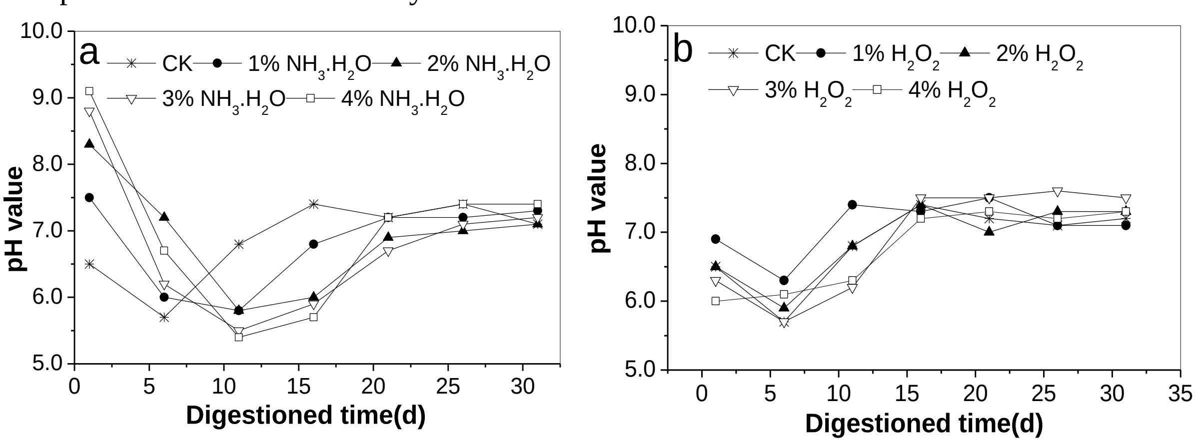

Fig. 5. Variations in pH value for each pretreatment. (a) $NH_3{\cdot}H_2O$ and (b) H_2O_2 pretreatments. CK: Rice straw without pretreatment

The VFA concentration of each pretreatment initially increased and subsequently decreased. This is contrary to the trend of the pH curve in Fig. 6. The VFA concentration varied in the range of 3000 mg/L to 14000 mg/L during AD. The daily biogas production increased when VFA concentration started to decrease. The peak value of the VFA concentration for each pretreatment was almost attained on days 6 to 8, in accordance with the greatest daily biogas production that occurred on days 5 to 10 (Fig. 2). The highest performance of VFA was observed in 1% $NH_3{\cdot}H_2O$ and 1% H_2O_2, and the lowest VFA concentration were observed in the control. The average VFA concentrations of the pretreatments during the AD period were (in mg/L) 7678 (1% $NH_3{\cdot}H_2O$), 10479 (2% $NH_3{\cdot}H_2O$), 8064 (3% $NH_3{\cdot}H_2O$), 4509 (4% $NH_3{\cdot}H_2O$), 7193 (1% H_2O_2), and 6331 (2% H_2O_2), in addition to 6249 (3% H_2O_2) and 6667 (4% H_2O_2). The average VFA concentration in 4% H_2O_2 was 4.9% higher than that in the 3% H_2O_2. However, no significant difference was observed in their biogas production (Fig. 3), because the excessively high concentration of H_2O_2 inhibited acetogenesis in the 4% H_2O_2.

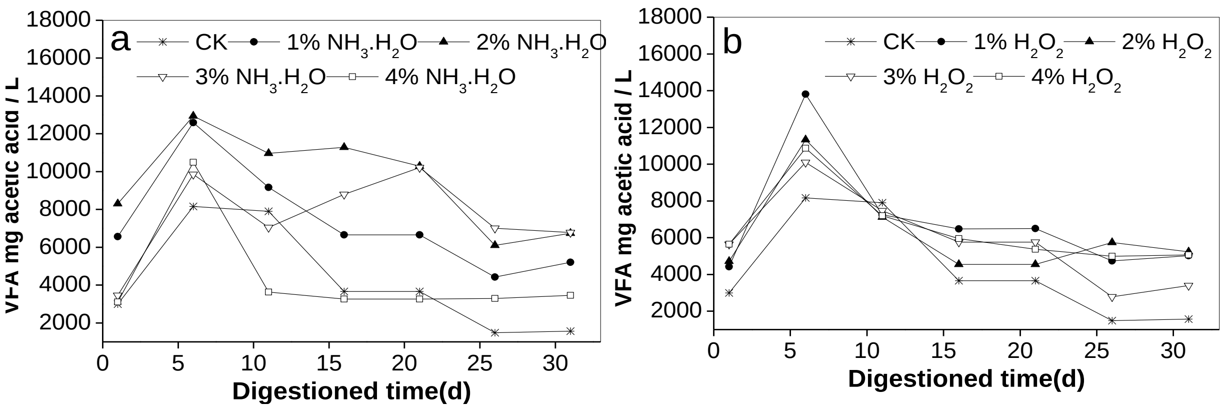

Fig. 6. VFA changes for each pretreatment. (a) $NH_3{\cdot}H_2O$ and (b) H_2O_2 pretreatment.
CK: Rice straw without pretreatment

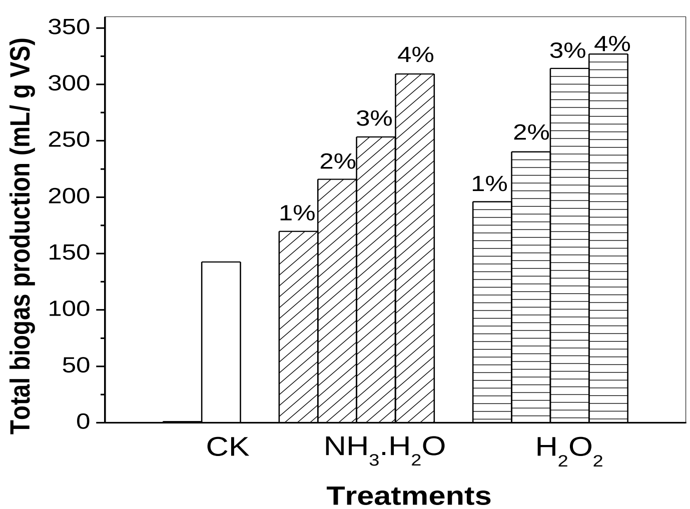

Fig. 7. Total biogas production for each pretreatment (mL/gVS).
CK: Rice straw without pretreatment

Comparison of $NH_3 \cdot H_2O$ and H_2O_2 Pretreatments on Biogas Production

The cumulative biogas productions obtained from treated rice straws were considered to compare the effects of $NH_3 \cdot H_2O$ and H_2O_2 pretreatments on AD. Among all the treatments, the rice straws treated with 4% and 3% H_2O_2 yielded 327.5 and 319.7mL/gVS, respectively, which were higher than that of the pretreatment with the same concentration of $NH_3 \cdot H_2O$ (Fig. 7). The 4% and 3% H_2O_2 also had higher efficiencies in decomposing hemicelluloses, cellulose, and lignin than that of the 4% and 3% $NH_3 \cdot H_2O$ (Table 1). Compared to the $NH_3 \cdot H_2O$, H_2O_2 pretreatment is easier to operate and manage compared because the pH value in the former need not be adjusted in feeding the anaerobic material before AD. In addition, H_2O_2 has a minor, but distinctive and sometimes annoying smell; however, a low concentration would not be harmful to the human health and environment. Third, H_2O_2 is cheaper than $NH_3 \cdot H_2O$. In China, H_2O_2 only costs 7 CNY (Abbreviation for Chinese Yuan, and a dollar is equivalent to 6.27 CNY, on Jan 1, 2012; Bank of China) per 500 mL, whereas a similar amount of $NH_3 \cdot H_2O$ costs 10 CNY. Hence, 4% and 3% H_2O_2 pretreatments are recommended for the biogasification of rice straw. However, the optimal hydrogen peroxide dosage was 3%, considering economic cost and biogas yield.

Recently, some researchers combined the different pretreatment methods to increase the biogas yield during the process of anaerobic digestion (Chang et al, 2001a; Chang et al, 2001b; Kang et al. 2012). Although both $NH_3 \cdot H_2O$ and H_2O_2 pretreatments significantly increased the anaerobic digestion efficiency in our study, combination of these two chemicals would probably not result in increased biogasification because there would be a reaction on the integration of $NH_3 \cdot H_2O$ and H_2O_2, with a production of NH_3 gas, which could weaken the oxidizability of H_2O_2 and biodegradation of $NH_3 \cdot H_2O$ on the hemicellulose and lignin. However, some researchers reported that the two-stage pretreatment operating sequentially under alkaline and acidic conditions would meet the purpose better than a single-stage process (Kim and Mazza, 2009). Kim et al. (2011) investigated the fractionation of rice straw by a two-stage pretreatment process consisting of aqueous ammonia followed by sulfuric acid. The first stage was intended for delignification, whereas the second stage is intended for hemicellulose removal. The results showed aqueous NH_3 used in the first stage removed lignin selectively but left most of cellulose (97%) and hemicellulose (77%). Dilute acid applied in the second stage removed most of the hemicellulose, partially disrupted the crystalline structure of cellulose, and thus enhanced enzymatic digestibility of cellulose in the solids remaining. Kim and Lee (2006) also conducted a two-stage pretreatment process on fermentation of corn stover using hot-water and aqueous ammonia percolation. Two-stage pretreatment may be a promising method for improving biogas production of agricultural wastes, but more effort should be made to investigate the integration of $NH_3 \cdot H_2O$ and H_2O_2 pretreatment to improve anaerobic digestion efficiency.

CONCLUSIONS

Anaerobic digestions were conducted to evaluate methane productivity when pretreating rice straw with $NH_3 \cdot H_2O$ and H_2O_2 prior to AD at retention times of 30 days at 37°C. Rice straw contains a high percentage of lignocelluloses, which is quite resistant to biodegradation by anaerobic bacteria. However, the main chemical compositions of rice straw, such as lignin, cellulose, and hemicellulose, were degraded considerably through chemical treatment using $NH_3 \cdot H_2O$ and H_2O_2. The results showed that biogas production was increased by all pretreatments, and that 4% and 3% H_2O_2 treatment were the optimal conditions for biogas production, with a biogas of 327.5 and 319.7mL/gVS, respectively. Moreover, rice straw pretreated using H_2O_2 was found to be better than that treated with the same concentration of $NH_3 \cdot H_2O$ for biogas production, ranked in an order of 4% ≈ 3% > 2% > 1%. Considering the economic cost and biogas yield, results of this study suggest that 3% H_2O_2 pretreatment is the optimal condition for rice straw biogas digestion. However, the AD process involved is rather complicated, and the current study can only provide limited evidence for this process. Therefore, additional studies should be performed to determine the optimal treatment time and temperature for enhancing biogas production.

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