Appl Biochem Biotechnol (2014) 172:1045–1054
DOI 10.1007/s12010-013-0584-5

Fungal Cellulase/Xylanase Production and Corresponding Hydrolysis Using Pretreated Corn Stover as Substrates

Liang Zhang · Xiaoqing Wang · Zhenhua Ruan · Ying Liu · Xiaorui Niu · Zhengbo Yue · Zhimin Li · Wei Liao · Yan Liu

Received: 16 July 2013 / Accepted: 3 October 2013 /
Published online: 19 October 2013

Abstract Three pretreated corn stover (ammonia fiber expansion, dilute acid, and dilute alkali) were used as carbon source to culture *Trichoderma reesei* Rut C-30 for cellulase and xylanase production. The results indicated that the cultures on ammonia fiber expansion and alkali pretreated corn stover had better enzyme production than the acid pretreated ones. The consequent enzymatic hydrolysis was performed applying fungal enzymes on pretreated corn stover samples. Tukey's statistical comparisons exhibited that there were significant differences on enzymatic hydrolysis among different combination of fungal enzymes and pretreated corn stover. The higher sugar yields were achieved by the enzymatic hydrolysis of dilute alkali pretreated corn stover.

Keywords *Trichoderma reesei* · Pretreated corn stover · Cellulase · Xylanase · Enzymatic hydrolysis

Introduction

Lignocellulose such as agricultural crop residues represents one of the most abundant renewable resources on the planet that provides an untapped resource for biofuel production [1–4]. Annually, 1.3 billion tons of lignocellulosic residues are available for biofuels production in the US [5]. It was estimated that approximate 80–90 billion gallons of biofuels could be produced from the lignocellulosic residues, which could replace up to 40 % of the national fuel consumption [5, 6]. In addition, lignocellulosic fuels as an alternative of fossil

L. Zhang · X. Wang · Z. Ruan · Y. Liu · X. Niu · Z. Yue · W. Liao · Y. Liu (✉)
Department of Biosystems and Agricultural Engineering, Michigan State University,
203 Farrall Hall, East Lansing, MI 48864, USA
e-mail: liuyan6@msu.edu

L. Zhang
School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China

Z. Li
State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology,
130 Meilong Rd., Shanghai 200237, China

fuels could also reduce greenhouse gas emission, avoid competition with food resources, stimulate rural economies, and provide a stable and secure source of energy production [7].

Enzymatic conversion of lignocellulose into fermentable sugars is one of the critical steps for lignocellulosic biofuel production. It has been widely reported that most of lignocellulose degrading enzymes are generated from fungal species such as *Trichoderma reesei*, *Aspergillus niger*, and *Phanerochaete chrysosporium* etc. [8–10]. Among which, the filamentous fungus *T. reesei* is a paradigm for commercial-scale production of cellulases and hemicellulases. Many strategies have been developed to improve enzyme activity and production [11, 12]. It has been reported that lignocellulose could be used as both inducer and substrate in the fermentation processes for cellulase production to significantly improve enzyme yields [13–15]. Our previous study also indicated that *T. reesei* cultivation on alkali pretreated lignocellulose had better cellulase/xylanase production than acid pretreated ones [16]. Enzymes from cultures on alkali pretreated samples were superior at the enzymatic hydrolysis of alkali pretreated samples, and enzymes from cultures on acid pretreated samples were good at hydrolysis of acid pretreated samples.

In the current study, the production of cellulase/xylanase was examined on different pretreated corn stover as carbon sources. The consequent enzymatic hydrolysis applying fungal enzyme complexes on pretreated corn stover samples was conducted in order to elucidate the relationship of fungal enzyme production, enzymatic hydrolysis, and different pretreatment methods.

Material and Methods

Substrates and Carbon Sources

The corn stover of yellow dent corn (zea mays indenata) was collected from a private farm at Muir, Michigan (43.005965, 84.975343) in October 2009. It was air-dried and ground using a mill (Willey Mill, standard model no. 3, Arthur H. Thomas, Philadelphia, PA) with 4 mm size opening at the MSU Crop and Soil Science Teaching and Research Field Facility. Three pretreatment methods (ammonia fiber expansion (AFEX), dilute alkali, and dilute acid) were applied on corn stover. The AFEX corn stover was pretreated in a high-pressure reactor under 130 °C and 15 min with a liquid ammonia-to-biomass ratio of 1:1(w w^{-1}). At the end of 15 min, the pressure was reduced to atmospheric level. The pretreated biomass was left under a fume hood overnight to ensure the residual ammonia being volatized. For dilute acid and dilute alkali pretreatment, sulfuric acid or sodium hydroxide at 1 % (w w^{-1}), reaction temperature of 120 °C, and pretreatment time of 2 h was applied, respectively. The pretreated corn stover was washed three times using deionized water. The washed corn stover samples were stored in a 4 °C refrigerator for further uses. The composition of pretreated corn stover were determined and exhibited in Table 1.

Table 1 Chemical compositions of differently pretreated corn stover

Substrates	Cellulose (%)	Hemicellulose (%)	Lignin (%)
AFEX corn stover	34.8	21.4	11.00
Alkali treated corn stover	53.1	23.0	5.5
Acid treated corn stover	59.9	0.2	19.74

Microorganism and Inoculum Preparation

The filamentous fungus *T. reesei* Rut C-30 (ATCC 56765) was obtained from the American Type Culture Collection (Manassas, VA). According to a previous study [17], the fungus was cultured on potato dextrose agar slants at 28 °C for 7 days to accumulate spores. The mature spores were then washed and suspended with sterilized distilled water and stored at 4 °C. One milliliter of spore suspension (1×10^9 spores mL^{-1}) was inoculated into 50 mL of potato dextrose broth medium in a 125 mL Erlenmeyer flask and cultured at 27 °C for 24 h on a rotary shaker at shaking speed of 2.32 Hz to obtain the inoculum.

Enzymes Production

Two types of media, chemical defined medium and media with three pretreated corn stover as carbon sources, were used for *T. reesei* culture to demonstrate the effects of the pretreated corn stover on cellulase and xylanase production. The chemical-defined medium was modified based on Mandels' medium [10] including 10 g L^{-1} of glucose, 10 g L^{-1} lactose, 10 g L^{-1} cellulose (Sigma 310697), 10 g L^{-1} xylan (Sigma 9014-63-5), 0.3 g L^{-1} yeast extract (Acumedia 7184A), 0.75 g L^{-1} peptone (Sigma P5905), 15 g L^{-1} KH_2PO_4 (J.T. Baker 3246), 5 g L^{-1} $(NH4)_2SO_4$ (CCI 0555AL), 1.23 g L^{-1} $MgSO_4{\cdot}7H_2O$ (J.T. Baker 2500), 0.8 g L^{-1} $CaCl_2{\cdot}2H_2O$ (J.T. Baker 1332), 4 g L^{-1} $CaCO_3$ (Sigma 310034), 0.5 g L^{-1} Tween (Sigma P1754), 0.0027 g L^{-1} of $FeSO_4{\cdot}7H_2O$ (Sigma F8048), 0.0016 g L^{-1} of $MnSO_4{\cdot}H_2O$ (Sigma M7634), 0.0014 g L^{-1} $ZnSO_4{\cdot}7H_2O$ (Sigma 221376), and 0.0036 g L^{-1} $CoCl_2{\cdot}6H_2O$ (Sigma 255599). The media with pretreated corn stover were composed of 15 g L^{-1} pretreated corn stover (from Section Substrates and carbon sources) to replace carbon substrates in the chemical-defined medium (glucose, cellulose, xylan, yeast extract, and peptone). Four grams per liter of calcium carbonate was used as pH buffer. Two grams per liter (dry basis) seed was inoculated into 100 mL sterilized medium in 250 mL Erlenmeyer flask to carry out the culture on a rotatory shaker (Thermal Scientific) at 2.33 Hz, and the temperature was maintained at 27 °C. Cellulase/xylanase activities were monitored during the course of cultivation till 96 h. After fermentation, the broth was centrifuged at 2,000 g for 5 min. The supernatant was kept as the enzyme solution for further enzymatic hydrolysis.

Enzymatic Hydrolysis

Cellulase/xylanase from section Enzymes production was used to carry out enzymatic hydrolysis of three pretreated corn stover by a completely randomized design with two replicates. A commercial enzyme (Accellerase 1500, Genencor, Palo Alto, CA) was used as the control. Wet pretreated samples (2 g dry matter) from section Substrates and carbon sources were mixed with deionized water to make 20 g mass in 125 mL Erlenmeyer flasks and autoclaved. After cooling down, 5 U cellulose per gram dry fiber (equivalent to 10 FPU per gram dry fiber) and sterilized 0.05 mol L^{-1} citrate buffer (pH 4.8) were added into the flasks to make 40 g slurry (solid concentration was 5 % (w w^{-1})). Each flask was placed on an incubator at 2.33 Hz and 50 °C. The enzymatic hydrolysis was done at 48 h. After hydrolysis, the samples were removed from the shaker and put on ice to stop the reaction; the hydrolysate was separated by centrifugation at 4,000 g for 5 min to obtain a clear sugar solution, which was then filtered through a 0.22 μm polyethersulfone membrane filter for HPLC analysis. The clear enzymatic hydrolysate solutions were stored at 4 °C for further use.

Analytical Methods

The pretreated corn stover samples were analyzed for cellulose, xylan, and lignin content according to the National Renewable Energy Laboratory's analytical procedure for determination of structural carbohydrates and lignin in biomass [18]. Cellulase and xylanase activity was analyzed as previously reported [16].

Cellobiose, glucose, xylose, and mannose in the enzymatic hydrolysate and fermentation broth were determined by high-performance liquid chromatography (Shimadzu Prominence, Kyoto, Japan), equipped with a Bio-Rad Aminex HPX-87P analytical column and a refractive index detector. The mobile phase was e-pure water from Barnstead E-pure Water System at a flow rate of 0.6 mL min^{-1}. The column temperature was 60 °C.

Statistical Analysis

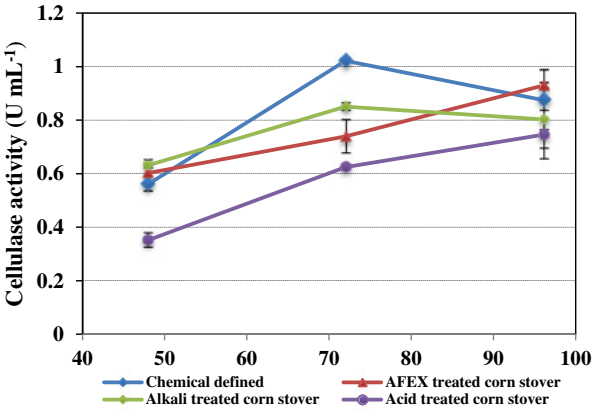
A general linear model using R software (R version 2.15.0, Vienna, Austria) was applied to the experimental data in order to perform an analysis of variance (ANOVA) and multiple comparisons. Tukey's test, using a comparison-wise type I error rate of 0.05, was adopted to compute honestly significant differences among different carbon sources regarding enzymes production and enzymatic hydrolysis.

Results and Discussion

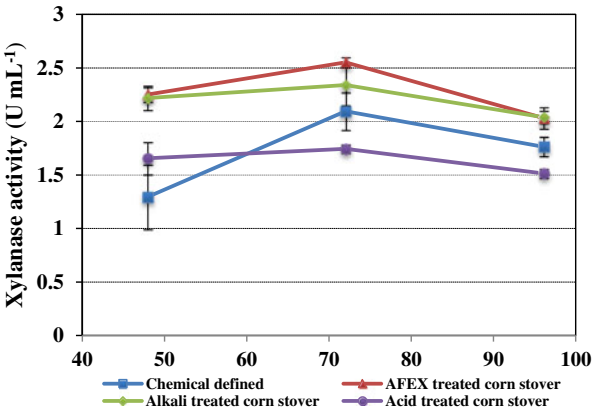
Enzyme Production

The *T. reesei* culture with 15 g L^{-1} pretreated corn stover (AFEX treatment, dilute alkali treatment, and dilute acid treatment) as carbon sources were compared with the culture on a chemical-defined medium to evaluate the effects of pretreated corn stover on cellulase and xylanase production (Fig. 1). At the end of the culture period of 96 h, the highest cellulase production was obtained from AFEX pretreated corn stover medium (0.93 U mL^{-1}), followed by chemical-defined medium (0.88 U mL^{-1}), dilute alkali pretreated corn stover medium (0.8 U mL^{-1}), and then dilute acid pretreated corn stover medium (0.74 U mL^{-1}) (Fig. 1a). However, ANOVA analysis indicated that there was no statistically significant difference in terms of cellulase production at type I error of 0.05. As for xylanase activity, AFEX and alkali pretreated corn stover generated equivalent xylanase activities of 2.03 and 2.04 U mL^{-1}, respectively ($p>0.05$), which were significantly higher than the xylanase produced from acid-pretreated corn stover medium (1.51 U mL^{-1}) ($p<0.05$) (Fig. 1b). Xylanase activities from chemical-defined medium were generally better than those from acid-pretreated stover medium, but worse than those from AFEX and dilute alkali pretreated stover media.

Compared with AFEX and dilute alkali pretreated corn stover, the culture on dilute acid pretreated corn stover had less cellulase and xylanase production at the end of the 96-h culture (Table 2). The reduced lignin content (Table 1), swelled fiber, and increased internal surface area in dilute alkali and AFEX pretreated corn stover [7] could be the reasons that made more cellulose and hemicellulose available to enhance the growth of the organism and induce synthesis of the related enzymes. In addition, acid pretreatment removed most of hemicellulose in the corn stover, while AFEX and dilute alkali pretreatment only removed part of hemicellulose (Table 1). Less hemicellulose led to the lack of xylanase inducer in the acid pretreated corn stover, which could be another cause that acid pretreated corn stover produced less xylanase.



(a) Cellulase



(b) Xylanase

Fig. 1 Fungal cellulase (**a**) and xylanase (**b**) production on pretreated corn stover. The cellulase and xylanase activities were the average of replicates with standard error

Cellulase and xylanase production by *T. reesei* in the four media varied during culture (Fig. 1). Cellulase production from dilute acid and AFEX pretreated corn stover continuously increased in 96-h culture period, and the cellulase activities reached 0.74 and 0.93 U mL^{-1}, respectively, at the end of the culture. Whereas the highest cellulase activities from chemical-defined and dilute alkali media of 1.02 and 0.85 U ml^{-1} were achieved at 72 h (Fig. 1a). The cellulase activities then leveled off with increase of the culture time. The chemical-defined medium had more mono-sugars and other available carbon sources than

Table 2 Enzyme activities at the end of the 96 h culture[a]

Substrates	Cellulase (U mL^{-1})	Xylanase (U mL^{-1})
Chemical-defined medium	0.88	1.77
AFEX treated corn stover	0.93	2.03
Alkali treated corn stover	0.80	2.04
Acid treated corn stover	0.75	1.52

[a]Data are the average of duplicates

the media with pretreated corn stover, which facilitated fungal metabolism of cellulase production and reached the highest enzyme activity in a shorter culture time. As for the cultures on pretreated corn stover, it has been reported that the oligosaccharide formation during cellulose hydrolysis of fungal cultivation plays an important role in cellulase induction [19]. Gradually releasing oligosaccharides during fungal cultivation on pretreated corn stover would lead to a slow induction of cellulase production, which could be a reason that the cellulase activities generally kept increasing with a lower productivity during the culture course. However, xylanase production exhibited different pattern from cellulase production. Xylanase activities of all cultures were peaked at 72 h (Fig. 1b). The highest xylanase activities of acid pretreated corn stover, chemical defined, alkali pretreated corn stover, and AFEX corn stover were 1.74, 2.10, 2.34, and 2.55 U mL^{-1} (Fig. 1b). As aforementioned, xylan content in the media played an important role on xylanase activity. Generally, less xylan in the acid pretreated corn stover led the less xylanase activities during the fungal cultivation.

Enzymatic Hydrolysis

Hydrolysis performance of the enzymes obtained from different media was evaluated by applying them back on the pretreated corn stover samples (AFEX, dilute alkali, and dilute acid pretreated corn stover) (Table 3). The enzymes from AFEX and dilute alkali pretreated corn stover media produced more sugars on all pretreated corn stover samples than the

Table 3 Sugars from enzymatic hydrolysis using Accellerase 1500 and enzymes derived from culture on differently pretreated corn stover [d, e]

Substrate	Enzyme source	Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)
AFEX-treated corn stover	Enzyme (AFEX)[a]	7.40	6.63	6.61	0.16	0.99
	Enzyme (alkali)[b]	6.46	6.21	6.88	0.16	1.01
	Enzyme (acid)[c]	4.50	6.48	4.88	0.00	0.86
	Chemical defined	5.41	4.50	5.13	0.11	0.47
	Accellerase 1500®	1.66	12.86	2.73	0	0.42
Alkali-treated corn stover	Enzyme (AFEX)[a]	12.64	14.83	12.94	0.13	0.99
	Enzyme (alkali)[b]	11.68	16.47	12.68	0.13	1.01
	Enzyme (acid)[c]	8.04	15.02	9.06	0.00	0.90
	Chemical defined	16.27	10.96	11.28	0.10	0.52
	Accellerase 1500®	0.95	19.60	5.05	0	0.30
Acid-treated corn stover	Enzyme (AFEX)[a]	14.69	6.04	1.25	0.00	0.00
	Enzyme (alkali)[b]	14.37	5.52	1.30	0.00	0.00
	Enzyme (acid)[c]	12.05	6.68	0.99	0.00	0.00
	Chemical defined	12.92	3.06	0.88	0.10	0.00
	Accellerase 1500®	0.00	20.95	1.01	0.00	0.00

[a] Enzyme (AFEX) is the enzymes from culture on AFEX-treated corn stover

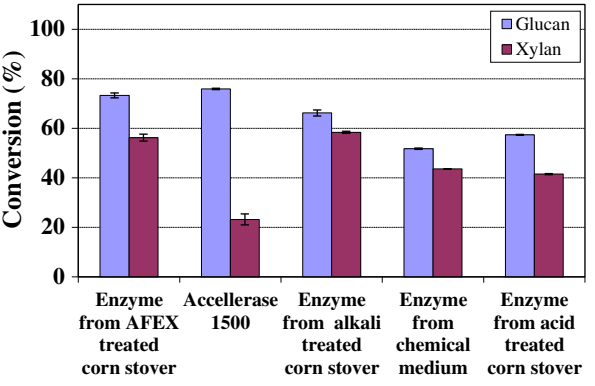
[b] Enzyme (alkali) is the enzymes from culture on alkali-treated corn stover

[c] Enzyme (acid) is the enzymes from culture on acid treated corn stover

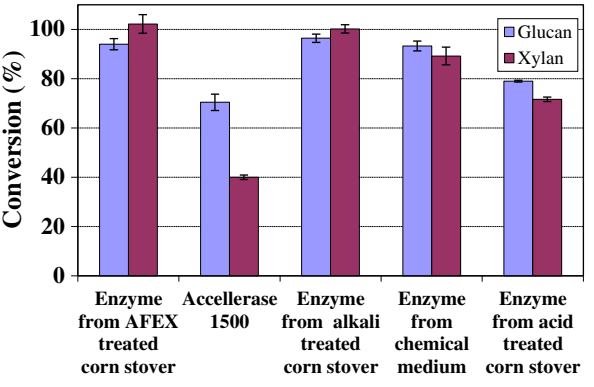
[d] 5 % (w/w) solid concentration and 5 U cellulase/g fiber (all based on dry matter), incubated at 50 °C for 48 h

[e] Data presented are the average of duplicates

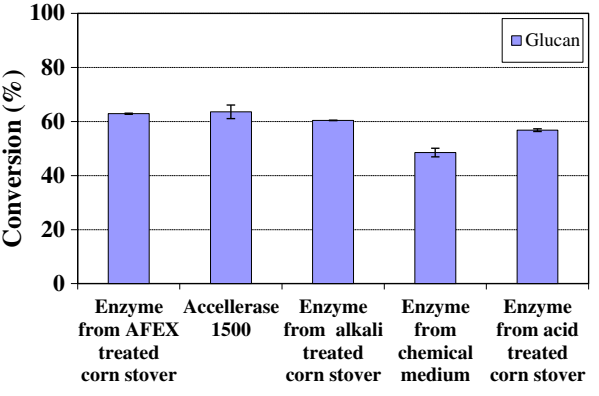
enzymes from the chemical-defined medium. AFEX and dilute alkali pretreated corn stover had higher conversion for all enzymatic hydrolysis than the acid pretreated corn stover (Fig. 2).



(a) AFEX treated corn stover



(b) Alkali treated corn stover



(c) Acid treated corn stover

Fig. 2 Enzymatic hydrolysis of pretreated corn stover using enzymes from different cultures media (**a**). AFEX pretreated corn stover (**b**). Alkali pretreated corn stover (**c**). Acid pretreated corn stover. Enzyme from AFEX treated corn stover meant enzyme was produced using AFEX pretreated corn stover as a substrate. Accellerase 1500 was a commercial cellulase. Enzyme from alkali-treated corn stover meant enzyme was produced using dilute alkali pretreated corn stover as a substrate. Enzyme from chemical medium meant enzyme was produced using chemical-defined medium. Enzyme from acid-treated corn stover meant enzyme was produced using dilute acid pretreated corn stover as a substrate. The enzymatic conversion were the average of replicates with standard error

Tukey's multiple comparison was used to interpret the differences of enzymatic hydrolysis on pretreated corn stover. For the hydrolysis of AFEX pretreated corn stover, ten groups of glucan conversion between five enzymes were compared. Significant differences were found between nine groups ($p<0.01$) except the group of enzymes derived from AFEX pretreated corn stover and Accellerase 1500 ($p>0.05$) (Fig. 2a, Table 4). The enzymes produced from AFEX, dilute alkali, and dilute acid pretreated corn stover presented better glucan conversion on AFEX pretreated corn stover than the enzyme from chemical-defined medium. Moreover, ten comparison groups of xylan conversion demonstrated that enzymes from AFEX, dilute alkali, dilute acid pretreated corn stover, and chemical-defined medium had better hydrolysis performance ($p<0.05$) than Accellerase 1500 (Fig. 2a, Table 5). There was no significant ($p>0.05$) difference on xylan conversion of AFEX pretreated corn stover between the enzymes from chemical-defined medium and from acid pretreated corn stover medium (Table 5). Xylan conversion using the enzyme from AFEX pretreated corn stover and the enzyme from alkali pretreated corn stover were significantly ($p<0.05$) higher than the enzymes from chemical-defined medium and acid-treated corn stover medium. There was also no significant difference on xylan conversion of AFEX-treated corn stover between two enzymes from alkali and AFEX-treated corn stove medium ($p>0.05$) (Table 5).

As for enzymatic hydrolysis of dilute alkali pretreated corn stover, no matter what kinds of enzyme was used, glucose concentration from enzymatic hydrolysis of dilute alkali pretreated corn stover were higher than those from AFEX and dilute acid pretreated corn stover (Table 3). In terms of both glucan and xylan conversion, all enzymes from treated corn stover and chemical-defined medium performed significantly ($p<0.05$) better than Accellerase 1500 (Fig. 2b, Tables 4 and 5). Based on the enzyme activity measurements, the activity ratio of xylanase and cellulase in Accellerase 1500 (0.65) was much lower than the enzymes from pretreated corn stover and chemical-defined medium (2.0–2.5). The

Table 4 Glucan conversion of differently pretreated corn stover using different enzyme complexes

Comparison groups for glucan conversion	AFEX	Dilute alkali	Dilute acid
Enzyme from acid medium[a]–Accellerase 1500	<0.0001	<0.05	<0.05
Enzyme from AFEX medium[b]–Accellerase 1500	>0.05	<0.001	>0.05
Enzyme from alkali medium[c]–Accellerase 1500	<0.001	<0.001	>0.05
Enzyme from Chemical medium[d]–Accellerase 1500	<0.00001	<0.001	<0.001
Enzyme from AFEX medium–Enzyme from acid medium	<0.0001	<0.01	<0.05
Enzyme from alkali medium–Enzyme from acid medium	<0.001	<0.01	>0.05
Enzyme from chemical medium–Enzyme from acid medium	<0.01	<0.01	<0.01
Enzyme from alkali medium–Enzyme from AFEX medium enzyme	<0.001	>0.05	>0.05
Enzyme from chemical medium–Enzyme from AFEX medium enzyme	<0.0001	>0.05	<0.001
Enzyme from chemical medium–Enzyme from alkali medium	<0.0001	>0.05	<0.01

[a] Enzyme from acid medium indicated enzyme complex was produced from the medium using dilute acid pretreated corn stover as carbon source

[b] Enzyme from AFEX medium indicated enzyme complex was produced from the medium using AFEX pretreated corn stover as carbon source

[c] Enzyme from alkali medium indicated enzyme complex was produced from the medium using dilute alkali pretreated corn stover as carbon source

[d] Enzyme from chemical medium indicated enzyme complex was produced from the chemical defined medium

Table 5 Xylan conversion of differently pretreated corn stover using different enzyme complexes

Comparison groups for xylan conversion	AFEX	Dilute alkali
Enzyme from acid medium[a]–Accellerase 1500	<0.001	<0.001
Enzyme from AFEX medium[b]–Accellerase 1500	<0.0001	<0.001
Enzyme from alkali medium[c]–Accellerase 1500	<0.0001	<0.001
Enzyme from Chemical medium[d]–Accellerase 1500	<0.0001	<0.001
Enzyme from AFEX medium–Enzyme from acid medium	<0.001	<0.01
Enzyme from alkali medium–Enzyme from acid medium	<0.001	<0.01
Enzyme from chemical medium–Enzyme from acid medium	>0.05	<0.01
Enzyme from alkali medium–Enzyme from AFEX medium enzyme	>0.05	>0.05
Enzyme from chemical medium–Enzyme from AFEX medium enzyme	<0.001	<0.05
Enzyme from chemical medium–Enzyme from alkali medium	<0.001	<0.05

[a] Enzyme from acid medium indicated enzyme complex was produced from the medium using dilute acid pretreated corn stover as carbon source

[b] Enzyme from AFEX medium indicated enzyme complex was produced from the medium using AFEX pretreated corn stover as carbon source

[c] Enzyme from alkali medium indicated enzyme complex was produced from the medium using dilute alkali pretreated corn stover as carbon source

[d] Enzyme from chemical medium indicated enzyme complex was produced from the chemical defined medium

synergistic action of cellulase and xylanase in the enzymes might contribute to the better hydrolysis performance.

Dilute acid pretreated corn stover was the only substrate that contains a negligible amount of xylan and was therefore a suitable substrate to investigate the conversion efficiency of cellulases from different culture media. The data in Fig. 2c showed that there was no significant difference between the enzymes from AFEX pretreated corn stover, dilute alkali pretreated corn stover medium, and Accellerase 1500 ($p>0.05$). However, there was significant difference between Accellerase 1500 and the enzymes from chemical-defined medium or dilute acid pretreated corn stover medium ($p<0.05$) (Table 4). The results demonstrated that the enzymes from AFEX and dilute alkali pretreated corn stover exhibited better performance on the enzymatic hydrolysis of dilute acid pretreated corn stover than the enzymes from chemical-defined medium or dilute acid pretreated corn stover medium.

Conclusions

Cellulase and xylanase were produced by *T. reesei* with four different substrates. The statistical analysis showed that the cellulase production was independent of the substrates, while xylanase production was dependent on the substrates. In terms of both cellulase and xylanase production, dilute acid pretreated corn stover was considerably less efficient substrate than AFEX and dilute alkali pretreated corn stover. Although different enzymes had different hydrolysis performance on pretreated corn stover, the enzymes from the media with AFEX and dilute alkali pretreated corn stover again demonstrated superior performance on glucan and xylan conversions compared to dilute acid treated corn stover. These results concluded that AFEX and dilute alkali pretreatment have great potential on biological conversion of corn stover for fuel and chemical production.

Acknowledgments This study was financially supported by the Northeast Sun Grant Initiative, US Department of Transportation (grant number NE08-013), and the Program of Introducing Talents of Discipline to Universities, the State Administration of Foreign Experts Affairs, P. R. China (grant number 111-2-06). The authors would like to thank Dr. Bruce Dale at Michigan State University for providing AFEX corn stover samples and Genencor Inc. for providing Accellerase 1500 samples.

References

1. Huber, G. W., Iborra, S., & Corma, A. (2006). *Chemical Reviews, 106*, 4044–4098.
2. Yue, Z., Teater, C., Liu, Y., Maclellan, J., & Liao, W. (2010). *Biotechnology and Bioengineering, 105*, 1031–1039.
3. Hofrichter, M. (2002). *Enzyme and Microbial Technology, 30*, 454–466.
4. Ruan, Z., Zanotti, M., Zhong, Y., Liao, W., Ducey, C., & Liu, Y. (2013). *Biotechnology and Bioengineering, 110*, 1039–1049.
5. Perlack, R. D., Wright, L. L., Turhollow, A. F., Graham, R. L., Stokes, B. J., & Erbach, D. C. (2005). OAK RIDGE NATIONAL LAB TN.
6. Zheng, Y., Pan, Z., Zhang, R., Labavitch, J. M., Wang, D., & Teter, S. A. (2007). *Applied Biochemistry and Biotechnology, 136–140*, 423–435.
7. Sun, Y., & Cheng, J. (2002). *Bioresource Technology, 83*, 1–11.
8. Fang, H., Zhao, C., & Song, X. Y. (2010). *Bioresource Technology, 101*, 4111–4119.
9. Linko, S. (1992). *Biotechnology Advances, 10*, 191–236.
10. Mandels, M., & Weber, J. (1969). *Advances in Chemistry Series, 95*, 391–414.
11. Bigelow, M., & Wyman, C. E. (2002). *Applied Biochemistry and Biotechnology, 98–100*, 921–934.
12. Cheng, Y., Song, X., Qin, Y., & Qu, Y. (2009). *Journal of Applied Microbiology, 107*, 1837–1846.
13. Szengyel, Z., Zacchi, G., Varga, A., & Reczey, K. (2000). *Applied Biochemistry and Biotechnology, 84–86*, 679–691.
14. Lee, Y. H., & Fan, L. T. (1982). *Biotechnology and Bioengineering, 24*, 2383–2406.
15. Lo, C. M., Zhang, Q., Callow, N. V., & Ju, L. K. (2010). *Bioresource Technology, 101*, 717–723.
16. Zhang, L., Liu, Y., Niu, X., Liu, Y., & Liao, W. (2012). *Biomass & Bioenergy, 37*, 16–24.
17. Ghose, T. (1987). *Pure and Applied Chemistry, 59*, 257–268.
18. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D.(2008). *Laboratory Analytical Procedure*.
19. Huang, A. A. (1975). *Biotechnology and Bioengineering, 17*, 1421–1433.