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Feasibility of Hydrothermal Pretreatment on Maize Silage for Bioethanol Production

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Abstract The potential of maize silage as a feedstock to produce bioethanol was evaluated in the present study. The hydrothermal pretreatment with five different pretreatment severity factors (PSF) was employed to pretreat the maize silage and compared in terms of sugar recovery, toxic test, and ethanol production by prehydrolysis and simultaneous saccharification and fermentation. After pretreatment, most of the cellulose remained in the residue, ranging between 85.87% by the highest PSF (185°C, 15 min) and 92.90% obtained at the lowest PSF (185°C, 3 min). A larger part of starch, varying from 71.64% by the highest PSF to 78.28% by the lowest, was liberated into liquor part, leaving 8.05–11.74% in the residues. Xylan recovery in the residues increased from 44.25% at the highest PSF to 82.95% at the lowest. The recovery of xylan in liquor changed from 20.13% to 50.33%. Toxic test indicated that all the liquors from the five conditions were not toxic to the Baker's yeast. Pretreatment under 195°C for 7 min had the similar PSF with that of 185°C for 15 min, and both gave the higher ethanol concentration of 19.92 and 19.98 g/L, respectively. The ethanol concentration from untreated maize silage was only 7.67 g/L.

Keywords Maize silage · Bioethanol · Hydrothermal pretreatment ·
Pretreatment severity factor (PSF) · Toxic test · Sugar recovery

Introduction

In recent years, interest in alternative, renewable bioethanol has grown with the recognition of the environmental deterioration resulted from the over-consumption of petroleum-derived products as well as the limited oil resources.

Bioethanol production has been promoted through policy decisions by many countries. The National Development Reform Commission of China initiated the Chinese national

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fuel ethanol program in 2002. Four fuel ethanol plants with a total annual production capacity of 1.02 million tons were then approved and put into operation in 2005 (http://www.biotech.org.cn/news/news/show.php?id=31419). The US government enacted the energy policy act of 2005 (EPAct 2005) at the national level [1]. A Renewable Fuels Standard included in Title XV of EPAct 2005 requires 4 billion gallons/year of ethanol to be blended into gasoline by 2006, increasing up to 7.5 billion gallons/year by 2012. Furthermore, beyond 2012, at least 0.25 billion gallons/year of this ethanol must come from cellulosic sources. The European directive has mandated the use of biofuels in the transportation sector corresponding to 5.75% by 20103/30/EC, 2003 [2]. In January 2007, the European Commission released the Renewable Energy Roadmap with a target of 10% biofuels by 2020 [3].

In 2007, the world ethanol production has reached about 13.1×10^3 millions of gallons (http://www.ethanolrfa.org/industry/statistics/). The USA and Brazil are the biggest producers, contributing 87.9% to the total ethanol production, followed by EU (4.4%) and China (3.7%). Most of this ethanol is from sugarcane, maize, wheat, and barley, which has not always been good for the environment and has had a major effect on food and feed prices and competed with food [4, 5]. Thus, new feedstocks are needed.

In Denmark, coastal climate does not allow maize to grow to its physiological maturity. Whole crop of maize, which consists of hardly degradable lignocellulosic material and starch, is harvested in October and ensiled as maize silage [6]. The area in Denmark with maize averaged 20,000 and 42,000 ha in the periods 1984–1993 and 1994–1999, respectively. By 2003, the area has increased to 118,000 ha, corresponding to 4.4% of the arable land [7].

In Denmark, maize silage is mainly used to provide all the year-round forage to domestic cattle with the production yield of 12–15 t dry weight (DW)/ha, but it has the potential of rising to 16–20 t dry matter (DM)/ha in the context of biorefining [8, 9]. Preliminary tests on maize silage had shown that it is a promising feedstock for bioethanol production [10, 11]. In the present study, hydrothermal pretreatment on maize silage was done with the aim of optimizing the bioethanol production.

Materials and Methods

Maize Silage Preparation

The maize silage was collected from a dairy farm with DM content at 27.5%. Before pretreatment, the maize silage was dried in oven at 60°C for 4 days until the DM was 95.9%.

The dried maize silage was milled to 2 mm particle size for the pretreatment experiments with Wiley mill, standard Model No.3.

Pretreatment of Maize Silage

The pretreatment was performed in a loop autoclave designed by Risø National Laboratory [12]. The autoclave was filled with 60 g milled maize silage (DW) and 1 L water. The autoclave was free of O_2 by flushing N_2. The pretreatment conditions are listed in Table 1. The pressure in the autoclave during the pretreatment was between 10 and 13 kg/cm^2. The pretreatment severity factor (PSF; R_0) was calculated as: $R_0 = t \cdot e^{\frac{(T-100)}{14.75}}$, where "$t$" is the pretreatment time in minutes and "T" is the temperature in °C [13]. In practice, $_{10}\log R_0$ is often used instead of R_0.

Table 1 Water-insoluble solid (WIS) recoveries, chemical composition of WISs, and raw maize silage.

Untreated/treated maize silage	Pretreatment severity factor (PSF) ($_{10}\log R_0$)	WIS (%)	Cellulose	Xylan	Klason lignin
Untreated			16.80±0.30	11.44±0.05	9.79±0.64
185°C, 3 min	2.98	46.25±0.21	33.76±2.37	20.52±0.27	15.96±0.95
195°C, 3 min	3.27	45.52±2.25	34.25±0.02	17.55±0.59	18.35±0.31
185°C, 7 min	3.35	41.84±0.15	36.26±1.92	18.86±1.07	16.90±0.43
195°C, 7 min	3.64	36.55±0.32	40.63±0.12	13.92±0.38	19.61±0.80
185°C, 15 min	3.68	35.85±0.57	40.26±2.81	14.13±0.70	19.92±0.08

After pretreatment, the slurry was filtered, and both the solid and liquid parts were collected and defined as water-insoluble solid (WIS) and liquor in the present work. WIS were dried and kept in a climate cabinet at 20°C and 65% relative humidity. The chemical compositions of raw material and WIS are presented in Table 1. Liquors were kept in the freezer at −4°C until analysis.

Toxic Test of the Liquors

One hundred milliliters liquor obtained from pretreatment or 100 ml water (as control) was mixed with 2 g pure glucose, 0.2 ml urea (24%, *w*/*v*), and 0.2 g dry yeast (Batch No. 2366) produced by De Danske Spritfabrikker A/S, Denmark. The gas phase of the bottles was exchanged with N_2, and the bottles were equipped with fermentation locks pre-filled with glycerol. Fermentation was then carried out at 32°C, and the amount of produced ethanol was determined as weight loss caused by CO_2 release. Fermentations were performed in duplicate.

Prehydrolysis and Simultaneous Saccharification and Fermentation

To examine the convertibility of the WIS into ethanol in simultaneous saccharification and fermentation (SSF), a mixture of dried WIS and water (pH adjusted to 4.8 with 0.1 M H_2SO_4) in a ratio of 13% (DW/*v*) was prepared. Prehydrolysis was performed at 50°C with an enzyme dosage of 15 FPU/g DW for 24 h. After cooling down to 32°C, another 15 FPU enzyme/g DW, 0.2 g dry yeast, and 0.2 ml urea (24%, *w*/*v*) were added into the fermentation bottle. The gas phase of the bottles was exchanged with N_2, and the bottles were equipped with fermentation locks pre-filled with glycerol. SSF was then carried out at 32°C, and the amount of produced ethanol was determined as weight loss caused by CO_2 release. Fermentations were carried out in duplicate.

Analysis Methods

DM content was determined by drying untreated and treated maize silage at 105°C until constant weight was obtained.

Two-step acid hydrolysis was employed to determine the composition of WIS and untreated maize silage. The first hydrolysis step was done at 30°C with 1.5 ml H_2SO_4 (72%) for 0.16 g DW. After 60 min, 42 ml water was added, and the second step was carried out in the autoclave at 121°C for 1 h. The mixture was then filtered, and the dried solid part subtracted the ash content was reported as Klason lignin. The amounts of sugars in the filtrate were determined by high-performance liquid chromatography (HPLC). Ash content was determined by heating the dried materials to 550°C for 3 h.

The liquor obtained after pretreatment was hydrolyzed by 4% H_2SO_4 at 121°C for 10 min. The sugar content was then determined by HPLC.

The amounts of sugar monomers in all the liquid fractions and the ethanol concentration were measured by HPLC (Shimadzu) using a Rezex ROA column (Phenomenex) at 63°C and 4 mm H_2SO_4 as eluent at a flow rate of 0.6 ml/min. A refractive index detector (Shimadzu Corp., Kyoto, Japan) was applied.

Recoveries and yields from pretreatment step were calculated as follows:

$$\mathrm{Recovery}_{\mathrm{WIS}} = \frac{\mathrm{Mass}_{\mathrm{Dry\ WIS}}}{\mathrm{Mass}_{\mathrm{Dry\ raw\ material}}} \times 100\% \tag{1}$$

$$\mathrm{Recovery}_{\mathrm{Cellulose}} = \frac{\mathrm{Mass}_{\mathrm{Cellulose\ in\ WIS}} \times \mathrm{Recovery}_{\mathrm{WIS}} + \mathrm{Mass}_{\mathrm{Cellulose\ in\ liquor}}}{\mathrm{Mass}_{\mathrm{Cellulose\ in\ raw\ material}}} \times 100\% \tag{2}$$

$$\mathrm{Recovery}_{\mathrm{Xylan}} = \frac{\mathrm{Mass}_{\mathrm{Xylose\ in\ WIS}} + \mathrm{Mass}_{\mathrm{Xylose\ in\ liquor}}}{\mathrm{Mass}_{\mathrm{Xylose\ in\ raw\ material}}} \times \mathrm{Yield}_{\mathrm{WIS}} \times 100\% \tag{3}$$

$$\mathrm{Recovery}_{\mathrm{Starch}} = \frac{\mathrm{Mass}_{\mathrm{Starch\ in\ WIS}} \times \mathrm{Recovery}_{\mathrm{WIS}} + \mathrm{Mass}_{\mathrm{Starch\ in\ liquor}}}{\mathrm{Mass}_{\mathrm{Starch\ in\ raw\ material}}} \times 100\% \tag{4}$$

Saccharomyces cerevisiae was used as the only fermenting microorganism in this work. So, ethanol yield was calculated according to the cellulose content in the raw maize silage.

$$\mathrm{Yield}_{\mathrm{Ethanol}} = \frac{\mathrm{Mass}_{\mathrm{Ethanol\ by\ CO_2\ weight\ loss}}}{\mathrm{Mass}_{\mathrm{Cellulose\ in\ WIS}} \times 0.51} \times 100\% \tag{5}$$

The theoretical ethanol production was calculated as the following:

$$\begin{aligned}\mathrm{Ethanol}_{\mathrm{Theoretical}} = {} & (\mathrm{Glucose}_{\mathrm{Raw\ material}} \times \mathrm{Yield}_{\mathrm{WIS}} \times \mathrm{Yield}_{\mathrm{Ethanol}} + \mathrm{Xylose}_{\mathrm{Raw\ material}} \times \mathrm{Recovery}_{\mathrm{Xylose}} \\ & + \mathrm{Starch}_{\mathrm{Raw\ material}} \times \mathrm{Recovery}_{\mathrm{Starch}}) \times 0.51.\end{aligned} \tag{6}$$

Results and Discussion

Recoveries of WIS, Cellulose, Hemicellulose, and Starch

Table 1 shows the recovery of WIS after pretreatment. At 185°C, when the pretreatment time increased from 3 to 15 min, the recovery of WIS, calculated using Eq. 1, decreased from 46.25% to 35.85%, while the recovery under 195°C decreased from 45.52% to 36.55% as the pretreatment time increased from 3 to 7 min. When the PSF was introduced to the present study, it was observed that the WIS recovery decreased as the PSF increased.

The slurry after pretreatment was separated into WIS and liquor. As shown in Table 2, most of the cellulose remained in the WIS after pretreatment. The highest cellulose recovery of 92.90%, based on Eq. 2, was found from the pretreatment with the lowest PSF, and the lowest of 85.87% was obtained by the highest PSF. There was still a small part of

Table 2 Sugar recoveries of maize silage at different pretreatment conditions.

Temp. (°C)	Time (min)	Cellulose (%)			Starch (%)			Xylan (%)		
		In WIS	In liquor	Total	In WIS	In liquor	Total	In WIS	In liquor	Total
185	3	92.90±6.11	4.09±2.78	97.00±3.33	9.95±1.59	78.28±2.24	88.23±3.84	82.95±0.70	20.13±1.10	103.08±0.40
195	3	92.79±4.54	3.15±4.60	95.95±0.06	11.74±2.82	76.09±0.43	87.83±2.40	69.78±1.10	36.00±1.29	105.78±0.19
185	7	90.29±4.47	7.85±3.36	98.14±1.12	9.50±1.10	76.09±2.14	85.59±1.03	68.97±3.67	38.99±0.66	107.95±3.02
195	7	88.38±0.51	9.18±0.22	97.57±0.73	8.05±0.03	76.32±0.96	84.37±0.93	44.47±0.82	50.33±0.31	94.80±0.51
185	15	85.87±4.62	10.34±2.45	96.22±2.17	8.18±1.21	71.64±7.58	79.82±6.37	44.25±1.50	47.70±5.13	91.95±6.63

cellulose dissolved into the liquor, ranging between 3.15% (195°C for 3 min) and 10.34% (185°C for 15 min), which could not cover all the cellulose loss from the WIS.

Xylan recovery in the WIS decreased from 82.95% to 44.25% as the PSF increased, which made xylan recovery in the liquor increase from 20.13% with PSF at 2.98 to 50.33% at 3.64. When the PSF was increased to 3.68, the xylan recovery in the liquor slightly decreased by 2.63% compared to that from the pretreatment with the PSF at 3.64. This was probably due to more xylan degradation under severe pretreatment condition. Some investigations indicated that the furfural and hydroxy methyl furfural (HMF), which were formed from degradation of xylose and glucose, increased as the PSF increased [14, 15]. The present study followed the same trend. As shown in Fig. 1, furfural concentration varied from 0.60×10^{-3} g/L by the lowest PSF to 0.051 g/L by the highest. HMF concentration increased as the PSF increased, ranging between 0.0047 g/L by the lowest PSF and 0.026 g/L by the highest.

As far as the total xylan recovery calculated according to Eq. 3 was concerned, the first three pretreatment conditions, which were 185°C for 3 min and 5 min, and 195°C for 3 min, gave the recoveries higher than 100%, and the lowest was 91.95% obtained by 185°C for 15 min, which was characterized by the highest PSF in all the pretreatments. Recoveries above 100% were obtained after some pretreatments, due to the underestimation in the analysis of the xylan content in the raw material. However, it is still comparable between different pretreatment conditions when the recoveries are expressed as grams recovered per 100 g raw material, which is not affected by the composition determination of the raw material.

For the starch, 71.64–78.28% was dissolved into liquor during the pretreatments, leaving only 8.05–11.74% in the WIS. The investigation on the thermal stability of cornstarch carried out by Stojanović showed that there was no degradation occurred on starch at

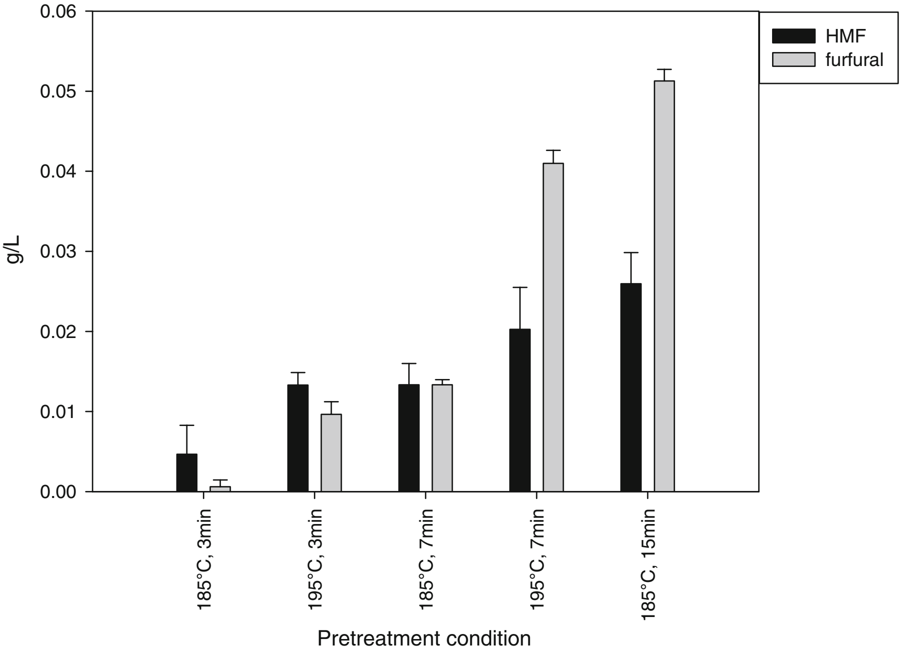

Fig. 1 Concentrations of hydroxy methyl furfural and furfural under different pretreatment conditions

temperature lower than 260°C [16]. In the present study, the maximum temperature used was 195°C, far below the starch degradation temperature at 260°C. Therefore, both the starch released into liquor and those that still remained in the WIS were mostly kept in the oligomer/polymer state. For all the pretreatments employed in the present study, the total starch recovery calculated from Eq. 4 showed not much difference, ranging between 79.82% and 88.23%.

Toxic Test of the Liquors

The toxic tests on the liquors were done in order to study the toxicity of the liquors to Baker's yeast. All liquors were fermentable, and the ethanol yields were slightly higher than that of the reference solution (62.52% of the theoretical, corresponding to 6.38 g/L; Fig. 2). This was probably due to the low concentration of the acetic acid present in the liquors which can increase the ethanol yield [17–21], and it might also be caused by the glucose introduced by the liquors themselves. All the liquors increased the final ethanol yield to 64.41% (6.64 g/L), 66.11% (6.85 g/L), 66.49% (6.85 g/L), 66.53% (6.85 g/L), and 65.89% (6.95 g/L) of the theoretical, respectively. Obviously, the liquors produced by all the five pretreatment conditions selected in this study could be fermented well and were non-toxic to the Baker's yeast. In addition, liquors obtained from all the pretreatments were showed to slightly increase the ethanol production.

Ethanol Production

To evaluate the fermentability of the WISs, prehydrolysis at 50°C for 24 h and then SSF at 32°C were carried out with *S. cerevisiae*. The untreated maize silage was also tested as control. Figure 3 shows the ethanol concentration for the five WISs compared to the untreated maize silage.

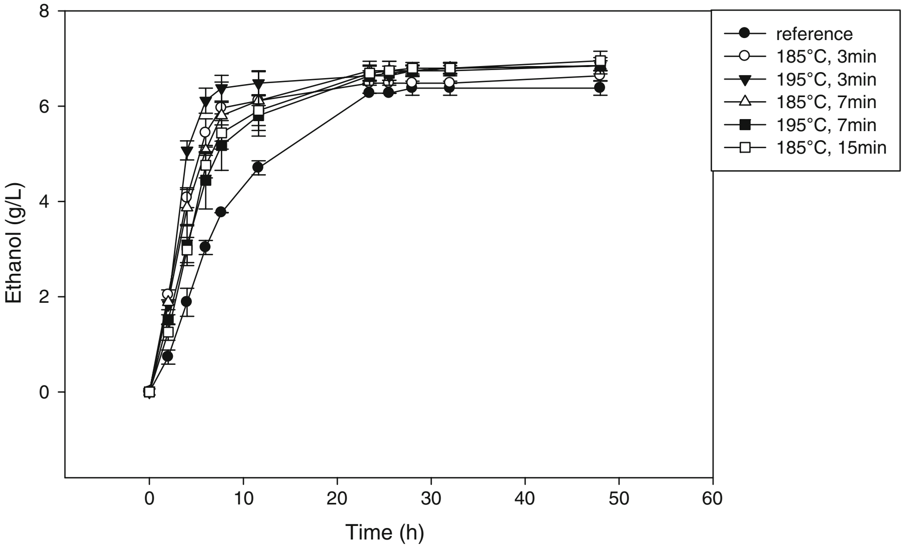

Fig. 2 Toxic tests of liquors obtained from pretreatments

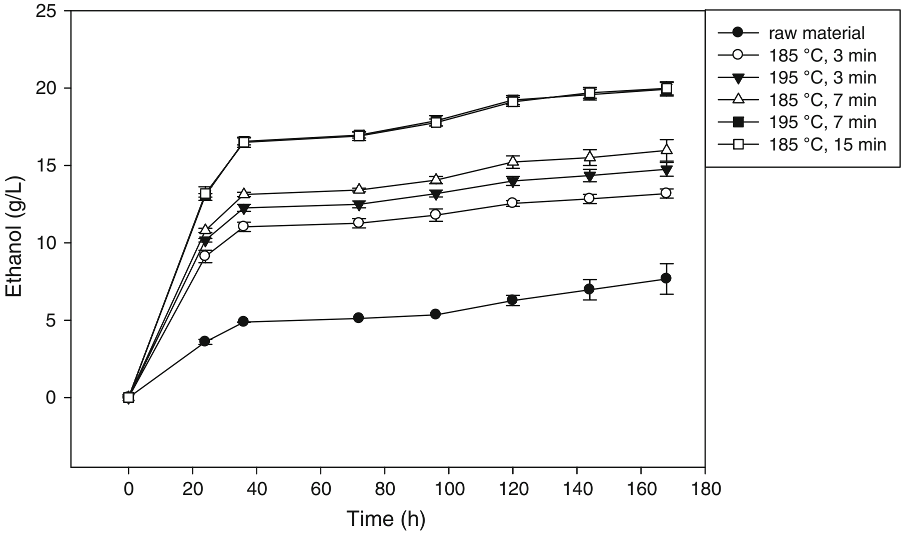

Fig. 3 Simultaneous saccharification and fermentation of the water-insoluble solids obtained from different pretreatment conditions

It can be seen obviously that all the WISs gave the higher ethanol concentration than that from the untreated material. The highest ethanol concentration of 19.92 g/L, corresponding to the yield of 78.00% of the theoretical, based on Eq. 5, was found by the pretreatment at 195°C for 7 min. The other pretreatment at 185°C for 15 min, which had the similar PSF factor with that of 195°C for 7 min, resulted in approximately the same ethanol concentration, which was 19.98 g/L with the yield of 77.23% of the theoretical. The ethanol concentration (yield of the theoretical) of the other three pretreatments ranged from 13.18 (55.21%) to 15.97 g/L (68.21%). The untreated maize silage gave only 7.67 g ethanol/L (42.54% of the theoretical ethanol yield). Piotr et al. [22] have done the ethanol production from wet-oxidized maize silage, and the ethanol production of 82% was achieved 5% higher than the optimal ethanol production in the present study, which was from pretreatment at 195°C for 7 min. The higher ethanol production in Piotr's investigation was probably due to the more harsh pretreatment condition (195°C, 2 g/l Na_2CO_3, 12 bar O_2) and pretreated anaerobically digested manure (20 min, 121°C) used as the nitrogen source which also contains micronutrients.

For any of the fermentations with WIS and liquor, there was no lag phase observed, which indicates that the level of inhibitors produced in all the pretreatments was low enough not to affect the yeast's activity under the present fermentation conditions.

Theoretical Ethanol Production

A theoretical ethanol production based on the achieved SSF yield and recoveries of the carbohydrates was calculated based on Eq. 6. For glucose from cellulose, the ethanol yield obtained in SSF from the present study was used. In order to evaluate the ethanol production potential, it was assumed that all the recovered hemicellulose and starch sugars could be converted to ethanol by the fermentation efficiency of 0.51. The results were compared to the maximum ethanol production from the available sugars in the raw material, which is 36.80 g ethanol/100 g dry maize silage. Pretreatment at 185°C for 7 min gave the highest theoretical ethanol production of 31.20 g/100 g dry maize silage, followed by 30.95 g/100 g dry maize silage at 195°C for 7 min. The theoretical ethanol yields from other pretreatments varied between 29.65 g and 30.14 g/100 g raw maize silage.

After pretreatment, there was only 0.41–0.48 g free glucose/100 g raw maize silage released into liquor, which was considered to be from cellulose in the present study. During SSF, cellulase was the only enzyme used to hydrolyze cellulose into glucose, and then glucose was fermented by the Baker's yeast. Therefore, xylan and starch were not utilized in this investigation. In order to evaluate the overall potential, the theoretical ethanol production was employed, which was based on all the carbohydrate components, which can be converted into ethanol.

Conclusions

Hydrothermal pretreatment, which degraded and/or solubilized the xylan fraction, was effective in improving the ethanol production from the cellulose in maize silage.

Among the five pretreatment conditions, the higher PSF yielded higher ethanol. In the present study, 195°C for 7 min and 185°C for 15 min had the similar PSF, which was also the highest in all the pretreatments, and these two pretreatments gave the highest ethanol production of 78.00% and 77.23% of the theoretical, respectively. In order to further increase the ethanol production, we will test more harsh pretreatment, i.e., adding weak acids, as we have done on corn stover [21, 23]. For the theoretical ethanol yields, all the five pretreatments gave the similar value based on the 100% fermentation efficiency of recovered xylan and starch. Although it is hard to get 100% fermentation efficiency in practice, the theoretical ethanol yields can still be employed to evaluate the effectiveness of the pretreatment technologies and predict the possibility of the ethanol production potential. The effect of different pretreatments on xylan and starch fermentation will be investigated in the near future.

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