Electronic supplementary information

~~Cellulolytic Enzyme-Aided Hemicellulose Extraction and Its Characteristics from Switchgrass~~

Jinhua Ding[†,§], *Chang Geun Yoo**[,¶], *Yunqiao Pu*[‡,x], *Xianzhi Meng*[§], *Samarthya Bhagia*[§,x], *Chongwen Yu**[,†,#], *Arthur J. Ragauskas**[,§,‡,x,//]

[†] College of Textiles, Donghua University, Shanghai, 201620, P.R. China
[§] Chemical & Biomolecular Engineering, University of Tennessee Knoxville, Knoxville, TN 37996, USA

[¶] Department of Paper and Bioprocess Engineering, State University of New York - College of Environmental Science and Forestry, Syracuse, NY 13210, USA
[‡] Center for Bioenergy Innovation (CBI), Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[x] UT-ORNL Joint Institute for Biological Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[#] Key Laboratory of Textile Science & Technology, Ministry of Education, China

[//] Department of Forestry, Wildlife, and Fisheries; Center for Renewable Carbon, The University of Tennessee Knoxville, Institute of Agriculture, Knoxville, TN 37996, USA

Corresponding Author:
[*]Chang Geun Yoo. Fax: 315-470-6945; Tel: 315-470-6516, E-mail: cyoo05@esf.edu
[*]Chongwen Yu. Fax: +8621-6779-2627; Tel: 13651603285, E-mail: yucw@dhu.edu.cn
[*]Arthur J. Ragauskas. Fax: 865-974-7076; Tel: 865-974-2042; E-mail: argauskas@utk.edu

Table S1. Carbohydrate Analysis of raw switchgrass, hemicellulose samples and residues (%).

Sample I.D.	Glc	Xyl	Ara	Gal	GalA	GlcA	Hemicellulose[1]	Lignin[2]
Raw-Swg	56.54	24.20	2.57	0.89	0.75	0.46	27.66	14.59
CEH	6.36	40.07	18.16	15.24	3.33	5.65	82.45	5.22
DMSOH	25.99	63.32	2.29	1.83	0	1.33	68.77	3.50
AEH	10.62	65.40	15.04	3.76	0	0	84.20	4.93
Residues of CEH	21.58	19.16	6.09	3.69	-	-	28.94	-
Residues of DMSOH	35.33	26.72	4.35	1.51	-	-	32.58	-
Residues of AEH	76.48	9.44	2.48	0.79	0.83	0.85	12.71	-

1 Hemicellulose include Xyl, Ara, Gal, GalA and GlcA.

2 Lignin includes acid soluble lignin and acid insoluble lignin.

Table S2. Assignment of ^{13}C-^{1}H hetero-correlated HSQC NMR spectra of hemicelluloses from switchgrass biomass.

Chemical shift δ_C/δ_H（ppm）	Assignment
22.7/1.9	Acetyl CH_3
55.6/3.8	Methoxyl
60.5/3.7	α-D-Glcp(6)+Aγ
62.3/4.2	(1-4)-*β*-D-Xyl*p* (5eq)
62.5/3.4	(1-4)-*β*-D-Xyl*p* (5ax)
72.2/3.2	(1-4)-*β*-D-Xyl*p* (2)
75.0/3.4	(1-4)-*β*-D-Xyl*p* (3)
75.7/3.8	(1-4)-*β*-D-Xyl*p* (4)
73.6/4.6	2-*O*-acetyl-*β*-D-Xyl*p* (2)
99.4/4.6	2-*O*-acetyl-*β*-D-Xyl*p* (1)
101.9/4.3	3-*O*-acetyl-*β*-D-Xyl*p* (1)
96.4/5.0	*α*-D-Man
97.5/5.3	4-*O*-Me-*α*-D-Glucuronic acid(C_1H_1)
81.7/3.23	4-*O*-Me-*α*-D-Glucuronic acid(C_4H_4)

102.1/4.5	(1-4)-*β*-D-Glc*p* (C_1H_1)
101.1/4.4	(1-4)-*β*-D-Xyl*p* (C_1H_1)
101.7/4.5	Xyl (C_1)-Uronic acid
84.3/4.2	C_β/H_β in *β*-*O*-4 linkage (A)
103.7/6.7	S (2/6)
115.2/6.8	G (5)/H (3/5)
128.9/7.2	H (2/6)
130.2/7.5	*p*-coumaric acid unit (2/6)
144.9/7.5	ferulic acid (7)
52.3/3.3	C_β/H_β in *β*-*β* resinol
84.2/4.3	C_β/H_β in *β*-*O*-4-H/G
87.3/4.1	C_β/H_β in *β*-*O*-4-S
71.3/4.8	C_α/H_α in *β*-*O*-4 linkage (A)

Table S3. The main functional groups assignment of hemicellulose from switchgrass in FTIR spectra.

Wave numbers (cm^{-1})	**Functional group**	**Compounds**	**References**
3343	O–H stretching	Hemicellulose	1
2950-2850	C–H stretching	Methyl group	2
1736	C=O stretching	Acetyl, uronic, and ferulic ester	2-8
1640	C=O stretching	Carboxylation and/or carbonylation	2-6
1562	Conjugated C-O	Glucuronic acid, Inorganic carboxylate	3, 9
1514	Aromatic skeletal vibration	Lignin	10
1462	$\delta_{as}CH_3$	Asymmetric bending in CH_3 of lignin	10
1413	–COO– symmetric stretching	Uronic acids	11
1375	C–H vibration of polysaccharides; C-CH_3 stretching	Cellulose	12
1252	–COOH vibration	Glucuronic acid	3
1164	C-O-C, C-OH	Arabinosyl side branches, pyranose ring skeletal	10, 11
1170-1000		Typical absorption peak of xylan	9
1091	C-O-C stretch vibration	Pyranose ring skeletal	11
1039-1049	C-O-C antisymmetric stretch vibration	Typical absorption peak of xylan	11
990	xylopyranosyl	Arabinosyl side branches	13
899	C1 group	*β*-1,4-glucosidic bond, *β*-D-xylose	14, 15

765-600	C-C stretch	The ring of sugar	11

The chemical shifts ($^{13}C/^{1}H$ ppm) of WCW and holocellulose from switchgrass in 2D NMR regions were presented in **Figure S1**. Three different plotted regions of NMR spectra provide the information of lignin sidechain and polysaccharides in aliphatic (**Figure S1**, a and b), polysaccharides in anomeric (**Figure S1**, c and d) and lignin aromatics in aromatic regions (**Figure S1**, e and f). The main ~~C1/H1~~ correlation peaks in this research, listed in **Table S2**, were assigned according to previous studies.[16-20]

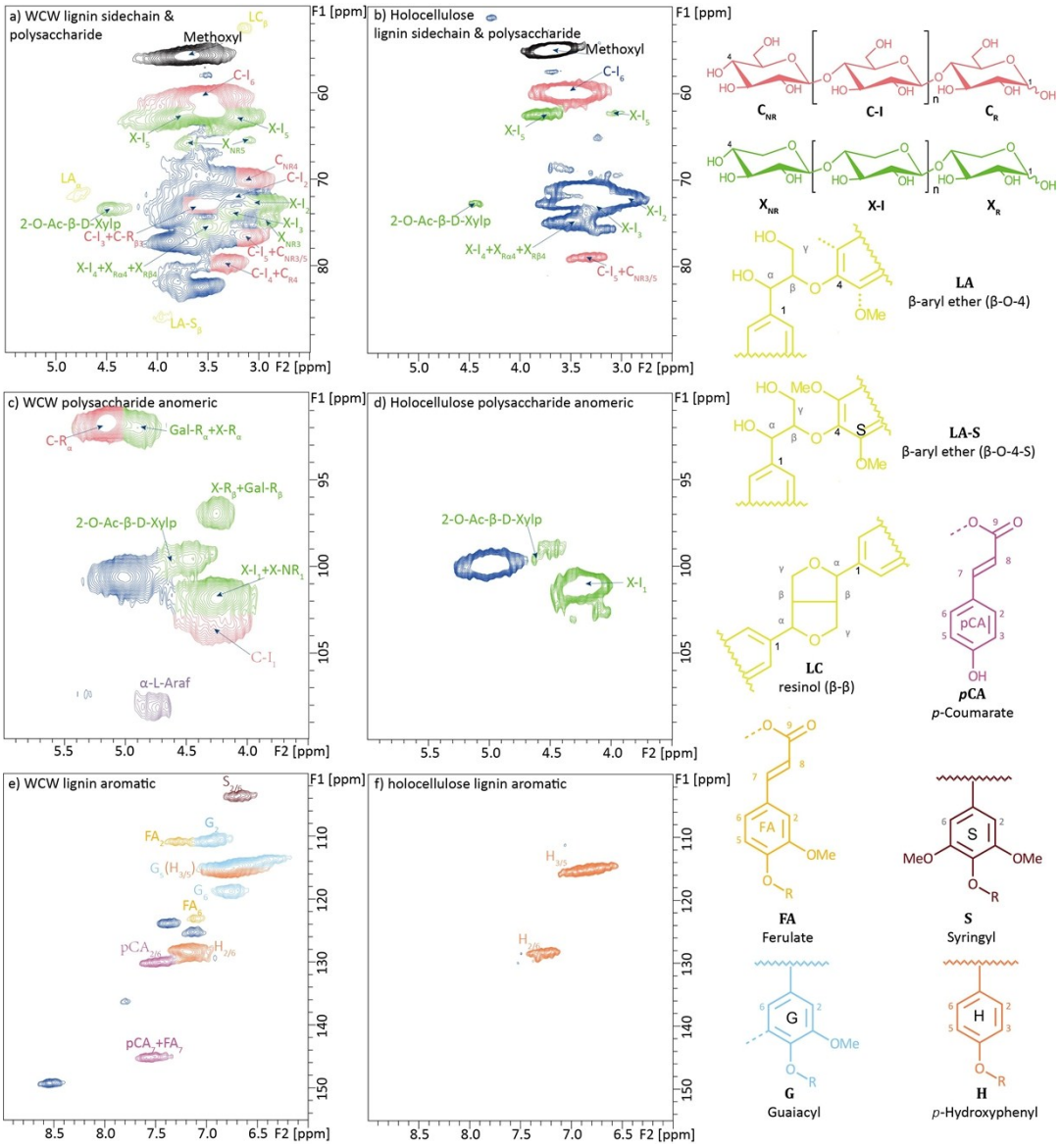

Figure S1. Assignments for the HSQC spectra of WCW and isolated holocellulose from switchgrass.

Two NMR spectra from WCW and holocellulose did not show significant differences in the structure and proportions of the 4-*O*-Methylglucurono-arabinoxylan before and after PAA treatment. Acetylated 4-*O*-Methyl-glucuronoxylan is a major hemicellulose component in the grass with the acetyl groups frequently attached to the C2 and C3 position. A correlation peak of C2/H2 of 2-*O*-acetyl-β-D-Xyl*p*(2) was present at δ_C/δ_H 73.6/4.58, while the peak of 3-*O*-acetyl-β-D-Xyl*p*(3) was not observed. In the polysaccharide anomeric region, C1/H1 signals of 2-*O*-acetyl-*β*-D-Xyl*p*(1) was also present at δ_C/δ_H 99.4/4.59 ppm, whereas, due to overlapping signals it was not obvious whether the C1/H1 peak of 3-*O*-acetyl-*β*-D-Xyl*p* was present at δ_C/δ_H 101.9/ 4.51ppm.

Details of the correlations corresponding to the non-anomeric carbohydrates were presented in **Figure S1** (a and b), which also indicate diagnostic lignin subunit structural type information. Notable differences were observed in the lignin aromatic region of the spectra, as described in **Figure S1** (e and f). To obtain NMR- based guaiacyl/ syringyl (S/G) value of switchgrass WCW, the volume integral of C-H pairs in a similar environment ($S_{2/6}$ and G_2) were used. The ratio quantified by volume integration of the appreciate contours is ~~0.478~~ for switchgrass WCW. The peaks corresponding to lignin *β*-*O*-4 (LA), *β*-*O*-4-S (LA-S) and *β*-*β* resinol (LC) were presented in WCW lignin sidechain and polysaccharides region, but were absent from holocellulose. In addition, the absence or decrease of contours for G-units, S-units, *p*-coumaric acid unit (*p*CA) and ferulic acid (FA) of holocellulose (**Figure S1**, g) compared to

the contours in WCW spectra indicated effective delignification (**Figure S1**, f). Interestingly, *p*-hydroxyphenyl (H) unit was clearly observed in lignin aromatic regions even after PAA delignification (**Figure S1**, g and f). Among the three main lignin components, the *p*-hydroxyphenyl unit was found to be more flexible, followed by the guaiacyl unit, whereas the syringyl group was the most refractory.[21] The high content of H-units in holocellulose of switchgrass possibly due to oxidation of *p*CA and FA, which could generate *p*-hydroxyphenyl (H) during PAA treatment.[22] The hydroxycinnamates (*p*CA and FA) widely occur in the grasses polymers, with *p*CA acylating the *γ*-OH of the lignin sidechains, and predominantly on S-units, whereas FA acylate arabinosyl residues of arabinoxylan chains and participate in both polysaccharide-polysaccharide and lignin-polysaccharide cross-coupling reactions.[23] Taken together, the results indicated that the treatment with PAA resulted in the removal of G-units, S-units, cleavage of lignin sidechains, and a considerable dissolution of lignin fraction.

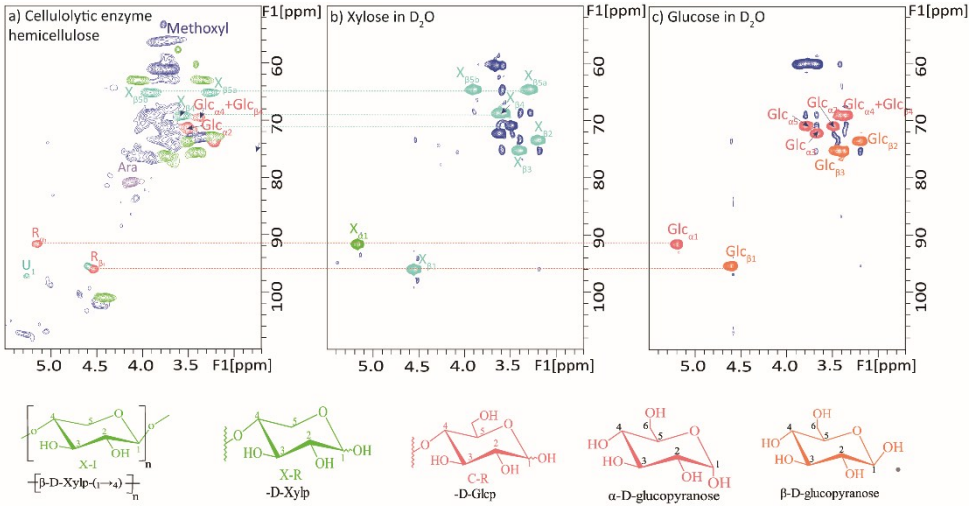

Figure S2. 2D 1H-13C HSQC spectrum of CEH and other saccharide models in D2O. a) cellulolytic enzyme hemicellulose, b) xylose, c) glucose.

2D HSQC NMR spectra of xylose and glucose were also obtained in the same condition as CEH. (Figure S2).

FTIR analysis.

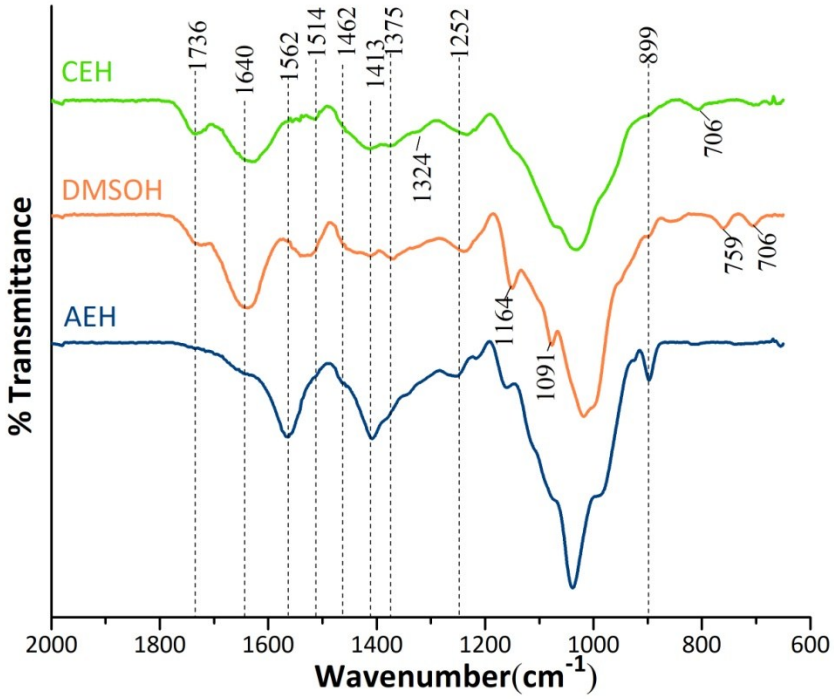

Figure S3. FTIR spectra of alkaline-extracted CEH, DMSOH, and AEH.

The band between 1175 and 1000 cm^{-1}, which are typical of xylan, reflected the stretching and bending vibrations of C-O, C-C, C-OH, and C-O-C asymmetric stretch vibration signal.[24]

In the CEH spectra, two peaks at 1736 cm^{-1} and 1413 cm^{-1} can be attributed to acetyl C=O or symmetric stretching -COO- (carboxyl) associated with hemicellulose.[8] This result reflected that hemicellulose extracted by enzyme-aided method retained acetyl group and uronic acid group, which was in agreement with carbohydrate analysis. The intensity of peaks at 1640 cm^{-1} in CEH and DMSOH were higher than that of AEH. The absorption at 1640 cm^{-1} was also observed in the previous study, which maybe attributed principally to the C=O stretching

vibration from carboxylate groups.[3, 5, 6, 15] The sugar analysis of the hemicellulose samples confirmed the presence of a small amount of 4-*O*-methyl glucuronic acid (MeGlcA) in the CEH and DMSOH, which was also seen in the FTIR spectra. (**Figure S3**). Meanwhile, a trace amount of signal at 1640 cm^{-1} indicates the absence of uronic acid in AEH. The peaks at 1462, 1324 and 1252cm^{-1} are related to C–H stretching, O–H bending and the stretching bands C–O and O–H in the hemicelluloses, respectively.[25]

The presence of the arabinosyl side-chains is documented by the two low-intensity shoulders at 1175 and 990 cm^{-1}, which have been reported to be attached only at positions of the xylopyranosyl constituents.[26] Increase in the number of branches was also associated with the disappearance or a significant decrease in the intensity of peaks at 1175-990 cm^{-1},[27] which are related to arabinosyl substituent contribution for the identification of arabinoxylan structures. The absence of these signals indicated abundant branches in the CEH, which is consistent with the HSQC NMR results.

References

1. F. K. Liew, S. Hamdan, M. R. Rahman, M. Rusop, J. C. H. Lai, M. F. Hossen and M. M. Rahman, *J. Chem.*, 2015, **2015**.
2. A. Monteiro, B. Leonardi, H. Savastano and J. Baruque-Ramos, *Green Mater.*, 2015, **3**, 120-131.
3. K. Bilba and A. Ouensanga, *J. Anal. Appl. Pyrolysis*, 1996, **38**, 61-73.
4. M. Grilc, B. Likozar and J. Levec, *Catal. Today*, 2015, **256**, 302-314.
5. Y. Zhao, C. Xu, C. Xing, X. Shi, L. M. Matuana, H. Zhou and X. Ma, *Ind. Crop. Prod.*, 2015, **65**, 96-101.
6. C. Saurabh, R. Dungani, A. Owolabi, N. Atiqah, A. Zaidon, N. A. S. Aprilia, Z. Sarker and H. Khalil, *Bioresources*, 2016, **11**, 6742-6755.
7. G. A. Lyons, C. McRoberts, H. S. Sharma, R. McCormack, E. Carmichael and R. D. McCall, *Bioresour. Technol.*, 2013, **146**, 184-191.
8. G. Xu, L. Wang, J. Liu and J. Wu, *Appl. Surf. Sci.*, 2013, **280**, 799-805.
9. S. Bhagia, Y. Pu, B. R. Evans, B. H. Davison and A. J. Ragauskas, *Bioresour. Technol.*, 2018, **269**, 567-570.

10. M. L. Fidalgo, M. C. Terrór, A. T. Martínez, A. E. González, F. J. González-Vila and G. C. Galletti, *J. Agric. Food. Chem.*, 1993, **41**, 1621-1626.
11. Y. Peng and S. Wu, *J. Anal. Appl. Pyrolysis*, 2010, **88**, 134-139.
12. C. Xu, A. S. Leppänen, P. Eklund, P. Holmlund, R. Sjöholm, K. Sundberg and S. Willför, *Carbohydr. Res.*, 2010, **345**, 810-816.
13. P. Robert, M. Marquis, C. Barron, F. Guillon and L. Saulnier, *J. Agric. Food. Chem.*, 2005, **53**, 7014-7018.
14. S. Gupta, R. N. Madan and M. C. Bansal, *Tappi J.*, 1987, **70**, 113-114.
15. D. Morais de Carvalho, A. Martínez-Abad, D. V. Evtuguin, J. L. Colodette, M. E. Lindström, F. Vilaplana and O. Sevastyanova, *Carbohydr. Polym.*, 2017, **156**, 223-234.
16. J. Ø. Duus, C. H. Gotfredsen and K. Bock, *Chem. Rev.*, 2000, **100**, 4589-4614.
17. H. Kim and J. Ralph, *RSC Adv.*, 2014, **4**, 7549-7560.
18. J. Rencoret, A. Gutierrez, L. Nieto, J. Jimenez-Barbero, C. B. Faulds, H. Kim, J. Ralph, A. T. Martinez and J. C. del Rio, *Plant Physiol.*, 2011, **155**, 667-682.
19. K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 1983, **41**, 27-66.
20. H. Kim and J. Ralph, *Org. Biomol. Chem.*, 2010, **8**, 576-591.
21. J. Long, W. Lou, L. Wang, B. Yin and X. Li, *Chem. Eng. Sci.*, 2015, **122**, 24-33.
22. U. Takahama, *Physiol. Plant.*, 1995, **93**, 61-68.
23. J. Ralph, *Phytochem. Rev.*, 2010, **9**, 65-83.
24. A. U. Buranov and G. Mazza, *Carbohydr. Polym.*, 2010, **79**, 17-25.
25. O. Chaikumpollert, P. Methacanon and K. Suchiva, *Carbohydr. Polym.*, 2004, **57**, 191-196.
26. R. C. Sun and J. Tomkinson, *Carbohydr. Polym.*, 2002, **50**, 263-271.
27. M. Kačuráková, P. S. Belton, R. H. Wilson, J. Hirsch and A. Ebringerová, *J. Sci. Food Agric.*, 1998, **77**, 38-44.