CHALLENGES OF CELLULOSIC ETHANOL PRODUCTION FROM XYLOSE-EXTRACTED CORNCOB RESIDUES

Lei Zhang,[a] Ji-Hong Li,[a] Shi-Zhong Li,[a,c,*] and Z. Lewis Liu [b,c,*]

Xylose-extracted corncob residue (X-ER), a byproduct from the xylose production industry, is a potential cellulose-rich energy resource. However, attempts to achieve large-scale production of cellulosic ethanol using X-ER have been unsatisfactory due to a lack of understanding of the substrate. This study presents the first characterization of the X-ER to evaluate its potential utilization in the sequential production of cellulosic ethanol. The current dilute acid treatment procedures used for the corncobs by the xylose-production industry were insufficient for efficient deconstruction of cellulose structure to release available sugars for subsequent cellulosic ethanol conversion. After a secondary dilute acid hydrolysis of the X-ER, an additional 30% hemicellulose was recovered. In addition, a more efficient enzymatic hydrolysis of X-ER was observed resulting in a significantly higher yield of glucose conversion compared with an untreated X-ER control. These results suggest X-ER can be utilized for cellulosic ethanol production. However, improved corncob pretreatment procedures are needed for economical cellulosic ethanol conversion.

Keywords: Cellulose acid hydrolysis; Enzymatic saccharification; Cellulosic ethanol production

*Contact information: a: Institute of New Energy Technology, Tsinghua University, Beijing, China; b: Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL USA; c: US-Sino Center for Biofuels Research; *Corresponding authors: ZLewis.Liu@ars.usda.go (ZLL); szli@mail.tsinghua.edu.cn (S-ZL)*

INTRODUCTION

Starch-to-ethanol is a classic method used to produce fuel ethanol; however, the use of starch for ethanol production threatens grain supplies and food security for humans and animals worldwide. Emerging interest in ethanol production using cellulosic biomass, such as agricultural crop residues, is a promising technology for the second generation of biofuels (Mosier et al. 2005; Outlaw et al. 2005; Taherzadeh and Karimi 2008; Vertes et al. 2010; Wall et al. 2008). Cellulosic biomass consists of cellulose, hemicelluloses, and lignin that need to be broken down for utilization by fermentative microorganisms. Conventional cellulosic biomass-to-ethanol conversion involves hydrolysis pretreatment, cellulose separation, and enzymatic saccharification. The multiple steps of processing procedures increase the cost of cellulosic ethanol production. Thus far, no economic process is available for the large-scale production of cellulosic ethanol. Overcoming complex technical barriers and reducing production costs have become significant challenges for low-cost cellulosic ethanol production at industrial scale (Kabel et al. 2007; Kim et al. 2006; Zhao et al. 2002).

Dilute acid hydrolysis is a widely used biomass pretreatment procedure with higher recovery rate of hemicellulose sugars and easy access for subsequent cellulose enzymatic saccharification (Emmel et al. 2003; Kabel et al. 2007; Lee et al. 1999). Acid hydrolysis pretreatment increases the porosity of the substrate and the available cellulosic surface for enzymatic reactions that facilitate efficient saccharification (Cara et al. 2008; Kabel et al. 2007; Liu et al. 2008). For a typical biomass acid pretreatment, enzymatic saccharification of the solid cellulose can reach over 70% (Cara et al. 2008; Emmel et al. 2003; Kabel et al. 2007). Commonly used dilute acid hydrolysis pretreatments for biofuel production apply from 0.05 to 2% H_2SO_4 (w/v) at between 120 and 220ºC for 2 to 90 min (Cara et al. 2008; Emmel et al. 2003; Galbe and Zacchi 2002; Kabel et al. 2007; Sun and Cheng 2005). In general, higher temperatures and longer reaction times of the pretreatment result in higher levels of hemicellulose sugar recovery. However, higher pretreating temperatures and longer pretreatment time cause dehydration of xylose and glucose, which leads to the production of inhibitory compounds such as furfural and 5-hydroxymethylfurfural (HMF). These toxic byproducts inhibit microbial growth and interfere with subsequent ethanol fermentation (Klinke et al. 2004; Liu and Blaschek 2010; Palmqvist and Hahn-Hägerdal 2000).

Xylose production using corncobs is an established industrial practice. The treatment process of corncobs for industrial xylose production is relatively mild compared with that used for lignocellulose-to-ethanol conversion. For example, corncobs used for xylose-production are treated with 1.2 to 1.5% H_2SO_4 at 125˚C to minimize the production of inhibitory compounds. After the utilization of hemicellulose for xylose production, cellulose and lignin are the main components remaining in the industrially processed corncob residues. The xylose-extracted corncob residue (X-ER) is usually burned, with byproducts released into the air, causing environmental contamination and becoming lost as a waste of energy resources. Utilization of byproduct X-ER for cellulosic ethanol production has been thought to improve industrial profitability and air quality; however, limited ethanol conversion yield was observed using X-ER. It is speculated that the deconstruction of the corncobs for the current industrial xylose production may be incomplete. To date, no detailed information on X-ER is available, and mechanisms of its enzymatic hydrolysis are not well known. The objective of this study was to evaluate essential characteristics of the X-ER and its potential use as a substrate for cellulosic ethanol production. Results of this study will aid research efforts and scale-up for potential development of combined productions of xylose using corncobs and subsequent cellulosic ethanol using its byproduct X-ER.

EXPERIMENTAL

Substrate Preparation and Chemicals

An industrial X-ER byproduct was supplied by Longlive Co., Ltd. (Yucheng, Shandong, China). Microcrystalline cellulose, Avicel®PH-200 NF (FMC Biopolymer Corp., Philadelphia, PA, USA), was used as the cellulose control. A control of lignaceous hydrolysis residue of corncobs was prepared by cellulase hydrolysis for 72 h, as previously described (Xu et al. 2008). An extensive washing procedure was applied to

the lignaceous residue control with 50 mM Na-acetate with a (pH of 5). The residue was then heated in boiling water for 5 min to inactivate the adsorbed cellulase and later dried at 60ºC in a drying oven until a constant weight was achieved. Standards used for liquid chromatography were HPLC grade and obtained from Sigma-Aldrich (USA). Tetracycline and cycloheximide were obtained from Merck (Whitehouse Station, New Jersey, USA). All other chemicals and reagents were of analytical grade, including sodium acetate and sodium hydroxide, and were purchased from Beihua Fine Chemical Co., Ltd (Beijing, China).

Composition Analysis of Corncobs and the X-ER

Compositions of the corncobs and the X-ER were determined using analytical methods for biomass based on standard biomass analytical procedures (Sluiter et al. 2007). Prior to testing, untreated raw corncobs and X-ER were extracted using water and ethanol, respectively. Cellulose and hemicellulose contents of the solid residue were determined based on monomer contents, which were fractionated by a two-step acid hydrolysis procedure. The hydrolysis was carried out using 72% (w/w) H_2SO_4 at 30ºC for 60 min, and the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121ºC for 1 h. Sugar contents of the hydrolysates were assayed using HPLC, and acid-soluble lignin was determined by absorbance at 205 nm using a UV spectrophotometer. Crucibles and acid-insoluble residues (AIR) were ashed in a muffle furnace at 575ºC for 6 h. The weight and content of the acid-insoluble lignin (AIL) were then measured by the difference before and after washing. HPLC was performed using a Shimadzu LC-20A liquid chromatograph with an evaporative light scattering detector. An Aminex HPX-87P carbohydrate analysis column (Bio Rad Labs) equipped with a guard column operated at 80ºC with ultrapure water as mobile phase (0.6mL/min) was used for the separation. Furfural, HMF, furoic acid, vanillin, and ethanol were determined by photo-diode array detection after separation on an Aminex ion exclusion HPX-87H cation-exchange column (Bio-Rad Labs) equipped with a guard column at 55ºC and 89% 5 mM H_2SO_4 and 11% acetonitrile as mobile phase at a flow rate of 0.7mL/min.

Acid Hydrolysis

The acid hydrolysis was carried out using a WX4000 microwave dissolver (EU instrument, China) with a pressure detector at a frequency of 2.45 GHz. The hydrolysis condition simulating the industrial process was performed using 1.5% (w/v) H_2SO_4 at 125ºC for 2 h with a liquid-solid (dry mass) ratio at 6:1. After the acid pretreatment, the resulting slurry was filtered to separate the liquid and solid. The liquid fraction was analyzed for concentrations of sugars, furfural, HMF, furoic acid, and vanillin. The water-insoluble solids (WIS) fraction was rinsed using water and analyzed for hemicellulosic sugars, glucose, and the AIL contents. The WIS fraction was treated with a secondary dilute acid hydrolysis under the same conditions. Components of the second acid hydrolysates were assayed as described above.

Scanning Electron Microscopy

The surface ultrastructure of the cell walls of the untreated corncobs and the X-ER were examined using a field emission scanning electron microscope with an FEI

Quanta 200 system (FEI, The Netherlands) operated at 15 kV, 10.7 mm. Samples were coated with gold/palladium by using a SC7640 auto/manual high resolution sputter coater (Quorum Technologies, UK).

Enzymatic Hydrolysis

Enzymatic saccharification of cellulosic materials was carried out using cellulase Accellerase 1000™ kindly provided by Genencor (Rochester, NY). For comparison of the enzymatic conversion efficiencies between X-ER and a microcrystalline cellulose control (Avicel), a series of cellulase loading doses from 0 to 59 filter paper units (FPU)/g of cellulose was applied. X-ER was ground into 0.3 to 1 mm particles similar to that of Avicel using a Wiley mill and passed through a sieve. For all other treatments, 2 g of X-ER solid loading was applied, and the enzyme was added at a loading dose of 13.4 FPU/g of cellulose. All enzyme treatments were performed in a 0.05M sodium acetate buffer (pH of 4.8) supplemented with 80 μg/mL of tetracycline and 60 μg/mL of cycloheximide. The hydrolysis was carried out at 50ºC on a rotary shaker at 140 rpm with a liquid/solid (dry mass) ratio of 8:1 (v/w). To test the efficiency of the enzyme treatment, a range of liquid-solid ratios from 5 to 15 was used. Samples were collected periodically for analysis of glucose concentrations.

Sugars Removal from Saccharification Solutions

Sugars released by enzymatic saccharification of X-ER were removed using a method as previously described (Azevedo et al. 2002). The liquid fraction was separated from the solids residues by centrifugation at 12,000 g for 10 min after the enzyme treatment at 12 and 24 h, respectively. Supernatant was then filtered through a Vivaspin 15R ultrafiltration tube (Sartorius, Germany) with a molecular mass cutoff of 10 kDa by centrifugation at 4,000 g for 30 min at 4ºC. The remaining solid residues were added with either a fresh sodium acetate buffer or an X-ER extract to the initial volume without additional enzymes. The X-ER extract was prepared following the method above without addition of Accellerase.

Non-productive Enzyme Adsorption

The adsorption of enzymes onto different substrates was compared in the enzyme treatment of Avicel, lignaceous residue, the X-ER, and the X-ER plus Tween 80. The enzymatic reactions were performed with 20 g/L of solids at the liquid-solid ratio of 8:1 (v/w). Tween 80 was added during hydrolysis of the X-ER with a final concentration of 2.5g/L. Accellerase 1000 was added at a rate of 10 FPU per gram of solid during the treatment. Supernatant was collected at various time points by centrifugation at 12,000 g for 10 min at 4ºC and stored at -20ºC until analyzed. The presence of Accelerase 1000 was detected by SDS-PAGE gel electrophoresis using a Liuyi system (Beijing, China) as described previously (Westermeier 2005). Gels were stained by Coomassie brilliant blue and bands were visualized using an Alphaimager HP system (Alpha Innotech Corp., San Leandro, California, USA). Total protein count was determined using the BCA method as previously described (Smith et al. 1985). To avoid interference from reducing sugars, deoxycholate-trichloroaetic acid (DOC-TCA) precipitation was used to extract and purify total proteins from a supernatant as previously described (Sugano et al. 1991).

RESULTS AND DISCUSSION

Composition and Deconstruction of Corncobs and the X-ER

Corncobs were measured to be approximately 22% cellulose and 29% hemicellulose. The majority of the hemicellulose was in the form of xylose, accounting for approximately 19% of the total (Table 1). As anticipated, cellulose was the predominant polymer, at about 50%, recovered from the X-ER. There was about 11% hemicellulose remaining in the residue, of which xylose accounted for 7%. Contents of both substrates were similar, except that the X-ER showed lower amounts of water extract and lignin. As revealed by the ultrastructure surface scanning, the fibrils showed a smooth surface for the untreated corncobs (Fig. 1a). On the other hand, a rough appearance was observed on the surface of microfibrils, the main ultrastructural elements of corncob cell walls, for the X-ER (Fig. 1b). Splits resulting from the deconstruction were apparent; however, the obvious structural collapse of fibrils was not observed.

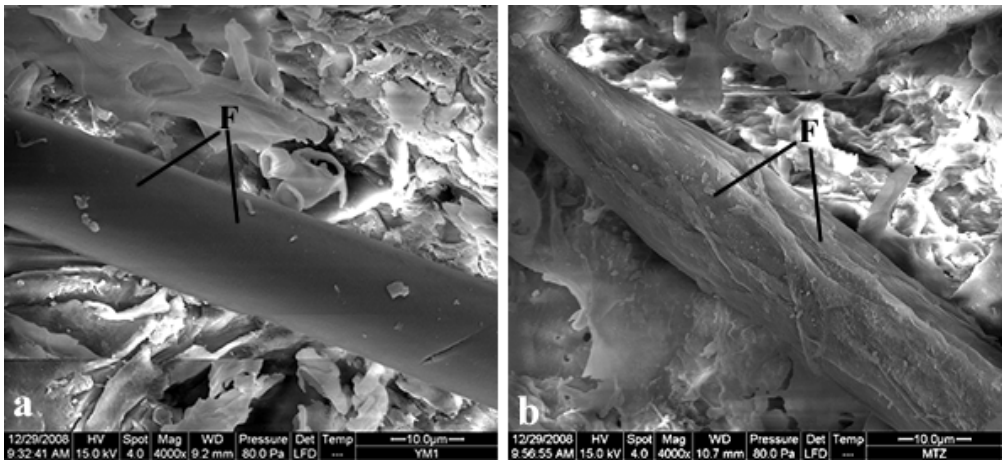

Fig. 1. Comparison of surface scanning ultrastructures of cell walls for an untreated corncob (a) and a post production of xylose-extracted corncob residue (b). Fibrils (F) on cell walls are labeled

As demonstrated by the surface scanning of the fibrils, decomposition of the corncob cell structure occurred during the acid hydrolysis treatment used for xylose production; however, the degree of the cell decomposition was incomplete, and approximately 11% of the hemicellulose remained in the X-ER (Table 1). This indicated that the current corncob acid treatment at relatively lower temperature did not release the maximum amount of xylose portion. It is known that dilute acid pretreatment of lignocellulose at higher temperatures, such as 170 to 210˚C used for experimental biofuels production, generates more inhibitory compounds including aldehydes, ketones, organic acids, and phenols (Antal et al. 1990, 1991; Klinke et al. 2004; Liu and Blaschek 2010; Palmqvist and Hahn-Hägerdal 2000). During a short period of acid treatment, most hemicellulose sugars released were in the form of polysaccharides or oligosaccharides (Kabel et al. 2007; Lloyd and Wyman 2003). It is generally believed that the current corncob acid pretreatment at 125˚C for 2 h facilitates simple sugar release with limited production of inhibitory compounds. Such a procedure may be sufficient for a single xylose production, although not all hemicellulose fractions were fully utilized. When the

byproduct X-ER is considered as a substrate for subsequent cellulose ethanol conversion, however, the incomplete decomposition of the corncobs prohibits efficient cellulose utilization for ethanol conversion.

Table 1. Comparison of Compositions of Untreated Corncobs and Xylose-extracted Corncob Residue (X-ER) as Measured by Percentage of Dry Weight

Elements	Corncobs (% dry mass)	X-ER (% dry mass)
Extract total	**19.63**	**15.74**
Water extracts	15.31 ± 1.5	11.21 ± 0.75
Ethanol extracts	4.32 ± 2.3	4.53 ± 3.6
Cellulose	**22.27***	**49.43**
Glucose	24.74 ± 0.9	54.92 ± 2.9
Hemicellulose	**28.30***	**11.06**
Xylose	18.99 ± 1.2	7 ± 2.4
Galactose	4.32 ± 3.8	1.22 ± 3.2
Arabinose	6.24 ± 4.1	2.36 ± 3.0
Mannose	2.46 ± 1.1	1.71 ± 2.1
Lignin total	**28.6**	**21.47**
Acid soluble	8.65 ± 1.9	5.23 ± 2.3
Acid nonsoluble	19.95 ± 2.7	16.24 ± 2.1
Ash	**6.58 ± 0.59**	**4.43 1.3**

*Due to supply of a water molecule to each broken glucosidic bond during hydrolysis of celluose or hemicellulose, the contents of monosugars were multiplied by a factors of 0.9 (hexose) or 0.88 (pentose) to convert into cellulose and hemicellulose, respectively. Data are means of three replications.

Enzyme Reactivity

In general, the yield of cellulose conversion to glucose was increased with an increased dose of cellulase loading from 0 to 59 FPU/g of cellulose for both micro-crystalline cellulose Avicel and the X-ER (Fig. 2). After 162 h of enzymatic treatment, however, higher levels of cellulase loading did not improve the glucose conversion for the X-ER, such as shown at 50.5 and 59 FPU/g levels (Fig. 2). In contrast, Avicel appeared to have an increased conversion yield at a higher enzyme loading. It also consistently showed higher levels of conversion efficiency than that of the X-ER.

Avicel, a microcrystalline cellulose, has often been chosen as a good model substrate, as it is considered to be highly ordered and commercially available in standardized form. Due to its compact structure, however, the enzymatic hydrolysis of Avicel is much slower compared with its swollen form when treated with phosphoric acid (Andersen et al. 2008). This study incorporated the use of Avicel as a reference substrate to evaluate the enzyme reactivity of X-ER. Within 96 h of enzyme hydrolysis, Avicel displayed higher glucose conversion levels than the X-ER, and its glucose yield was significantly higher than X-ER at all enzyme loading levels at 162 h. The highest glucose yield of 82 g/L was observed for Avicel at a cellulase loading of 59 FPU/g. In contrast, the X-ER yielded only 56 g of glucose under the same conditions. These results suggest that the

incompletely deconstructed hemicellulose and lignin structures in the X-ER limited the accessibility of the enzymes. Unlike degradation in a layer-by-layer fashion for Avicel, the enzymatic hydrolysis of the X-ER is much slower and needs to be further improved.

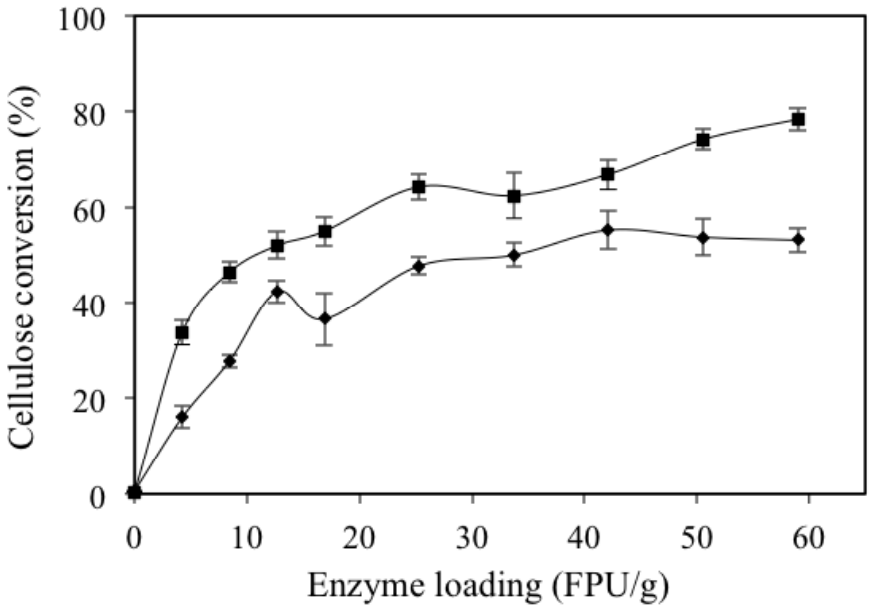

Fig. 2. Comparison of glucose conversion response of microcrystal cellulose Avicel (■) and xylose-extracted corncob residue (♦) treated with various cellulase loading doses of Acellerase 1000 after 162 h reactions

Liquid-Solid Ratio

Among numerous factors affecting efficiency of the X-ER enzyme treatment, the ratio of hydrolysis liquid to cellulosic solid is a significant element. The yield of cellulose conversion was increased with increasing liquid-solid ratio from 5 to 15 (Fig. 3a); however, glucose yield obtained from the cellulose conversion was decreased with increasing liquid-solid ratio. When the rate of cellulose conversion was examined, it was obvious that most of the cellulose conversion happened during the first 24 h with a highest conversion rate of 1.6% per hour (Fig. 3b).

Removal of Sugars Released during Saccharification

Inhibition of end products affects cellulose conversion significantly. Removal of converted sugars such as glucose and cellobiose during continued enzymatic saccharification in this study showed improved yield of subsequent glucose conversion. Compared to an untreated control, treatments of earlier removal after 12 h by buffer extract and the X-ER extract displayed a significant increase in glucose conversion yield of approximately 30 and 42% when examined after 24 and 36 h, respectively (Fig. 4). No significant difference was observed in efficiency of enzymatic hydrolysis supplemented with either X-ER extract or a sodium acetate buffer. A late removal treatment at 24 h by buffer extract also showed an increase of approximately 20% in glucose yield for continued hydrolysis after an additional 12 h (Fig. 4).

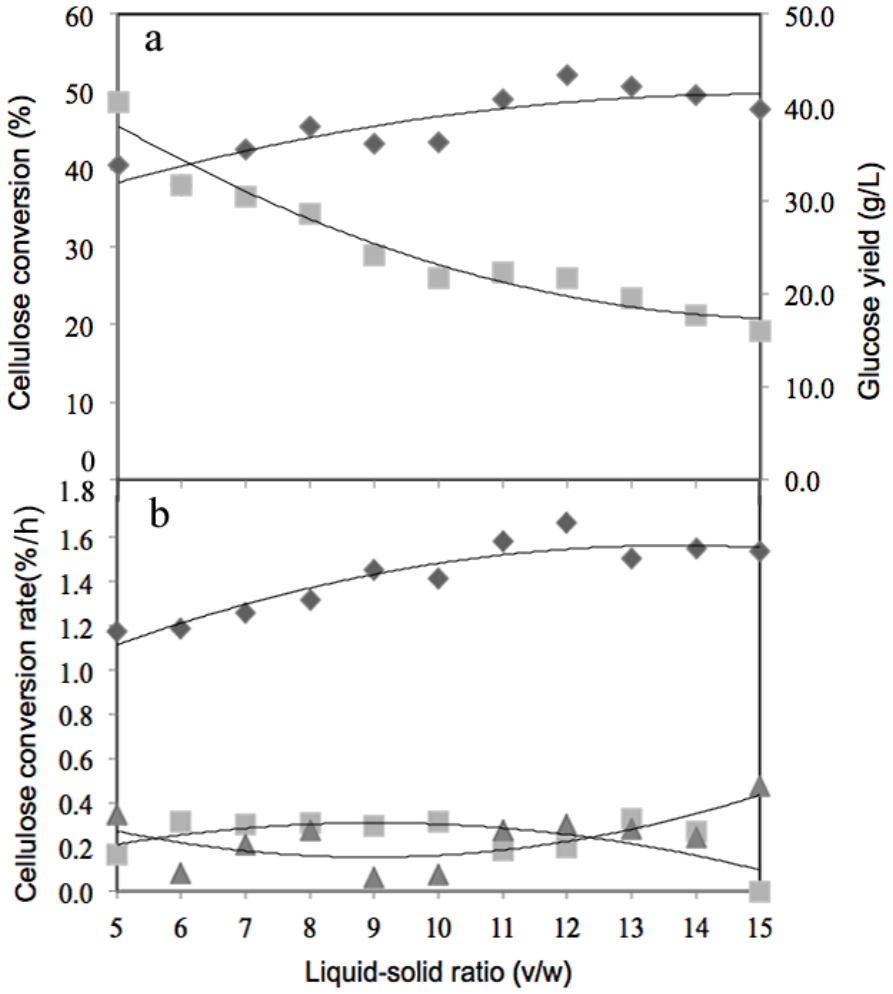

Fig. 3. Effects of liquid-solid X-ER ratio on yield of (a) cellulose conversion (♦) and glucose yield (■); and cellulose conversion rate (b) at 24 (♦), 48 (■) and 72 h (▲) in enzymatic saccharification reactions from liquid-solid ratios 5 to 15. The cellulose conversion rate was defined as percentage of cellulose conversion per hour within 24 h.

Inhibition of enzyme hydrolysis efficiency by cellobiose and glucose released during the hydrolysis has been observed (Azevedo et al. 2002; Gusakov and Sinitsyn 1992). In this study, removing sugars from the hydrolysate increased the glucose conversion yield up to approximately 30% compared with a non sugar-removal control. The presence of the X-ER extract did not appear to affect the efficiency of enzymatic hydrolysis significantly.

As mentioned earlier, the cellulose conversion yield was slightly improved with the increased liquid-solid ratio. In fact, the addition of buffer or X-ER extract after the sugar removal increases liquid-solid ratio. Thus, the decreased concentrations of the end products attributed to the increased cellulose conversion rate as demonstrated in this study.

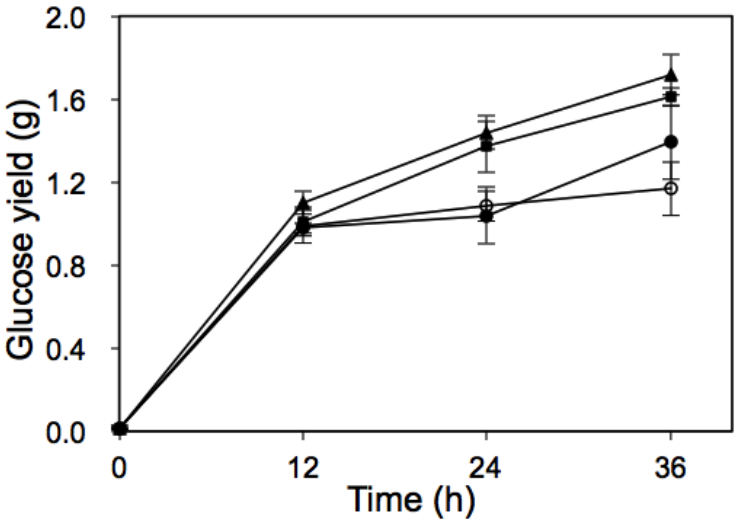

Fig. 4. Effects of sugar removal on subsequent glucose release during continued enzymatic saccharification for samples removed at 12 h and refilled with a buffer, B12, (■), at 12 h and refilled with an extract of xylose-extracted corncob residue, E12, (▲), at 24 h and refilled with a buffer, B24, (●) compared with a none removal control (○) over time.

Adsorption of Cellulose onto Cellulosic Substrates

Most cellulolytic enzymes are able to attach to cellulosic or non-cellulosic materials by means of a cellulose binding domain (CBD). Higher accessibility levels of cellulose facilitate cellulose adsorption for efficient enzymatic hydrolysis. However, non-productive adsorption reduces efficiency of enzymatic saccharification. The endoglucanase in the projected enzyme profile of the Accellerase was not detected from 10 to 60 min after addition of substrate microcrystal cellulose, a control, and the enzyme was partially recovered 3 days after the substrate addition as examined by SDS gel electrophoresis (Fig 5d). This indicated efficient cellulose binding to a great amount of accessible surface areas of Avicel for cellulase. The lignaceous residue sample displayed a significant loss of soluble enzyme recovery over time (Fig. 5c). On the other hand, substrates X-ER+Tween80 and X-ER showed significantly higher amounts of enzyme recovery from 10 min to 3 days after the substrate addition (Fig. 5a, b). It was obvious that most non-productive adsorption occurred during the earlier time of the hydrolysis (around 60 min) except for Avicel. Quantitative protein analysis further confirmed these observations by the BCA method (Table 2). On the other hand, glucose released from the total enzyme treatment was the highest for microcrystal Avicel, followed by lignaceous residue, X-ER+Tween80, and the X-ER (Table 2).

The X-ER showed a lower non-productive adsorption rate of cellulase comparing with the cellulose control and the Avicel and lignaceous hydrolysis residues, especially within the first hour of treatment. It appeared that the incomplete deconstruction of the X-ER structures with fewer exposed binding sites might be related to the limited non-productive adsorption. Surfactant treatment has been suggested to reduce non-productive adsorption of enzymes (Eriksson et al. 2002).

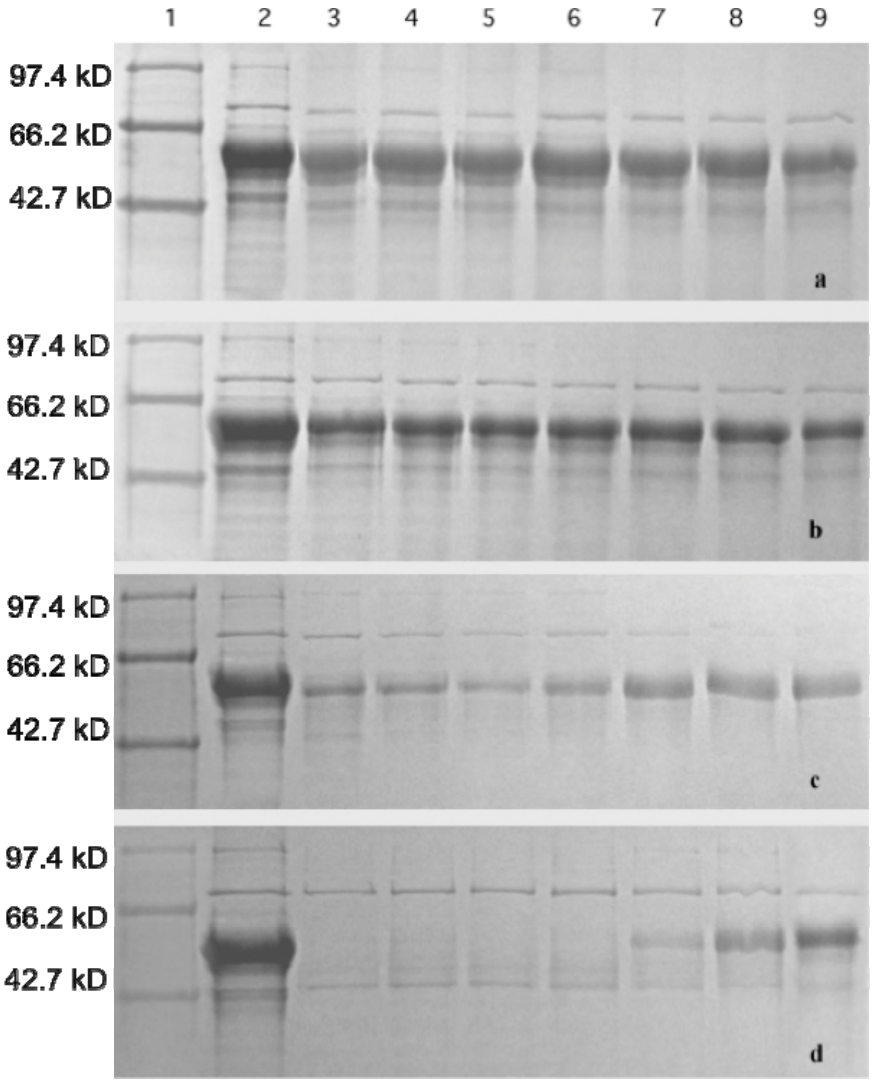

Fig. 5. SDS-PAGE gels showing the recovery of cellulose Accellerase 1000 after enzyme treatment for substrate xylose-extracted corncob residues (X-ER)+Tween80 (a), X-ER (b), lignaceous hydrolysis residue (c), and microcrystal cellulose Avicel (d). Lanes are marked as follows: 1, molecular marker; 2, a control sample containing an enzyme without a substrate (the intensive band close to 55 kD serves as a reference of anticipated endoglucanase); 3, samples taken after reactions at 10 min; 4, 20 min; 5, 35 min; 6, 60 min; 7, 1day; 8, 2day; and 9, 3 day.

In this study, the addition of Tween 80 did not affect the non-productive adsorption significantly. On the other hand, the rate of cellulose conversion by the enzyme treatment of X-ER was significantly lower than that of Avicel and lignaceous hydrolysis residues.

The relatively lower levels of enzyme adsorption explained the slower cellulose conversion rate of X-ER; thus, the limited enzyme accessibility was the major cause of the low enzyme reactivity in the X-ER saccharification process.

Table 2. Glucose Conversion and Soluble Protein Detected from Four Substrates Compared with a Cellulase Hydrolysis Control without X-ER during the Hydrolysis Treatment over Time

Time (min)	Glucose yield (g/L)				
	Avicel	Ligaceous residue	X-ER	X-ER + Tween80	Control
0	0.01	0.00	0.16	0.14	0.04
10	3.60	1.70	1.40	1.90	0.03
20	6.70	2.00	2.10	1.90	0.05
35	7.70	2.70	2.20	2.20	0.04
60	9.20	3.90	2.50	2.70	0.03
1440	27.70	15.80	7.20	9.00	0.06
2880	44.00	23.70	9.80	12.50	0.05
4320	59.00	30.60	14.20	15.20	1.00
	Soluble protein (μg/mL)				
0	ND[a]	ND	ND	ND	ND
10	18.07	30.16	38.79	54.34	64.88
20	22.24	29.21	46.91	58.68	67.18
35	20.75	32.52	45.93	52.36	64.40
60	21.56	30.43	40.09	58.54	67.87
1440	29.12	29.76	37.51	53.54	67.88
2880	50.93	31.72	42.52	56.86	65.03
4320	53.76	30.71	46.57	58.08	69.60

[a] Not determined.

Secondary Dilute Acid Hydrolysis

After a secondary dilute acid hydrolysis of the X-ER, additional xylose was recovered at 11.5g/L (Table 3). Small amounts of glucose, arabinose, and galactose were also recovered from the additional acid hydrolysate. As expected, the concentration of furfural was also increased by the additional acid treatment. Cellulose obtained following this second acid treatment demonstrated significantly higher levels of glucose conversion compared with that of the untreated X-ER at all examined time points from 24 to 72 h (Fig. 6).

Table 3. Composition of Water Insoluble Solid and Hydrolyzates of Corncobs and Xylose Extracted Corncob Residue (X-ER) by Dilute Acid Hydrolysis Pretreatment

	Water insoluble solids			Composition of hydrolyzates				
	Total gravimetric recovery (%)	Cellulose (%)	Hemicellulose (%)	Glucose (g/l)	Xylose (g/l)	Galactose (g/l)	Arabinose (g/l)	Furfural (g/l)
Corncobs	60.3 ± 1.5	47.2 ± 3.1	10.2 ± 2.1	2.9 ± 0.3	36.4 ± 0.5	2.1 ± 0.7	3.9 ± 0.4	0.9 ± 0.1
X-ER	82.2 ± 4.6	45.1 ± 1.9	8.2 ± 0.9	1.3 ± 0.6	11.5 ± 1.1	0.6 ± 0.1	1.2 ± 0.1	1.3 ± 0.5

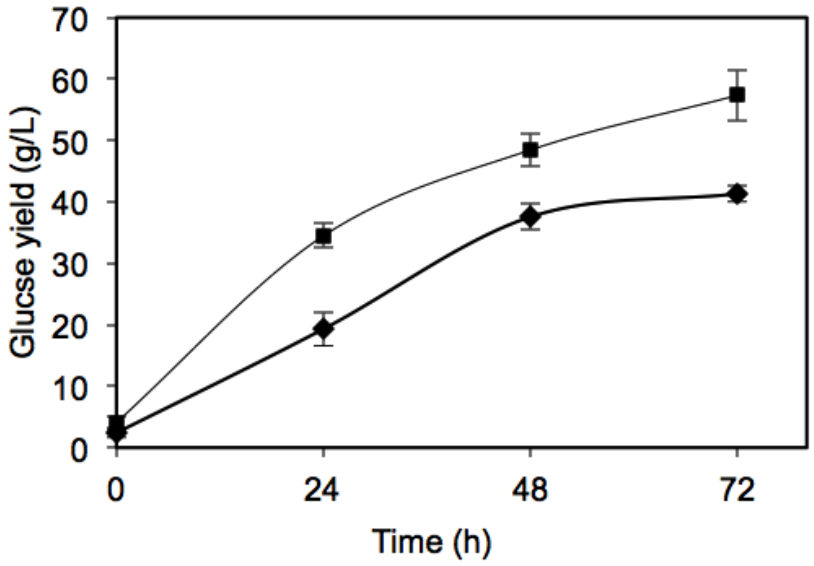

Fig. 6. Comparison of glucose conversion between a conventional processed xylose-extracted corncob residue (X-ER) (♦) and an X-ER treated with a secondary dilute acid hydrolysis (■) over time

Using a secondary acid treatment, more than 20% yield of xylose was recovered from the X-ER. The results also showed that glucose conversion yield was higher than non-treated X-ER from 24 to 72 h after the secondary acid hydrolysis of the X-ER. It appeared that the current processed X-ER is not well-degraded and not adequate for immediate utilization of efficient cellulosic ethanol production. A secondary acid hydrolysis pretreatment of the X-ER improved sugar release but would increase process steps and cost. For a combined production of xylose and cellulosic ethanol production, it is desirable to utilize a single acid pretreatment procedure that is acceptable for both economic xylose production and the subsequent efficient cellulosic ethanol conversion. The challenge is to develop a more efficient corncob pretreatment procedure to meet the requirements of economic utilizations of corncobs and the X-ER. Results obtained from this study will aid further investigation and decision-making toward improved and integrated processing procedures.

CONCLUSIONS

This study presents the first characterization of the X-ER as a potential substrate for cellulosic ethanol production. A substantial amount of cellulose was found remaining in the X-ER that can be utilized for cellulosic ethanol production; however, the X-ER obtained by the current acid treatment for the first step of xylose production was not effective for immediate application of efficient cellulosic ethanol conversion. Due to its incomplete deconstruction for efficient sugar release, further improved corncob pretreatment procedures and fermentation strategies are needed for the combined productions of xylose and cellulosic ethanol using corncobs and its byproduct X-ER.

ACKNOWLEDGMENTS

This work was supported in part by awards of Ministry of Science and Technology, China Projects of 2009AA10Z406 and 2010DFA61200, and the U.S. Department of Agriculture 2008 International Research Project 3620-41000-123-124. The authors thank Michael A. Cotta for critical reading of the manuscript. The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Article submitted: June 22, 2011; Peer review completed: August 17, 2011; Revised version received and accepted: Sept. 5, 2011; Published: Sept. 8, 2011.