ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Physical Insight into Switchgrass Dissolution in Ionic Liquid 1-Ethyl-3-methylimidazolium Acetate

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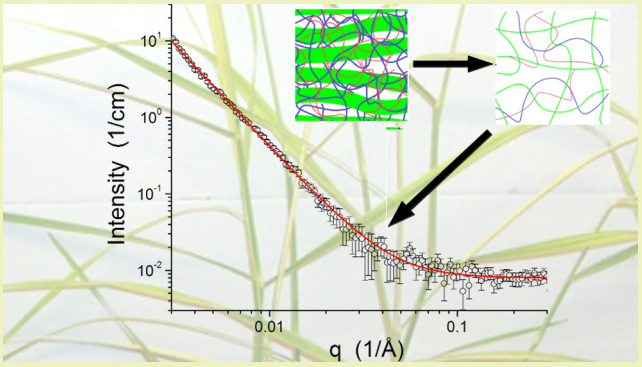
ABSTRACT: Small-angle neutron scattering was used to characterize solutions of switchgrass and the constituent biopolymers cellulose, hemicellulose, and lignin, as well as a physical mixture of them mimicking the composition of switchgrass, dissolved in the ionic liquid (IL) 1-ethyl-3-methylimidazolium acetate. The results demonstrate that the IL dissolves the cellulose fibrils of switchgrass, although a supramolecular biopolymer network remains that is not present in solutions of the individual biopolymers and that does not self-assemble in a solution containing the physical mixture of the individual biopolymers. The persistence of a network-like structure indicates that dissolving switchgrass in the IL does not disrupt all of the physical entanglements and covalent linkages between the biopolymers created during plant growth. Reconstitution of the IL-dissolved switchgrass yields carbohydrate-rich material containing cellulose with a low degree of crystallinity, as determined by powder X-ray diffraction, which would impact potential downstream uses of the biopolymers produced by the process.



KEYWORDS: *Ionic liquids, Switchgrass, Small-angle neutron scattering, Biopolymers, Physical entanglements, Biomass deconstruction*

INTRODUCTION

A great deal of attention continues to be paid to the development of renewable resources for sustainable industries to meet the problems associated with depletion of the world's petroleum supply.[1] Lignocellulosic biomass, a potential sustainable energy source and chemical feedstock, poses an incredible challenge as a result of its naturally occurring variability and inherent recalcitrance to breakdown. Separation and purification of biopolymers from biomass generally requires the use of energy-intensive processes and hazardous chemicals.[2] The replacement of traditional chemical systems with ionic liquids (ILs, defined as salts that melt below 100 °C [3]) in biomass processing has been suggested as a possible way toward realizing its full potential for a variety of applications.[4−7]

Complete dissolution of both softwoods and hardwoods was demonstrated in 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl)[8,9] and 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]),[10] with the latter being a better solvent for wood dissolution. After dissolution, carbohydrate-rich material (CRM or pulp) and carbohydrate-free lignin can be regenerated by adding precipitating solvents, such as an acetone/H_2O mixture (1:1, v/v).[8,10] In addition, the three major biopolymers in lignocellulosic biomass, cellulose, hemicellulose, and lignin, can be co-dissolved in [C_2mim][OAc] and separated during reconstitution.[11]

Our results in biomass dissolution using ILs suggested that any carbohydrates not bonded to lignin or with fewer lignin bonds will be preferentially dissolved,[10] and the use of a catalyst or reagent that selectively cleaves lignin−carbohydrate bonds would enhance dissolution and decrease the lignin content of the recovered pulp.[12] To better understand the nature of the IL-dissolved lignocellulosic biomass and how this relates to biopolymer separation and the reconstituted materials, we dissolved switchgrass (*Panicum virgatum*), a perennial crop of key interest for biofuel production,[13,14] in [C_2mim][OAc] and analyzed the solution using small-angle neutron scattering (SANS), which probes structures in materials over length scales spanning from 10 to 1000 Å.[15] For comparison, we also investigated IL solutions of isolated xylan (a major component of hemicellulose), lignin, and cellulose, as well as a mixture of these biopolymers approximating their composition in switchgrass. We further analyzed the crystallinity of the CRM reconstituted from the switchgrass/IL solution using powder X-ray diffraction to understand the impact of the process on the structure of the product material. The results provide new physical insight into the dissolution of biomass in ILs.

Received: February 11, 2014
Revised: April 8, 2014
Published: April 13, 2014

MATERIALS AND METHODS

Materials and Chemicals. Microcrystalline cellulose (MCC) with degree of polymerization 270, xylan (from beechwood), and all organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and were used as received. Indulin AT lignin (from the kraft pulping process) was provided by MeadWestvaco Corporation (Glen Allen, VA, U.S.A.). The IL, [C_2mim][OAc], was purchased from Iolitec USA (Ionic Liquids Technologies, Inc., Tuscaloosa, AL, U.S.A.). Deionized (DI) water was obtained from a commercial deionizer (Culligan, Northbrook, IL, U.S.A.) with specific resistivity of 16.82 MΩ cm at 25 °C.

Switchgrass Sample Preparation. Dried lowland cultivar Alamo switchgrass (*Panicum virgatum*) was obtained as described previously.[16] Samples harvested at Oak Ridge National Laboratory (ORNL, Oak Ridge, TN, U.S.A.) were shipped to the National Renewable Energy Laboratory (NREL, Golden, CO, U.S.A.) for drying at room temperature and size reduction by Wiley milling to pass through a 20-mesh screen (0.841 mm upper size limit). The samples were stored in a freezer to maintain moisture content prior to being dissolved in [C_2mim][OAc].

Dissolution and Biopolymer Recovery. Dissolution and recovery of the biopolymers and of switchgrass in [C_2mim][OAc] followed the approach described previously by Sun et al.[10] Five different samples were prepared: switchgrass, cellulose, xylan, Indulin AT lignin, and a physical mixture of cellulose (37%), xylan (28%), and Indulin AT lignin (22%), which is consistent with switchgrass composition.[17] Briefly, 0.5 g of each sample was added to 10.0 g of IL in a 20 mL vial and put in an oil bath preheated to 110 °C while being subjected to vigorous magnetic stirring. Aliquots of the solution were taken out to determine if all of the particles were dissolved by viewing it through an optical microscope (25× magnification). Complete dissolution was reported as the point in time at which a homogeneous solution free of undissolved materials was observed. It took 1 h to completely dissolve the cellulose and xylan and 1.5 h to completely dissolve the lignin and the biopolymer mixture. In contrast, the switchgrass required 44 h to completely dissolve.

For reconstitution and material recovery, the switchgrass/IL solution was poured into a 300 mL beaker containing 100 mL of a 1:1 (v/v) mixture of acetone and water and stirred at room temperature for 1 h followed by centrifugation. The supernatant was transferred to a 300 mL beaker for lignin recovery. The precipitated CRM was further washed with an acetone/water (1:1 v/v) mixture twice more and then with DI water to ensure all of the lignin and IL were washed out before subjecting the material to vacuum filtration using a ceramic funnel with nylon filter paper (20 μm). The lignin was precipitated from the supernatant and combined wash solutions by allowing the acetone to evaporate while the material was stirred. The recovered lignin was separated from the remaining aqueous IL solution by vacuum filtration as above, but 0.8 μm nylon filter paper was used because of the smaller lignin particle size. The lignin and CRM were dried overnight in an oven (Precision Econotherm Laboratory Oven, Natick, MA, U.S.A.) at 90 °C.

Small-angle neutron scattering (SANS). SANS data were collected using the Bio-SANS instrument at the High Flux Isotope Reactor of Oak Ridge National Laboratory.[18] The liquid samples were loaded into cylindrical quartz cuvettes (Hellma U.S.A., Inc.; Plainview, NY, U.S.A.) having a 1 mm path length (280 μL capacity). Three sample-to-detector distances were employed for the measurements of the switchgrass, namely 2.5 m, 6.8 m, and 15.3 m, while only the 2.5 and 6.8 m configurations were used for the measurements of solutions of the individual biopolymers. The wavelength, λ, was set to 6 Å and the wavelength spread, $\Delta\lambda/\lambda$, was set to 0.14. Data reduction followed standard procedures to account for detector sensitivity, dark current (electronic noise), and solvent background prior to azimuthally averaging the data to produce $I(q)$ *vs* q, where $q = 4\pi \sin(\theta)/\lambda$ and 2θ is the scattering angle from the incident beam. Data fitting was accomplished using the multilevel unified model for small-angle scattering data[19−21] as implemented in the IRENA tools[22] in IgorPro (Wavemetrics, Inc.; Lake Oswego, OR, U.S.A.). In cases where it was appropriate, the program ELLSTAT[23] was used to model the data.

Powder X-ray Diffraction (PXRD). The reconstituted CRM was ground using a mortar and pestle and deposited as a thin film onto a glass microscope slide for PXRD analysis, while the untreated switchgrass was directly deposited on a glass microscope slide for the measurements. PXRD data were collected using a PANalytical X'Pert Pro diffractometer (PANalytical B.V., Almelo, The Netherlands) using a 2θ-ω scan from 5° to 90° in 0.017° increments using an RTMS detector. The incident beam was conditioned using a Ni filter, 0.02° Soller slit, 0.5° anti-scatter slit, and variable divergence slit that provided a 2 mm irradiated length on the sample. The instrument resolution was measured using a National Institute of Standards and Technology (Gaithersburg, MD, U.S.A.) reference material (#660a, lanthanum boride) peak position and line-width diffraction standard and was found to be negligible compared to the width of the diffraction peaks of the sample. Data from the sample and an empty glass slide were collected to allow for direct subtraction of the instrumental background for data analysis.

PXRD data analysis followed the amorphous background subtraction approach described by Park and co-workers.[24] Briefly, after subtraction of the instrumental background, the PXRD pattern was treated as a set of diffraction peaks and an amorphous material background that results from amorphous cellulose and any remaining hemicellulose and lignin. For the data presented here, the amorphous material background was modeled as a single Gaussian curve. Five Gaussian functions were used to describe the diffraction pattern from the CRM, while it was possible to use only four Gaussian functions to model the diffraction pattern from the untreated switchgrass. To define the amorphous material background, the signal estimated to lie outside the diffraction peaks was fit using the single Gaussian function. After subtracting this amorphous background signal from the instrumental background-corrected data, the remaining diffraction pattern was fit using the set of Gaussian functions. The crystallinity index (CI) of the sample was calculated as the ratio of the area under the diffraction peaks divided by the area under the amorphous material background.[24]

RESULTS AND DISCUSSION

Dissolution and Reconstitution. Pretreatment of switchgrass using [C_2mim][OAc] demonstrates that high temperatures (e.g., 160 °C) can increase delignification of the lignocellulosic biomass and, therefore, the subsequent hydrolysis of the cellulosic material to sugars.[25,26] However, our previous work indicated that temperatures above the glass transition temperature of lignin results in significant degradation of the IL and the biopolymers (e.g., 15% of [C_2mim][OAc] was degraded after heating at 185 °C for only 10 min).[27] In this study, the switchgrass was heated at 110 °C for 44 h to ensure complete dissolution of the biomas without degradation of the IL and component polymers. The individual commercially obtained biopolymers and the mixture that mimicked the composition of switchgrass required much less time to dissolve than the switchgrass, as indicated in the Materials and Methods. The possible limitation of using these model biopolymers (beechwood xylan for hemicellulose and Indulin AT for lignin) is that they may not exactly mimic the real lignocellulosic biomass because of the different structures of the biopolymers that depend on the biomass resources. This variation in solubility can be expected based on the known differences between the chemical structures of these isolated biopolymers and those of switchgrass hemicellulose and lignin. Hardwoods such as beech produce glucuronoxylans composed of a xylan backbone with glucuronic acid substituents. As a result of the commercial isolation methods that remove the native acetylation and methylation from the glucuronxylan, the

isolated biopolymer contains anionic uronic acid groups that increase its solubility in polar solvents. Switchgrass produces an arabinoxylan with arabinose side groups on the xylan, which is less soluble as it does not have these acid groups. The most abundant inter-unit linkage in both wood and switchgrass is reported to be the β−O−4 aryl ether bond, so the chemistry of lignin solubilization is expected to be similar. However, switchgrass lignin is distinguished from wood lignin by its lower molecular weight and greater *p*-hydroxyphenyl (H lignin) content, as well as the esterification of the *p*-hydroxyphenyl groups with ferulic and coumaric acid groups of the hemicellulose.[28−31] Reconstitution of the switchgrass yielded 0.2455 g of CRM (mainly containing cellulose, with some hemicellulose and lignin[26]) and 0.0236 g of lignin, equating to 49.1% and 4.7% of the starting mass, respectively. Although not further studied, it is likely that the 44 h of heating required to dissolve the material caused some chemical breakdown of the biopolymers into material that is not retained during the post-dissolution reconstitution process.[10]

Small-Angle Neutron Scattering. The SANS data collected for switchgrass dissolved in [C_2mim][OAc] are shown in Figure 1. The data display a single, well-defined,

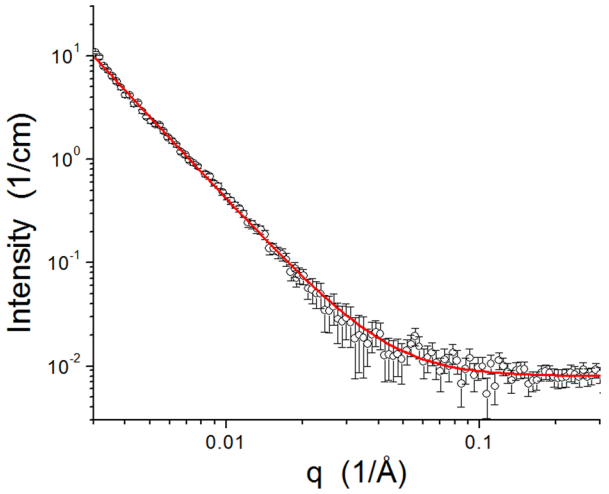


Figure 1. SANS data from switchgrass dissolved in [C_2mim][OAc] (open circles) and the power law fit to the data (red line).

power law character. Fitting of the data yields a power law exponent of 2.64 ± 0.02, consistent with a branched polymer network.[32] Unlike previous SANS studies of solutions and slurries of biomass and cellulose in conditions mimicking dilute acid pretreatment,[16,33−35] there is no indication in the present SANS data of a characteristic length scale, which would manifest as a knee in the data and would correspond to a particle size, cylindrical cross-section, or some other regular feature in the sample. Previous studies of cellulose and lignocellulosic biomass found such scattering features in the range $q > 0.06$ Å^{-1}, which was attributed to the cellulose fibril diameter.[16,34] The lack of a feature consistent with the cellulose fibril diameter indicates that the cellulose fibrils are dissolved into individual chains or into considerably smaller bundles by the IL. The lack of a larger characteristic length scale (0.006 Å^{-1} < q < 0.06 Å^{-1}), also observed previously, further suggests that the lignin has not aggregated as it does in response to dilute acid pretreatment.[16]

The data in Figure 1 also indicate that large structures consistent with smooth cell walls are not present.[16] Instead, the branched polymer network spans all of the length scales measured (up to ∼1000 Å). The origin of the branched network in the samples is presumably residual disordered branched polymeric lignin and hemicellulose networks that are interwoven with IL-dissolved cellulose fibrils. Covalent lignin−hemicellulose complexes, which exist in plants with a relatively low frequency,[36] may also be present. The SANS data suggest that [C_2mim][OAc] does not break all the covalent bonds between the biopolymers or disentangle them, resulting in a swollen interconnected network. As noted in previous studies, employing temperatures higher than the lignin glass transition can facilitate the separation of carbohydrate and lignin in lignocellulosic biomass.[25,27] However, even under these conditions, the carbohydrate-rich material regenerated from the biomass/[C_2mim][OAc] solution still contains lignin,[25,26] suggesting that dissolution of switchgrass in [C_2mim][OAc], even at high temperature, does not disrupt all of the physical entanglements and covalent linkages between the biopolymers. One can postulate that if the pretreatment process were to be carried out at high temperature, the SANS results would reflect the smaller volume fraction of the sample composed of the biopolymer network through a decrease in the strength of the scattering signal and would reflect any changes in the interconnectivity of the network through a change in the power law exponent observed.

SANS data for the MCC, xylan, and Indulin AT lignin dissolved in the IL are shown in Figure 2, along with the SANS

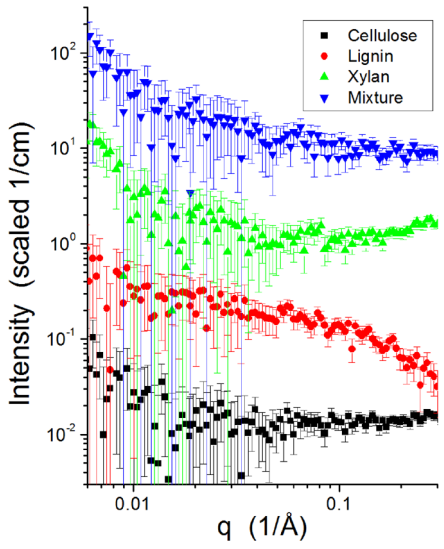


Figure 2. SANS data for purified cellulose, lignin, and xylan, as well as a mixture prepared to mimic the biopolymer composition of switchgrass.

data from the mixture of the three components that was prepared to mimic the composition of switchgrass. The scattering signal is weak, in spite of measurement times being as long as the beam time allocated to the work would reasonably allow (∼5 h maximum for any given data set). A weak signal is expected because of the limited scattering length density contrast between the materials that make up biomass and the [C_2mim][OAc]. The scattering length density of [C_2mim][OAc] is 1.13 × 10^{-6}/Å^2, while those of cellulose, lignin, and xylan are 1.78 × 10^{-6}, 2.21 × 10^{-6}, and 1.52 × 10^{-6}/Å^2, respectively. Only large structures would scatter strongly

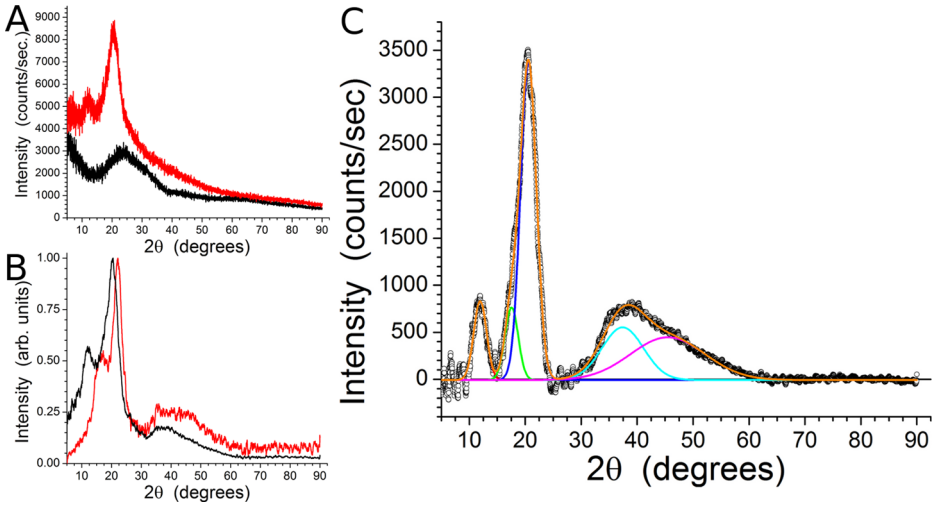


Figure 3. (A) Raw PXRD data from the recovered CRM (red line) and associated instrument background (black line). (B) Instrument background-corrected PXRD data from the recovered CRM (black line) and untreated switchgrass (red line) scaled to a maximum of 1 for easy comparison. (C) Five Gaussian function fit (orange line) and individual Gaussian functions (red [not visible], green, blue, cyan, and magenta lines) to the CRM PXRD data (circles) after final removal of the amorphous scattering background.

under such contrast conditions at the material concentrations employed.

The data in Figure 2 indicate that the MCC and xylan components possess a great deal less supramolecular structure than the dissolved switchgrass. In the case of Indulin AT lignin, the samples scatter in a manner consistent with a solution of small particles, in contrast to previous work in aqueous solution.[35] The Indulin AT lignin SANS data can be modeled as a cigar-like triaxial ellipsoid having semi-axes of 26.9, 3.8, and 3.0 Å. The data collected from the solution of the physical mixture of the three materials in the proportions found in switchgrass are more consistent with a superposition of the SANS intensity profiles of the individual components than the dissolved switchgrass. This key result demonstrates that the large scale network structure observed in the IL-dissolved biomass data does not result from the self-assembly of the component biopolymers when mixed together. Instead, the SANS data suggest that the biopolymer network present in the IL-dissolved biomass results from residual entanglements and linkages that form during plant growth that are not disrupted by the IL. It is also possible that the observed differences result from the compositional and structural differences between switchgrass and wood lignins and the presence of other biomass components, such as pectin and proteins, in the switchgrass sample.[29] The Indulin AT lignin is also sulfated, unlike native lignins.

Powder X-ray Diffraction. The PXRD data collected for the CRM reconstituted from the IL-dissolved switchgrass and the data collected from the untreated switchgrass are shown in Figure 3A. The results of the fitting the CRM data for the amorphous material background and the subsequent fitting of the diffraction peaks are provided in Table 1, while the results obtained from fitting the untreated switchgrass data are presented in Table 2. The positions of the diffraction peaks in the untreated switchgrass PXRD data are consistent with cellulose I.[10,26,37] The fitting of the diffraction peaks in the CRM data, shown in Figure 3C, demonstrates that the material has transitioned to cellulose II, consistent with previous work.[10,26,37] The CI of the CRM is 0.363 ± 0.010, indicative of considerably disordered cellulose, while that of the untreated

Table 1. Results of Fitting CRM PXRD Data as Described in Materials and Methods.

peak position (deg)		area of peak (arbitrary units)
amorphous background		
15.76		51824.39 ± 113.42
diffraction peaks	D-spacing (Å)	
11.95	7.41	2416.47 ± 18.97
17.56	5.05	2297.86 ± 81.46
20.58	4.32	12024.83 ± 83.02
37.44	2.40	5218.86 ± 412.18
45.51	1.99	7583.01 ± 447.06
	total area	81365.42 ± 629.67

Table 2. Results of Fitting Untreated Switchgrass PXRD Data As Described in Materials and Methods.

peak position (deg)		area of peak (arbitrary units)
amorphous background		
21.96		876.05 ± 10.15
diffraction peaks	D-spacing (Å)	
16.62	5.33	546.69 ± 3.38
22.10	4.02	732.98 ± 3.17
36.26	2.48	145.61 ± 7.93
44.52	2.04	827.60 ± 11.74
	total area	3128.84 ± 18.04

switchgrass is 0.720 ± 0.009. For comparison, the CI of untreated switchgrass was found by NMR to be ~0.63, and it increased to ~0.72 after dilute acid pretreatment.[38] Similarly, the CI of commercially available Avicel PH-101, a highly crystalline form of cellulose, was measured to be 0.777.[24] Here, the continued entanglement of cellulose and the other biopolymers may be partially responsible for preventing the reconstituted cellulose in the CRM from adopting a highly crystalline state. However, the most likely cause of the decrease in crystalline order is the fact that the cellulose is truly dissolved in the IL and that the reconstitution process employed does not favor recrystallization of the cellulose. The low crystallinity of the regenerated CRM would facilitate its subsequent hydrolysis into sugars.

CONCLUSIONS

This work provides new molecular-level physical insight into the nature of switchgrass lignocellulosic biomass dissolved in an IL and the reconstituted CRM produced by the process. The SANS data from the switchgrass dissolved in IL $[C_2mim][OAc]$ demonstrates that dissolution does not completely disrupt the entangled cellulose−hemicellulose−lignin network that forms as the plant grows, although the cellulose fibrils are dissolved. Instead, the results show that an interconnected network remains that spans a wide range of length scales, which supports a hypothesis put forward by Sun and co-workers when dissolution of biomass in $[C_2mim][OAc]$ was first demonstrated.[10] Dissolution of the isolated biopolymers results in much smaller structures, and the mixture does not self-assemble into a large-scale network in the IL. It should be noted that the Indulin AT lignin is sulfated as a result of the kraft process used to produce it, which may impact its interactions with the IL and the other biopolymers in the solution made to mimic the composition of switchgrass.

The reconstituted CRM is much less crystalline than is typical for highly purified commercial celluloses, which would facilitate the hydrolysis of the regenerated cellulose but make it poorly suited for applications requiring highly crystalline cellulose feedstock. The newly obtained physical insight presented here suggests a way forward toward improving CRM reconstituted from IL-dissolved biomass. Specifically, development of more suitable ILs or the use of chemical additives, particularly those that break bonds that exist between the lignin and hemicellulose, would improve the purity of the resulting product but may do little to disrupt the highly physically entangled network of biopolymers to facilitate their separation.

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Notes

The authors declare the following competing financial interest(s): Robin D. Rogers is co-owner of 525 Solutions, Inc., a start-up of The University of Alabama funded by a DOE-SBIR grant, and Gabriela Gurau is a part-time employee of the company.

ACKNOWLEDGMENTS

The authors thank J. Keum for assistance collecting PXRD data and P. Langan and D. Sawada for discussions of the interpretation of the PXRD data. Switchgrass samples were obtained through a collaborative agreement with the Bioenergy Science Center (BESC) located at the Oak Ridge National Laboratory, Oak Ridge, TN. Portions of this research were funded by the Genomic Science Program, Office of Biological and Environmental Research, U.S. Department of Energy, under FWP ERKP752. This research at Oak Ridge National Laboratory's Center for Structural Molecular Biology (CSMB) was supported by the Office of Biological and Environmental Research (FWP ERKP291). A portion of this research at the High Flux Isotope Reactor and Spallation Neutron Source was supported by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy. A portion of this research was conducted at the Center for Nanophase Materials Sciences, which is sponsored at Oak Ridge National Laboratory by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy. The work at UA was financially supported by 525 Solutions, Inc. as part of DOE SBIR Grant DE-SC0004198.

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