Influence of Particle Size and Alkaline Pretreatment on the Anaerobic Digestion of Corn Stover

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The influence of particle size and an alkaline pretreatment on the anaerobic digestion of corn stover was studied. Four particle sizes, 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm, were used. The highest and lowest methane yields were obtained from untreated corn stover at particle sizes of 0.25 to 1.0 and 5.0 to 20.0 mm, respectively. 4% NaOH and 2% $Ca(OH)_2$ (combined alkaline pretreatment, CAP) were then used together to pretreat corn stover at these two particle sizes, compared with 6% NaOH pretreatment (single alkaline pretreatment, SAP). The cumulative methane yields from particle sizes 0.25 to 1.0 mm after CAP, 0.25 to 1.0 mm after SAP, 5.0 to 20.0 mm after CAP, and 5.0 to 20.0 mm after SAP were 286.9, 287.0, 268.7, and 272.6 mLg^{-1} VS, respectively. The particle size barely influenced the final cumulative methane yield of alkali-treated corn stover. Moreover, the cumulative methane yield of the corn stover after CAP was comparable with that of the corn stover after the SAP under the same conditions. These results provide us with a promising substitute of NaOH pretreatment for corn stover bioconversion in the future.

Keywords: Corn stover; Particle size; Alkaline pretreatment; Combination of sodium hydroxide and lime; Anaerobic digestion

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INTRODUCTION

China is a large agricultural country that produces a great quantity of crop wastes every year. Corn is one of the major crops and is mainly cultivated in central and north China (Pang *et al*. 2008). According to the China Agriculture Statistical Report (Ministry of Agriculture, PRC 2010), the nationwide yield of corn in 2010 was 177 million tons, and around 350 million tons of the byproduct, corn stover, was generated. Although there are a few methods available for corn stover reutilization, over 50% of the corn stover is usually thrown away or burnt due to its large quantities and low price, resulting in resource wastage and environmental pollution (Zheng *et al*. 2009). Thus, it is imperative to find an environmental friendly method to treat and reutilize corn stover to minimize pollution.

Anaerobic digestion technology has been widely used for the conversion of organic wastes such as sewage, food waste, energy crops, and crop residues to biogas (He *et al*. 2009). However, in lignocellulosic biomass, such as corn stover, cellulose and hemicellulose are densely packed by lignin, which protects them against enzymatic hydrolysis (He *et al*. 2008; Xie *et al*. 2011). Therefore, the digestibility of biomass highly depends on the degree of lignin solubilization.

Alkaline pretreatments can greatly improve the digestibility of lignocellulosic biomass through lignin solubilization, hemicellulose removal, and the modification of cellulose crystallinity (Mosier *et al*. 2005; Zhu *et al*. 2010). Sodium hydroxide (NaOH)-based alkaline pretreatments have been extensively studied and shown to be efficient for improving anaerobic digestion (Chandra *et al*. 2012; Liew *et al*. 2011; Zhu *et al*. 2010). However, high chemical loading, causing toxicity to methanogens and a high chemical expense, were also reported (Gumersindo *et al*. 1995; Pang *et al*. 2008). Lime ($Ca(OH)_2$) is a weak base that cannot effectively improve the biomass digestibility alone in a reasonable time frame at ambient temperatures (Kim and Holtzapple 2005; Xu *et al*. 2010), but it is much cheaper than NaOH (Chang *et al*. 1998; William and Holtzapple 2000; Xu and Cheng 2011). Furthermore, due to its poor solubility in water (1.73 g L^{-1} at 20 °C) (Xu and Cheng 2011), a great part of $Ca(OH)_2$ exists as a solid that will gradually dissolve to supplement the alkali consumed by the biomass. Therefore, $Ca(OH)_2$, although weak by itself, potentially could be used as a supplement to the strong but expensive NaOH to achieve a cost-effective pretreatment. However, there has been no report about the influence of a pretreatment with the combination of $Ca(OH)_2$ and NaOH on the anaerobic digestion of crop residues.

The particle size is an important factor that influences biogas production. Smaller particles increase the surface area available to the microorganisms and make organic material more accessible for biodegradation, thus increasing the biogas yield (Mshandete *et al*. 2006). Moorhead and Nordstedt (1993) reported that the cumulative biogas production of water hyacinth was higher for a particle size of 6.4 mm than for particles of 12.7 mm. However, little research has been conducted to date concerning the influence of the particle size of crop residues on an alkaline pretreatment and methane yield.

The objectives of this study were as follows: (1) to investigate the possibility of using the combination of NaOH and $Ca(OH)_2$ as a substitute for an NaOH pretreatment and a more cost-effective method for high efficiency anaerobic digestion of corn stover; and (2) to study the influence of particle size on methane production of untreated and alkali-treated corn stover.

EXPERIMENTAL

Feedstock and Inoculum

The corn stover used in this study was collected from a farm in DQY Agriculture Technology Co. Ltd, Beijing, China. The air-dried corn stover was rubbed by a kneading machine and then sieved and separated into four fractions (0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm) with standard test sieves (Shangyu Gonglu Instrument Co., Zhejiang, China). The samples were stored in airtight plastic bags at room temperature for further use. The activated sludge from a mesophilic anaerobic digester in Xiaohongmen Wastewater Treatment Plant in Beijing, China, was used as the inoculum. The inoculum was pre-incubated for seven days and fully degassed at 37 °C to minimize the background biogas production. More precisely, the inoculum was added to batch anaerobic digester (1-L serum bottle), to reach a final working volume of 800 mL. The digester was then checked for leakage and flushed with 99.0% pure argon to ensure anaerobic conditions. Finally, the digester was sealed with a rubber stopper and placed in an incubator for anaerobic digestion at 37 °C for 7 days until no gas produced. The gas produced by inoculum during the degassing progress was exhausted out once a day by displacement of water. All of the digesters were manually shaken once a day for 1 min.

Alkaline Pretreatment

The pretreatment experiments were carried out in 50-mL beakers. Two kinds of alkali solutions, 4% NaOH combined with 2% $Ca(OH)_2$, and 6% NaOH, based on dry matter of corn stover, were used according to a previous study in our lab (data not shown). The pretreatment experiment was therefore identified as two groups, the combined alkaline pretreatment (CAP) and the single alkaline pretreatment (SAP). In each beaker, 3.62 g of corn stover (3 g volatile solids-VS) and 28.7 mL of alkali solution were added to adjust the moisture content to 90%, which was calculated according to Zheng *et al*. (2009) (Eq. 1).

$$\text{Moisture contents}(\%) = \left(1 - \frac{\text{dry matter weight of corn stover}}{\text{weight of corn stover+water added}}\right) \times 100\% \qquad (1)$$

Then, all of the prepared beakers were covered with plastic films and placed in a 20 °C incubator for 3 days. All of the beakers were manually agitated once a day for 1 min. The pH value of the pretreatment liquor was tested every 12 h with an le438 pH electrode (Mettler Toledo, USA). At the end of the pretreatment, the pretreated corn stover in each beaker was directly used for the anaerobic digestion experiment without any washing or detoxification. The structure of the untreated and alkali-treated corn stover was observed with a scanning electron microscope (SEM) (Hitachi S-4700, Japan) at an accelerating voltage of 20.0 kV. Chemical analyses of the pretreated corn stover were carried out.

Anaerobic Digestion

The untreated and alkali-treated corn stovers were digested in batch anaerobic digesters, which were 1-L serum bottles. Each digester was seeded with activated sludge to keep the substrate to inoculum (S/I) ratio (based on VS) at 1 (Owen *et al.* 1979). Tap water was then added to each digester to reach a final working volume of 500 mL. The digesters were checked for leakage and flushed with 99.0% pure argon to ensure anaerobic conditions. Finally, the digesters were sealed with rubber stoppers and placed in an incubator for anaerobic digestion at 37 °C for 20 days. All of the digesters were manually shaken once a day for 1 min. The biogas production due to biomass decay was calculated by subtracting blank control, which contained the same amount of inoculum and water.

Analytical Methods

The total solids (TS) and volatile solids (VS) contents of corn stover, inoculum, and digestate were measured according to standard methods (APHA 1998). The total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (Vario ELcube, Germany). The lignin, cellulose, and hemicellulose contents were determined according to the Van Soest method (Van Soest *et al*. 1991) using an A2000 fiber analyzer (ANKOM Technology, USA).

The daily biogas production was calculated by measuring the pressure in the headspace using a 3151WAL-BMP-Test system pressure gauge (WAL Mess-und Regelsysteme GmbH, Germany) with an accuracy of 0.1% (based on the full gauge range). The daily biogas yield was calculated according to the equation reported by El-Mashad and Zhang (2010), and the biogas volume was normalized to standard conditions. The biogas composition in the anaerobic digestion progress was measured daily for the first 7 days and every 3 days afterwards using a 7890A gas chromatograph (Agilent Company, USA) equipped with a thermal conductivity detector and a TDX-01 column. Argon was used as the carrier gas at a flow rate of 35 mL min^{-1}. The temperatures of the injection inlet, oven, and detector were 150, 120, and 150 °C, respectively. The yield of CH_4 was calculated from the volume of the biogas and the CH_4 content. A standard gas (BeiWen Co., Beijing) consisting of 34.9% (V/V) CO_2, 10.06% (V/V) H_2, 5.07% (V/V) N_2, and 49.97% (V/V) CH_4 was used for the calibration.

Statistical Analysis

All the experiments such as pretreatment, anaerobic digestion, and chemical analyses were prepared based on triplicates. The significant differences were determined with *t*-test using PASW Statistics 18.0 software (IBM, USA). Differences were considered statistically significant when $p < 0.05$ for statistical analysis.

RESULTS AND DISCUSSION

Characteristics of Corn Stover and Inoculum

The characteristics of corn stover and inoculum are shown in Table 1.

Table 1. Characteristics of Corn Stover and Inoculum

Parameter	Corn Stover	Inoculum
TS (%)	89.31	3.44
VS (%)	82.91	1.61
TC (% TS)	46.37	39.18
TN (% TS)	0.63	4.48
C/N ratio	73.60	8.75
pH value	ND	7.68
Cellulose (% TS)	42.34	ND
Hemicellulose (% TS)	29.78	ND
Lignin (% TS)	13.41	ND
* ND = not determined		

Influence of Particle Size on Anaerobic Digestion of Untreated Corn Stover

The daily methane yields of untreated corn stover with particle sizes of 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm are shown in Fig. 1. Methane was generated rapidly after inoculation and kept increasing until it reached the maximal peak. It then began to decline as the digestion process proceeded. Similar daily methane yield trends were observed for all four particle sizes. The peak values of the daily methane yields were 30, 23, 18, and 17 mLg^{-1} VS at particle sizes of 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm, respectively. It can be seen that the peak values of the daily methane yields increased as the particle size was reduced from 20.0 mm to 0.075 mm. The results could be explained by the fact that a smaller particle made a larger surface area available to the microorganisms, thereby enhancing the methane production rate (Izumi *et al*. 2010).

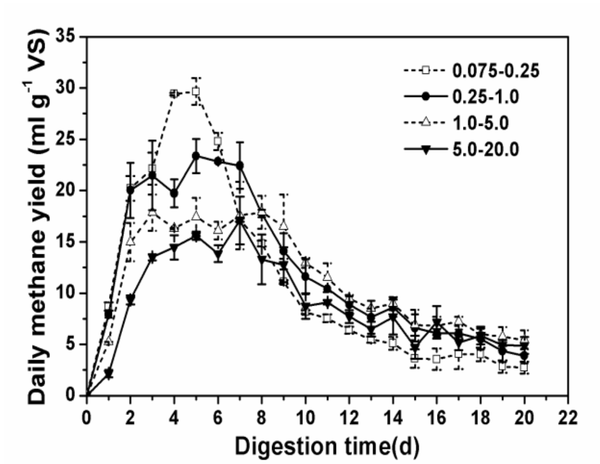

Fig. 1. Daily methane yields of untreated corn stover at the four particle sizes of 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm

The cumulative methane yields of the untreated corn stover at the four particle sizes are shown in Fig. 2. The cumulative methane yields were 231, 249, 229, and 184 mL g^{-1} VS for corn stover at particle sizes of 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm, respectively. The cumulative methane yields increased as the particle size was reduced from 20.0 mm to 0.25 mm. Significant differences between methane yields were detectable only between the 5.0 to 20.0 mm variant and the shorter variants, but not between the variants with particle sizes less than 5.0 mm.

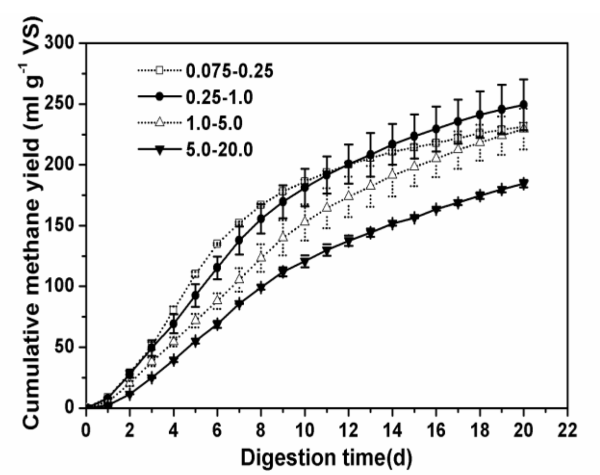

Fig. 2. Cumulative methane yields of untreated corn stover at the four particle sizes of 0.075 to 0.25, 0.25 to 1.0, 1.0 to 5.0, and 5.0 to 20.0 mm

The results obtained in this study clearly illustrated that the particle size reduction from 5.0-20.0 to 0.075-5.0 mm could significantly increase the substrate utilization and hence enhance the final methane yield. This finding agrees with results published in other studies. Menardo *et al*. (2012) reported that barley straw methane yields increased from 19.2% to 54.2% as the particle size was reduced from 5.0 mm to 0.5 mm, compared to the raw samples without any reduction. Mshandete *et al*. (2006) reported that the methane yield was inversely proportional to the particle size with sisal fiber waste sizes ranging from 2 to 100 mm. In this study, for particle size of 0.075 to 0.25 mm, the cumulative methane yield was only slightly lower than that of the 0.25 to 1.0 mm corn stover and both appeared to have no significant difference ($p > 0.05$) from that of 1.0 to 5.0 mm corn stover. This finding is in agreement with previously published results. Menardo *et al*. (2012) reported that the main effect of the particle size reduction (0.2 mm) of maize stalk was speeding up the hydrolytic phase, but not significantly improving the final methane yield.

Changes of pH Value, Characteristics, and Structure during the Alkaline Pretreatment

The corn stover at particle sizes of 0.25 to 1.0 and 5.0 to 20.0 mm, which produced the highest and the lowest cumulative methane yield, respectively, were chosen as the substrates for the pretreatment.

Variation of pH value

The pH value of the pretreated sample is a common stress indicator used for indirectly reflecting the consumption of alkali during the pretreatment process, according to Zheng *et al*. (2009). In this study, the initial pH values of the combined alkaline pretreatment (CAP) and the single alkaline pretreatment (SAP) of corn stover were 11.99 and 12.35, and then they were decreased sharply to a final pH of 6.80 and 7.80, respectively, by the end of the pretreatment. The pH value of the CAP samples in the early phase (48 h) decreased more slowly than that of the SAP. Due to the poor solubility of $Ca(OH)_2$ in water, a significant amount of $Ca(OH)_2$ was present as a solid, which could gradually dissolve to supply alkali consumed by the biomass (Xu and Cheng 2011), thus stabilizing the pH at a high level; this might be beneficial to a highly efficient alkaline pretreatment of corn stover.

SEM analysis

The physical structure of the corn stover was imaged by a scanning electron microscope with a magnification of 250. As shown in Fig. 3A, the untreated corn stover exhibited a compact and smooth texture. When pretreated with 4% NaOH combined with 2% $Ca(OH)_2$, the surfaces of all of the pretreated samples became rough and partially broken (Figure 3B). Some holes appeared on the surface, indicating that the lignin was broken down or dissolved, and degradable fractions were likely to be more exposed. This observation was confirmed by the lignin degradation data given in Table 2.

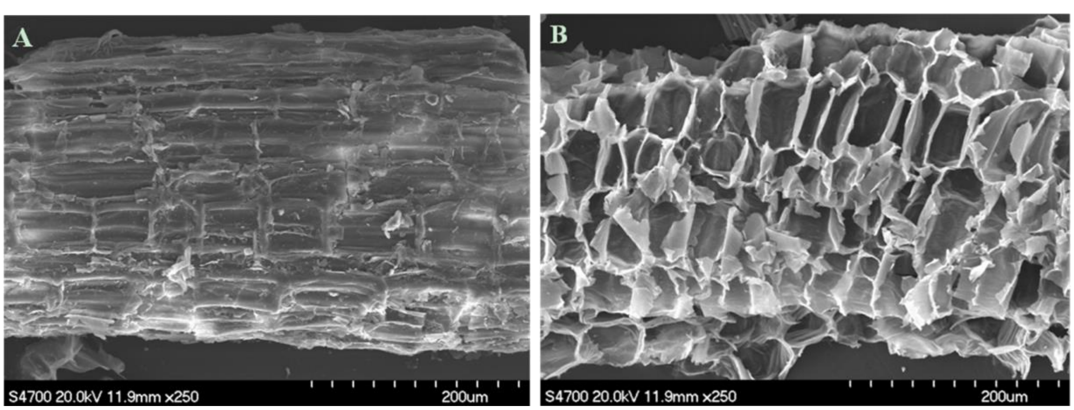

Fig. 3. Scanning electron microscope images of corn stover at 0.25 to 1.0 mm particle size (250×). (A) Untreated corn stover; (B) corn stover pretreated with 4% NaOH combined with 2% $Ca(OH)_2$, based on dry matter of corn stover

Changes in characteristics

The degradations of lignin, cellulose, and hemicellulose in the corn stover before and after the pretreatment were analyzed and calculated according to Equation 2, and the results are presented in Table 2,

$$\text{Component degradation}(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

where W_1 represents the dry matter weight (g) of lignin, cellulose, or hemicellulose in the material before alkaline pretreatment; W_2 refers to the dry matter weight (g) of lignin, cellulose, or hemicellulose in the material after alkaline pretreatment.

In each pretreatment condition, the degradation percentage of lignin in the corn stover was the highest among the main constituents (cellulose, hemicellulose, and lignin). The cellulose degradation percentage was less than 7% for all of the tests. Sodium hydroxide could modify the cellulose crystallinity through destroying cellulosic crystalline areas and enlarging the pore ratio and inner surface areas, rather than directly dissolving cellulose (He *et al.* 2008; Hendriks and Zeeman 2009). The degradation percentages of lignin and hemicellulose at 0.25 to 1.0 mm were higher than those of 5.0 to 20.0 mm corn stover in both the CAP and the SAP groups. The results clearly implied that a particle size reduction increased the surface area available to the alkali and thus improved the degradation of the biomass. The CAP treatment resulted in less lignin degradation, but a slightly higher hemicellulose degradation in the corn stover than that of the SAP at the same particle size. The final pH values of the CAP samples were lower than SAP group, and a number of studies have also reported that $Ca(OH)_2$ is too weak to degrade lignin by the interaction between calcium ions and lignin within the biomass structure (Kim and Holtzapple 2005; Xu and Cheng 2011; Xu *et al*. 2010). However, the decreased lignin degradation of the CAP group did not compromise the improvement of the methane yield, as will be shown below.

Table 2. Effect of the Combined Alkaline Pretreatment (CAP) and the Single Alkaline Pretreatment (SAP) on Corn Stover Degradation

Particle Size (mm)	Degradation (%)					
	Lignin		Cellulose		Hemicellulose	
	CAP	SAP	CAP	SAP	CAP	SAP
0.25-1.0	39.0±0.27	43.2±0.36	5.3±0.12	6.8±0.04	15.5±0.07	14.2±0.15
5.0-20.0	21.7±0.40	25.0±0.06	6.2±0.03	5.5±0.02	13.7±0.01	12.4±0.02

Influence of Alkaline Pretreatment on Anaerobic Digestion of Corn Stover

Methane Yield

Figure 4 shows the effect of the alkaline pretreatment on the daily methane yields of the corn stover at two different particle sizes during 20 days of anaerobic digestion. The daily methane yields after the CAP and the SAP of corn stover with particle size of 0.25 to 1.0 mm had peak values of 37 and 40 mL g^{-1} VS, respectively. The daily methane yield after the CAP and the SAP of corn stover with particle size of 5.0 to 20.0 mm reached 30 and 29 mL g^{-1} VS, respectively. It was found that, compared with the untreated corn stover (Fig. 1), the alkali-treated samples obtained higher peak values of the daily methane yield during a shorter time period at the same particle size. This finding is in agreement with the results obtained by previous researchers (Pang *et al*. 2008; Zheng *et al*. 2009). The daily methane yield of corn stover at 0.25 to 1.0 mm at the early stage of digestion (on days 2 to 7) was higher than that of corn stover at 5.0 to 20.0 mm in both the CAP and the SAP groups. This could be explained by the fact that a particle size reduction increased the surface area where the alkalis and microorganisms could reach and adhere, resulting in an increased methane production rate. As seen from the results in Fig. 4, the CAP and SAP caused a similar effect on the trends and the peak values of the daily methane yield at the same particle size.

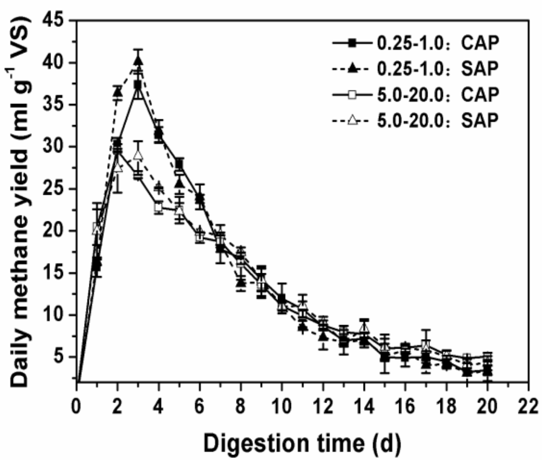

Fig. 4. Daily methane yields of the combined alkaline pretreatment (CAP) and the single alkaline pretreatment (SAP) of corn stover at the particle sizes of 0.25 to 1.0 and 5.0 to 20.0 mm

The effect of the alkaline pretreatment on the cumulative methane yields of corn stover at two different particle sizes during 20 days of anaerobic digestion is shown in Fig. 5. For particle size of 0.25 to 1.0 mm, the cumulative methane yields after the CAP and the SAP were 286.9 and 287.0 mL g^{-1} VS, respectively, causing a significant ($p < 0.05$) improvement of 15.0% and 15.1%, respectively, compared with the untreated corn stover. For particle size of 5.0 to 20.0 mm, the cumulative methane yields after the CAP and the SAP were 268.7 and 272.6 mL g^{-1} VS, causing a very significant ($p < 0.01$) improvement of 45.6% and 47.8%, respectively, compared with the untreated corn stover. It was found that the highest cumulative methane yield was obtained with the SAP at size of 0.25 to 1.0 mm. Moreover, the cumulative methane yield of corn stover at a 0.25 to 1.0 mm particle size showed no significant difference ($p > 0.05$) from that of 5.0 to 20.0 mm at the same pretreatment conditions.

The particle size barely influenced the final cumulative methane yield of alkali-treated corn stover. Furthermore, the cumulative methane yield of the corn stover pretreated using the combination of 4% NaOH and 2% $Ca(OH)_2$ was found to be comparable with that of the corn stover pretreated using 6% NaOH under the same conditions, while the chemical expense could be remarkably decreased due to the lower cost of lime and the reduced loading of NaOH. Pretreatment with the combination of NaOH and $Ca(OH)_2$ was found to be a promising method for the highly efficient anaerobic digestion of corn stover and might be a useful substitute for NaOH pretreatment in future applications.

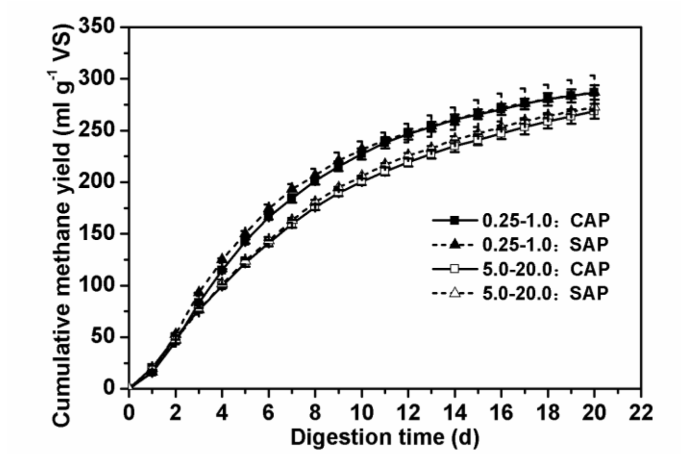

Fig. 5. Cumulative methane yields of combined alkali-pretreated (CAP) and single alkali-pretreated (SAP) corn stover at particle sizes of 0.25 to 1.0 and 5.0 to 20.0 mm

TS and VS Reduction

The TS and VS reduction of the untreated and alkali-treated corn stover after 20 days of anaerobic digestion was determined, and the results are shown in Table 3. It can be seen that the alkali-treated corn stover obtained a higher TS and VS reduction compared to the untreated biomass for both particle sizes. The results showed that the biodegradability of the alkali-treated corn stover was improved and that more components were digested (Pang *et al*. 2008). A greater improvement in the TS and VS reduction was achieved from the alkali-treated corn stover at 5.0 to 20.0 mm than that of 0.25 to 1.0 mm, compared with the untreated corn stover. Additionally, the TS and VS reduction after the CAP was close to that of the SAP under the same particle size. Moreover, there was no significant ($p > 0.05$) difference in the TS and VS reduction between the SAP at 0.25 to 1.0 mm and CAP at 5.0 to 20.0 mm. These results were consistent with the methane yield data at the same conditions (Fig. 4 and Fig. 5).

Table 3. TS and VS Reduction of the Combined Alkali-pretreated (CAP) and the Single Alkali-pretreated (SAP) Corn Stover after 20 Days of Anaerobic Digestion

Particle Size (mm)	TS Reduction (%)			VS Reduction (%)		
	Untreated	CAP	SAP	Untreated	CAP	SAP
0.25-1.0	55.5±1.19	63.4±0.27	64.3±1.31	62.1±0.81	71.9±0.41	73.6±0.76
5.0-20.0	45.3±1.64	61.1±1.41	63.2±0.33	53.7±0.97	67.8±0.14	69.8±1.26

CONCLUSIONS

1. A particle size reduction from 5.0-20.0 to 0.075-5.0 mm could significantly enhance the final methane yield from corn stover that was subjected to alkaline pretreatment. The highest and lowest methane yields of the untreated corn stover were 249 and 184 mL g^{-1} volatile solids (VS) at particle sizes of 0.25 to 1.0 and 5.0 to 20.0 mm, respectively.

2. The highest cumulative methane yield of 287.0 mL g^{-1} VS was attained with a single alkaline pretreatment (SAP) treatment with 6% NaOH at a size of 0.25 to 1.0 mm. The particle size barely influenced the final cumulative methane yield of alkali-treated corn stover.
3. The cumulative methane yield of the corn stover after the combined alkaline pretreatment (CAP), 4% NaOH combined with 2% $Ca(OH)_2$, was found to be comparable with that of the corn stover after the SAP at the same conditions, while the chemical expense could be remarkably decreased due to the lower cost of lime and the reduced loading of NaOH. Pretreatment with the combination of NaOH and $Ca(OH)_2$ was found to be a promising method for the highly efficient anaerobic digestion of corn stover and might be a useful substitute for NaOH pretreatment in future applications.

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