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Lignin Extraction from Straw by Ionic Liquids and Enzymatic Hydrolysis of the Cellulosic Residues

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Lignocellulose is a promising starting material for bioproducts, ranging from biofuels to specialty chemicals; however, lignocellulose is resistant to enzymatic degradation. Overcoming this resistance is therefore an important priority for the development of the lignocellulosic biorefinery concept. In this work, 1-ethyl-3-methylimidazolium acetate ([emim]Ac) was selected from six ionic liquid candidates for the extraction of lignin from triticale and wheat straw and flax shives. Lignin extractability, composition, and cellulose enzymatic digestibility of the residues after extraction by [emim]Ac were determined at various temperatures (70−150 °C) and time intervals (0.5−24 h). The optimal result (52.7% of acid insoluble lignin in triticale straw) was obtained at 150 °C after 90 min, yielding >95% cellulose digestibility of the residue. Little cellulose was extracted, and the extracted lignin was recovered by acid precipitation. Selective extraction of lignin by ionic liquids is a potentially efficient technique for the comprehensive utilization of lignocellulose.

KEYWORDS: Lignocellulose; biomass; delignification; ionic liquids; enzymatic digestibility; polysaccharides; lignin; cellulase; pretreatment; × *Triticosecale*; *Linum usitatissimum*; *Triticum aestivum*

INTRODUCTION

Alternative and renewable energy sources are being developed as replacements for fossils fuels. Lignocellulosic biomass from agricultural residues, forestry wastes, waste paper, and energy crops has come under intense research scrutiny due to its potential use as a starting material for bioenergy/biofuels and other bioproducts such as bioplastics and biochemicals (*1*). Lignocellulosic biomass is a renewable, relatively carbon-neutral source of energy that is readily available, with a yearly supply of approximately 200 billion tonnes worldwide (*2*, *3*). The biological conversion of lignocellulose into biofuels typically includes three main steps: pretreatment of lignocellulose to liberate cellulose and hemicellulose from their complex with lignin; depolymerization of the carbohydrates to produce fermentable reducing sugars; and fermentation of the sugars to ethanol or other products (*4*). Transition from a fossil-fuel-based economy to a more renewable carbohydrate−lignin economy is envisioned to take place in the foreseeable future (*3*, *5*, *6*).

The complex and rigid structure of lignocellulose, a natural composite with three main biopolymers, cellulose, hemicellulose, and lignin, causes its notorious resistance to biological and chemical degradation. Effectively overcoming the recalcitrance is an important and urgent research and development priority for the development and implementation of the lignocellulosic biorefinery concept (*7*−*9*). Cellulose in lignocellulose is highly crystalline, and this property protects it from chemical and biological degradation. Lignin is a highly cross-linked aromatic polymer based on phenylpropanoid units acting as a "glue" that binds cellulose and hemicellulose, imparting rigidity and microbial resistance to the cell wall (*10*). Not surprisingly, the majority of lignocellulosic pretreatment strategies have focused on removal of lignin and reduction in cellulose crystallinity.

A number of approaches have been proposed for lignocellulose pretreatment, some of which are under intensive investigation at both the laboratory-scale and pilot-plant levels. They can be categorized as biological, chemical, physical, and thermal processes (*11*). Biological pretreatment, such as lignin degradation by white-rot fungi (*12*), offers the benefit of low chemical and energy use, but a controllable and sufficiently rapid system has not been found yet. The performance of physical pretreatment such as milling is relatively poor (*13*, *14*). Steam explosion has the advantage of being very simple, but yields of cellulose and xylan are too low (*15*). Passing hot water through biomass at high flow rate is effective in removing over half of the lignin and producing highly digestible cellulose, but the energy requirement is relatively high (*16*, *17*). Chemical pretreatment is the most promising option so far. Approaches being investigated include dilute acid hydrolysis (*18*, *19*), ammonia fiber explosion (*20*), ammonia recycle percolation (*21*), and lime (*22*) and organosolv (*23*, *24*) processes; however, all of these processes suffer from relatively low sugar yields, severe reaction conditions (high temperature and/or high pressure), and high processing costs.

Recently, ionic liquids (ILs) have received attention as promising green solvents for lignocellulose pretreatment or fractionation. ILs are organic salts that usually melt below 100 °C. They are nonflammable and recyclable solvents with extremely low volatility and high thermal stability (*25*, *26*). It has been reported that some hydrophilic ILs can dissolve cellulose, for example,

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1-butyl-3-methylimidazolium chloride ([bmim]Cl) (*27*), 1-allyl-3-methylimidazolium chloride ([amim]Cl) (*28*), 1-ethyl-3-methylimidazolium acetate ([emim]Ac) (*29*, *30*), and 1-allyl-3-methylimidazolium formate ([amim]Fo) (*31*). Highly digestible cellulose can be reconstituted by adding an antisolvent such as water. Recently, several research groups have discovered that wood can completely dissolve in some ILs (*32*, *33*), and it has been reported that lignin can also dissolve in some ILs (*34*). On the basis of these reports, more practical investigations are underway on the pretreatment and fractionation of lignocellulose using ILs (*35*−*38*).

In this study, we first determined the solubility of cellulose, xylan, and lignin, the extractability of lignin from triticale straw, and cellulose digestibility of the extraction residues using six ILs: 1-butyl-3-methylimidazolium chloride ([bmim]Cl); 1-ethyl-3-methylimidazolium acetate ([emim]Ac); *N*,*N*-dimethylethanolammonium formate (DMEAF); *N*,*N*-dimethylethanolammonium acetate (DMEAA); *N*,*N*-dimethylethanolammonium glycolate (DMEAG); and *N*,*N*-dimethylethanolammonium succinate (DMEAS). On the basis of the results from the screening experiments, we selected the ionic liquid [emim]Ac for further research and studied the extraction of lignin from triticale straw, flax shives, and wheat straw at 70−150 °C for 0.5−24 h. Lignin extractability and composition and cellulose digestibility of the residues were determined. The extracted lignin was recovered by acid precipitation.

MATERIALS AND METHODS

Reagents. Cellulase from *Trichoderma reesei*, xylan from birch wood, citric acid, sodium citrate, sulfuric acid, [bmim]Cl, and [emim]Ac were purchased from Sigma-Aldrich (Oakville, ON, Canada). DMEAF, DMEAA, DMEAG, and DMEAS were purchased from Bioniqs (York, U.K.). Sodium hydroxide was supplied by BDH (Toronto, ON, Canada). Microcrystalline cellulose was from Applied Science Laboratories (Bedford, MA). Kraft lignin Indulin AT was provided by MeadWestvaco Corp. (Glen Allen, VA). All of the ILs were predried at 80 ± 1 °C in a vacuum oven for 24 h and cooled in a desiccator.

Triticale (× *Triticosecale* cv. AC Ultima) straw was provided by Agriculture Agri-Food Canada, Lethbridge, AB, Canada. Flax (*Linum usitatissimum* cv. CDC Bethune) shives, a byproduct of flax fiber production, were provided by Biolin Research Inc., Saskatoon, SK, Canada. Canada Prairie Spring wheat (*Triticum aestivum*) straw was obtained from CBioN ABIP Network, Saskatoon, SK, Canada. These straw samples were initially ground with a Retsch SM 2000 cutting mill (Newtown, PA), using a 2 mm screen before being passed through a Wiley cutting mill (Swedesboro, NJ) using a 0.5 mm screen. The ground samples were kept in sealed bags (reclosable poly bag, 9 × 12 in., Great Little Box Co., Kelowna, BC, Canada) at room temperature prior to use.

Solubility of Cellulose, Xylan, and Lignin in Ionic Liquids. To determine cellulose, xylan, and lignin solubility, 2 mg of the sample was added to a glass vial containing 2 g of each ionic liquid at 90 °C under N_2 with stirring for up to 24 h and visually checked as to whether it was soluble. If it was, 20 mg of the sample was introduced into the solution every time after it became homogeneous, until the ionic liquid could not dissolve more material within 24 h. The dynamic method described here is similar to previously reported procedures (*28*, *32*, *33*).

Lignin Extraction from Straw. Lignin extraction from straw was carried out as described by Lee et al. (*35*), and sample preparation for extracted lignin determination was modified with acid precipitation and redissolving of the extract. Five hundred milligram samples of straw were incubated in 10 g of various ILs under N_2 with magnetic stirring at a constant temperature for a preset time period. After incubation, the suspension was diluted by 100 mL of 0.1 mol/L NaOH and centrifuged at 11600*g* for 20 min. The supernatant was decanted to a plastic vial (Histoplex Container, 120 mL, Starplex Scientific Inc., Etobicoke, ON, Canada), and the residue was washed by 500 mL of distilled water using a Buchner funnel. After washing, the residue was dried in a vacuum oven at 55 °C for 24 h and then kept in a sealed bag (reclosable poly bag, 3 × 5 in., Great Little Box Co.) in a freezer at −15 °C prior to analysis. One and a half milliliters of the supernatant was transferred into a 2 mL centrifuge tube, and the pH was adjusted to 2 with sulfuric acid (*39*). The centrifuge tube was stored in a refrigerator at 4 °C overnight for complete precipitation of lignin. The suspension was then centrifuged at 8030*g* for 5 min. The supernatant was discarded, the lignin precipitate was redissolved with 0.1 mol/L NaOH, and the volume of the redissolved lignin solution was 1.5 mL. The absorbance was measured at 280 nm after the solution was centrifuged at 8030*g* for 5 min. If necessary, the solution was diluted to adjust the absorbance into the linear range. Kraft lignin Indulin AT was pretreated by acid precipitation (pH 2), washed, and dried prior to use as a standard and preparation of the calibration curve (**Figure 1**).

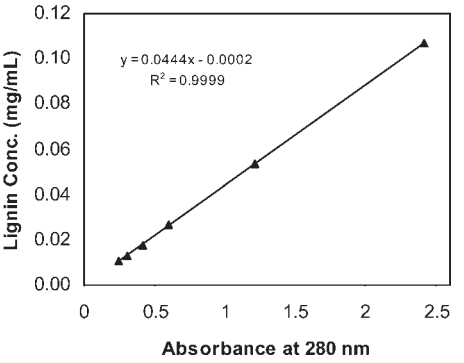

Figure 1. Calibration curve of Kraft lignin Indulin AT pretreated by acid precipitation (pH 2), washing, and drying.

Compositional Analysis of Straw and Residues. Cellulose, xylan, and lignin contents of all the samples were determined by quantitative saccharification upon acid hydrolysis and subsequent HPLC and gravimetric analysis, based on the standard NREL procedure (*40*). Before acid hydrolysis, water and ethanol extractives of native straw were removed and quantified by Soxhlet extraction for 24 h with water and for 7 h with ethanol, according to the standard NREL procedure (*41*). The samples were treated with 72% sulfuric acid at 30 °C for 1 h, followed by diluted acid (4%) at 121 °C for 1 h. The hydrolysates were neutralized by calcium carbonate and analyzed by HPLC for sugar content. The HPLC system (Agilent 1100 series) was equipped with a Bio-Rad Micro-Guard Deashing cartridge, a Bio-Rad Aminex HPX-87P column operated at 75 °C, and a refractive index detector. The mobile phase consisted of deionized water with a flow rate of 0.5 mL/min. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively (*42*). The acid insoluble lignin after acid hydrolysis was determined gravimetrically using filtering crucibles. The acid soluble lignin was measured by UV−vis spectrophotometry at 320 nm using the extinction coefficient value of 30 L/g cm. Most analyses were carried out in duplicate, but a few analyses were done with one replicate due to insufficient residue amount; these results are reported without standard deviations.

Enzymatic Hydrolysis. Enzymatic hydrolysis reactions were performed in 25 mL sealed vials on a shaking incubator at 100 rpm and 50 °C in volumes of 3.5 mL with a biomass sample size of 10 mg and a cellulase concentration of 35 U/mL in 50 mM citrate buffer (pH 4.8) (*35*, *43*). Samples (300 μL) were periodically removed and boiled for 3 min to quench the enzymatic reaction. After centrifugation at 8030*g* for 5 min and neutralization by NaOH solution, glucose concentration was measured by HPLC, as described above. All reactions were carried out in duplicate.

RESULTS AND DISCUSSION

Composition of Three Native Straws. The contents of structural carbohydrates, lignin, ash, and water−ethanol extractives in the three native straws (i.e., triticale straw, flax shives, and wheat straw) were determined according to the standard NREL procedures (*40*, *41*) described above. The results are shown in **Table 1**. As shown, cellulose, hemicellulose, and lignin are the three main constituents of all the straws, whereas the proportion of these

components varied. Triticale straw and wheat straw were similar in composition, and this was expected as triticale is a hybrid crop developed by crossing wheat and rye (*44*). The composition of flax shives was considerably different, with a much higher content of lignin (23.22%) and a lower content of cellulose (26.14%). The composition of wheat straw determined in this work is comparable to that reported in the literature (*45*−*47*). The minor variations among data provided by different groups are reasonable, because there were differences in cultivar of wheat straw, sample preparation procedure, or analytical approach. The composition of flax shives is also similar to reported values (*48*).

Table 1. Composition of Triticale Straw, Flax Shives, and Wheat Straw As Determined by the Analysis of a Water−Ethanol-Extracted Preparation

component	triticale straw	flax shives	wheat straw
total glycans	55.05 (0.35)[a]	41.36 (0.46)	55.81 (0.26)
glucan	32.20 (0.26)	26.14 (0.22)	34.48 (0.10)
xylan	19.29 (0.06)	11.77 (0.18)	17.85 (0.13)
galactan	1.14 (0.01)	1.46 (0.01)	1.05 (0.02)
arabinan	2.25 (0.03)	0.64 (0.03)	1.72 (0.01)
mannan	0.40 (0.00)	1.35 (0.02)	0.70 (0.00)
total lignin	15.02 (0.08)	23.22 (0.09)	17.46 (0.02)
acid insoluble lignin	13.97 (0.09)	22.39 (0.09)	16.44 (0.03)
acid soluble lignin	1.05 (0.01)	0.83 (0.00)	1.02 (0.00)
protein	3.03 (0.02)	2.35 (0.03)	3.42 (0.09)
uronic acids	1.50 (0.09)	3.55 (0.06)	1.56 (0.22)
acetyl groups	1.97 (0.04)	2.82 (0.03)	2.25 (0.04)
ash	4.14 (0.13)	10.00 (0.13)	2.68 (0.10)
water−ethanol extractives	17.62	13.45	14.94
total	98.55 (0.35)	96.75 (0.40)	98.11 (0.45)

[a] Results are expressed as a percentage of the native, oven-dried basis. All values in parentheses represent standard deviations.

Table 2. Solubility and Extraction Efficiency of Lignin in Various Ionic Liquids

	solubility at 90 °C (g/100 g)			
ionic liquid[a]	microcrystalline cellulose	xylan from birch wood	Kraft lignin Indulin AT	extracted lignin content[b] (g/100 g)
[emim]Ac	22	2	30	0.21
[bmim]Cl	14	nd[c]	10	0.11
DMEAF	nd	2	28	0.02
DMEAA	nd	nd	19	0.03
DMEAG	nd	nd	17	0.05
DMEAS	nd	nd	10	0.08

[a] Abbreviations of ionic liquids: [emim]Ac, 1-ethyl-3-methylimidazolium acetate; [bmim]Cl, 1-butyl-3-methylimidazolium chloride; DMEAF, *N,N*-dimethylethanolammonium formate; DMEAA, *N,N*-dimethylethanolammonium acetate; DMEAG, *N,N*-dimethylethanolammonium glycolate; DMEAS, *N,N*-dimethylethanolammonium succinate. [b] 500 mg of triticale straw was incubated in 10 g of ionic liquids at 90 °C for 24 h under N_2. Lignin content was determined using Indulin AT as standard. [c] nd indicates <0.1 g/100 g.

Selection of Ionic Liquids. Six ILs, [bmim]Cl, [emim]Ac, DMEAF, DMEAA, DMEAG, and DMEAS, were used in the screening of a solvent for delignification and pretreatment of straw. Three aspects were investigated: solubility of cellulose, xylan, and lignin; extraction efficiency of lignin; and cellulose digestibility of the residues.

Solubility values for cellulose, xylan, and lignin in the six ILs at 90 °C are presented in **Table 2**. Extractability of lignin determined by using the UV method described above is also listed in the table for comparison. All six ILs dissolved lignin (≥10 g/100 g); however, good solubility of the Indulin AT lignin does not imply an efficient extraction of lignin from straw. For example, the solubility of Indulin AT lignin in DMEAF (28 g/100 g) was similar to that in [emim]Ac (30 g/100 g), whereas [emim]Ac could extract 10 times more lignin from straw than DMEAF under the same conditions (90 °C, 24 h and a biomass/IL ratio of 1:20). [emim]Ac was much more effective in dissolving cellulose (22 g/100 g) than DMEAF (<0.1 g/100 g), which provided a clue to explain the difference in lignin extraction. As is known, lignin is highly cross-linked and also linked to both cellulose and hemicellulose in lignocellulosic materials (*10*), and the complex structure of lignocellulose is an inhibitor for the diffusion of ionic liquid into its interior (*32*). A common property of the cellulose-dissolvable ILs is their ability to break down the extensive inter- and intramolecular hydrogen bonding network (*32*, *37*). With the dissolution of cellulose, more and more lignin was exposed and became accessible to the solvent and, thus, dissolved (*33*), resulting in more efficient extraction of lignin. The ILs [emim]Ac and [bmim]Cl, which can dissolve both cellulose and lignin, performed more efficiently in lignin extraction, and the other four ILs, DMEAF, DMEAA, DMEAG, and DMEAS, performed relatively poorly. Kraft lignin Indulin AT solubility values in [emim]Ac and [bmim]Cl determined in this work are similar to the data reported by Lee et al. (*35*). Cellulose solubility in [bmim]Cl determined in this work is comparable to the data provided by Barthel et al. (*49*), and the minor variation was probably caused by the difference in degree of polymerization of cellulose.

The extraction efficiency of lignin by the six ILs is shown in **Table 3**. The quantity of acid insoluble lignin extracted was determined by using the UV method with sample preparation of acid precipitation and redissolving of the extract, as described under Materials and Methods. Acid precipitation and redissolving of lignin before absorbance measurement at 280 nm were

Table 3. Lignin Extraction from Triticale Straw by Various Ionic Liquids and Enzymatic Hydrolysis of the Cellulosic Residues

	lignin extraction[a]		composition of the residue[b] (%)				enzymatic hydrolysis of the residue[c]	
ionic liquid[d]	extracted lignin[e] (%)	residue recovery[f] (%)	cellulose	xylan	AIL	ASL	released glucose (mg)	digestibility (%)
untreated	0.0	100	32.20 (0.26)[g]	19.29 (0.06)	13.97 (0.09)	1.05 (0.01)	0.59 (0.02)	16.5 (0.5)
[emim]Ac	30.3 (1.3)	66.0 (2.9)	29.91 (0.70)	13.36 (0.34)	7.18 (0.20)	1.00 (0.03)	4.91 (0.09)	97.6 (1.8)
[bmim]Cl	15.3 (2.1)	76.9 (1.8)	30.79 (1.11)	17.13 (0.25)	10.57 (0.42)	0.92 (0.00)	2.88 (0.02)	64.8 (0.5)
DMEAF	3.4 (0.2)	80.4 (0.2)	30.14 (0.82)	17.83 (0.33)	12.40 (0.20)	1.10 (0.02)	1.20 (0.00)	28.9 (0.1)
DMEAA	4.4 (0.7)	81.6 (0.8)	29.12 (0.22)	17.56 (0.15)	12.92 (0.12)	1.05 (0.02)	1.00 (0.09)	25.1 (2.2)
DMEAG	7.6 (0.3)	80.0 (1.2)	28.83 (0.16)	17.21 (0.14)	12.96 (0.02)	1.05 (0.00)	1.12 (0.07)	27.9 (1.8)
DMEAS	11.9 (0.0)	83.6 (1.4)	29.92 (0.16)	17.54 (0.14)	12.72 (0.20)	1.09 (0.02)	0.83 (0.05)	20.9 (1.2)

[a] 500 mg of triticale straw (0.5 mm screen) was incubated in 10 g of ionic liquids at 90 °C for 24 h. [b] Determined by NREL protocol (LAP version 2007). Results are expressed as a percentage of the native, oven-dried basis. AIL, acid insoluble lignin; ASL, acid soluble lignin. [c] Reaction conditions: 10 mg of recovered triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm, 11 h. [d] Abbreviations of ionic liquids: [emim]Ac, 1-ethyl-3-methylimidazolium acetate; [bmim]Cl, 1-butyl-3-methylimidazolium chloride; DMEAF, *N,N*-dimethylethanolammonium formate; DMEAA, *N,N*-dimethylethanolammonium acetate; DMEAG, *N,N*-dimethylethanolammonium glycolate; DMEAS, *N,N*-dimethylethanolammonium succinate. [e] Acid insoluble lignin extracted was determined by UV−vis spectrometry at 280 nm after acid precipitation (pH 2) and redissolving in alkali solution, with the Indulin AT standard. Results are expressed as a percentage of extracted lignin relative to the original AIL in the straw. [f] Percent of recovered triticale straw (residue) relative to untreated triticale straw, oven-dried basis. [g] Values in parentheses are standard deviations.

crucial to eliminate the strong interference that was found in our preliminary experiments. Furthermore, 100% of the pretreated lignin standard was recovered after another acid precipitation and redissolving, which indicated that there was no loss in acid insoluble lignin using this approach. It has been reported that the UV method to determine lignin content in herbaceous plant samples is problematic because of the frequently high amounts of non-lignin phenolics contained in these plants (*50*). Specifically, triticale straw contains 359.2 mg/100 g phenolics (*44*). A minor drawback of the acid-precipitated and redissolved preparation was that acid soluble lignin (ASL) could not be quantified because ASL could not be separated from the interference. As shown, [emim]Ac provided the best extractability of lignin. Specifically, 30.3% of the initial acid insoluble lignin was extracted from triticale straw by [emim]Ac at 90 °C for 24 h. It was reported (*35*) that 51.8% of the initial lignin was extracted from maple wood flour by [emim]Ac under the same conditions. Although it was possible the difference was the result of the different lignocellulosic materials used, we suspect that the reported value was overestimated because there could be significant interference if the acid-precipitated and redissolved preparation was not used. Lignin mass balance calculation from their data also indicated the overestimation. [bmim]Cl was the second best among the six ILs evaluated, with a lignin extraction efficiency approximately half that of [emim]Ac under the same conditions. Lignin content in the residues reflected the same tendency. [emim]Ac displayed a dramatic drop in lignin content of the residue, but there was only a slight decline for DMEAF, DMEAA, DMEAG, and DMEAS.

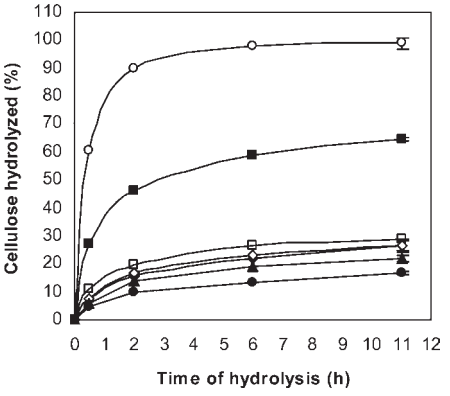

Figure 2. Enzymatic hydrolysis of triticale straw extracted by various ionic liquids at 90 °C for 24 h: (●) untreated; (○) [emim]Ac; (■) [bmim]Cl; (□) DMEAF; (◆) DMEAA; (◇) DMEAG; (▲) DMEAS. Reaction conditions: 10 mg of extracted triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm.

In addition to solubility and lignin extractability, we evaluated the enzymatic hydrolysis of native triticale straw and the residues extracted by various ILs (**Figure 2**). ILs with stronger capacity for lignin extraction also provided higher cellulose digestibility of the residue. The trend was the same as has been reported (*32*, *35*), although the lignocellulosic materials and ILs varied. [emim]Ac extracted 30.3% of the original lignin from triticale straw at 90 °C for 24 h, and the cellulose digestibility of the residue was 97.6%; [bmim]Cl extracted 15.3%, and the digestibility was 64.8%; lignin extraction with DMEAF, DMEAA, DMEAG, and DMEAS was even more inferior, and the digestibility was < 30%. Addition of fresh cellulase after 11 h did not increase the hydrolysis yield, indicating that the termination of the reaction was not due to loss of cellulase activity. The limited enzymatic hydrolysis of untreated straw resulted from the incomplete accessibility of the crystalline cellulose and the hindrance of lignin (*10*, *11*). Decrystallization of cellulose proved by XRD and FTIR (*32*, *35*, *37*, *38*) and removal of lignin, via treatment with [emim]Ac, therefore increased the accessibility and then enhanced the digestibility of cellulose in lignocellulose. Simply placing lignocellulose in an ionic liquid without sufficient capability for lignin extraction cannot produce readily biodegradable cellulose.

As a result of these determinations, ionic liquid [emim]Ac was chosen as the solvent for further study on the selective extraction of lignin from triticale straw, flax shives, and wheat straw.

Influence of Extraction Temperature. With an increase in temperature, increasing amounts of lignin were extracted from triticale straw by [emim]Ac, and the lignin content in the residues decreased accordingly (**Table 4**). This is consistent with previously reported results (*35*, *36*), although biomass, ionic liquid, and temperature range were not identical. Of the initial acid insoluble lignin (AIL) 52.7% was extracted after 90 min at 150 °C, approximately 5 times the amount extracted at 70 °C. Higher temperatures increased the solubility of cellulose and lignin in various ILs including [emim]Ac (*34*, *51*) and also accelerated the diffusion of ILs into lignocellulose (*32*, *51*), resulting in more lignin dissolved in the ionic liquid and more efficient extraction of lignin. The summation of extracted lignin and lignin remaining in the residue varied from 83 to 93%. The inability to reconcile the lignin mass balance was likely due to the difference in analytical methods and slight degradation of the lignin. Extracted lignin was determined by UV−vis spectrometry with an Indulin AT lignin standard, whereas AIL remaining in the residue was determined gravimetrically by the standard NREL procedure, and lignin could also be degraded slightly during the extraction process (*32*, *33*). Furthermore, there was a roughly linear relationship between extracted lignin and residue recovery (**Figure 3**), for all of the data related to lignin extraction from triticale straw using [emim]Ac. A similar relationship can be found in literature

Table 4. Effect of Extraction Temperature on Composition and Enzymatic Hydrolysis of the Triticale Straw Residues

lignin extraction[a]			composition of the residue[b] (%)				enzymatic hydrolysis of the residue[c]	
temperature (°C)	extracted lignin[d] (%)	residue recovery[e] (%)	cellulose	xylan	AIL	ASL	released glucose (mg)	digestibility (%)
untreated	0.0	100	32.20 (0.26)[f]	19.29 (0.06)	13.97 (0.09)	1.05 (0.01)	0.59 (0.02)	16.5 (0.5)
70	11.4 (1.8)	82.0 (3.0)	32.41	17.77	11.18	1.04	3.16 (0.05)	71.9 (1.2)
90	20.0 (1.9)	76.6 (2.4)	30.85 (0.97)	16.66 (0.28)	10.15 (0.56)	1.00 (0.03)	3.76 (0.03)	84.0 (0.6)
110	21.7 (0.3)	73.3 (0.8)	30.49 (0.51)	15.52 (0.44)	8.62 (0.11)	0.99 (0.03)	4.18 (0.14)	90.5 (3.1)
130	41.0 (0.8)	62.0 (1.8)	29.45	11.88	6.39	0.81	5.09 (0.07)	96.5 (1.3)
150	52.7 (0.4)	51.2 (3.2)	27.71	6.86	5.11	0.58	6.11 (0.10)	101.6 (1.7)

[a] 500 mg of triticale straw (0.5 mm screen) was incubated in 10 g of the ionic liquid [emim]Ac (1-ethyl-3-methylimidazolium acetate) at various temperatures for 1.5 h. [b] Determined by NREL protocol (LAP version 2007). Results are expressed as a percentage of the native, oven-dried basis. AIL, acid insoluble lignin; ASL, acid soluble lignin. [c] Reaction conditions: 10 mg of recovered triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm, 11 h. [d] Acid insoluble lignin extracted was determined by UV−vis spectrometry at 280 nm after acid precipitation (pH 2) and redissolving in alkali solution, with the Indulin AT standard. Results are expressed as a percentage of extracted lignin relative to the original AIL in the straw. [e] Percent of recovered triticale straw (residue) relative to untreated triticale straw, oven-dried basis. [f] Values in parentheses are standard deviations.

data (*35*). The correlation is actually part of the mass balance of the whole lignocellulosic material. In addition to lignin, there were other soluble molecular species in the alkali solution after IL extraction; however, these compounds were not identified.

With increasing temperature, cellulose content (expressed as a percentage of the native) decreased slightly but hemicellulose content declined distinctly, especially at 150 °C. Quantitatively, 14% of cellulose and 64% of hemicellulose were removed during the extraction at 150 °C, with hemicellulose removal being calculated on the basis of xylan content. Substantial loss of hemicellulose is common in many lignocellulose pretreatment techniques (*10*, *11*), as hemicellulose in plants is slightly cross-linked, relatively amorphous, and more easily hydrolyzed into sugars than cellulose (*47*). Pre-extraction of hemicellulose (*52*) is a potential way to get more fermentable sugars.

Figure 4 shows the course of glucose released via the enzymatic hydrolysis of triticale straw extracted by [emim]Ac for 1.5 h at temperatures ranging from 70 to 150 °C. Cellulose digestibility of triticale straw was largely enhanced by ionic liquid [emim]Ac extraction at various temperatures. The results are similar to the

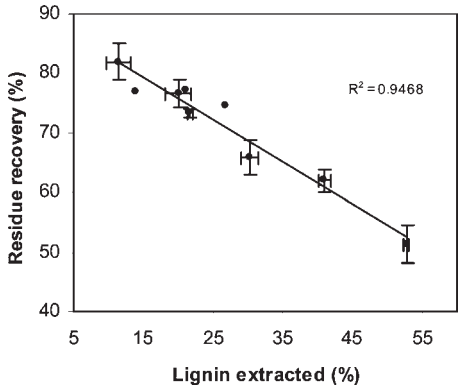

Figure 3. Relationship of lignin extracted (by [emim]Ac) and residue recovery of triticale straw.

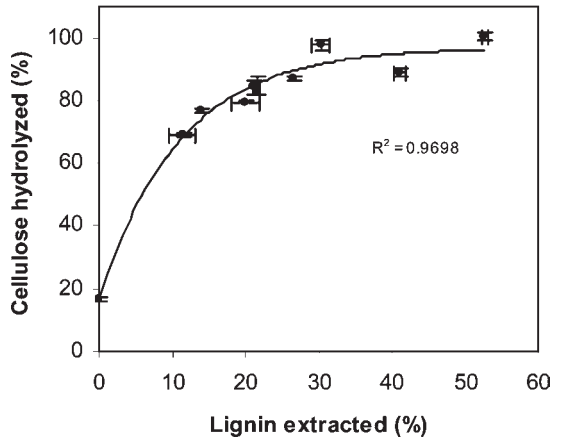

Figure 5. Relationship of lignin extracted (by [emim]Ac) and cellulose digestibility of triticale straw residues.

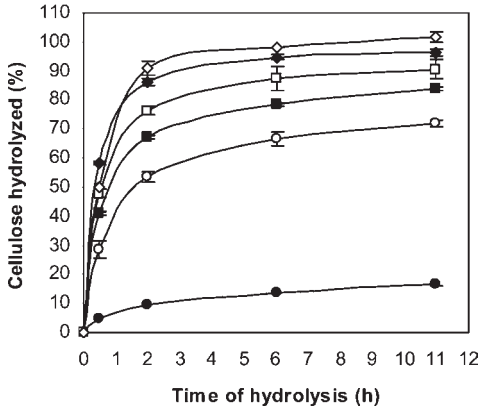

Figure 4. Influence of ionic liquid extraction temperature (for 1.5 h) on enzymatic hydrolysis of recovered triticale straw: (●) untreated; (○) 70 °C; (■) 90 °C; (□) 110 °C; (◆) 130 °C; (◇) 150 °C. Reaction conditions: 10 mg of extracted triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm.

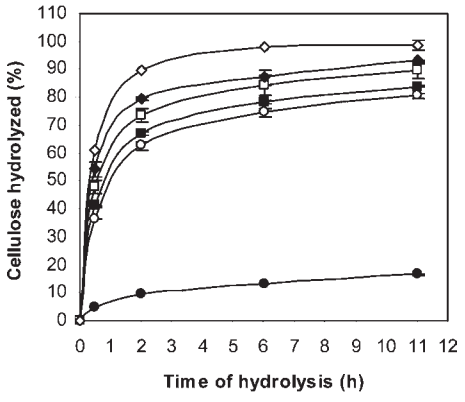

Figure 6. Influence of ionic liquid extraction time (at 90 °C) on enzymatic hydrolysis of recovered triticale straw: (●) untreated; (○) 0.5 h; (■) 1.5 h; (□) 5 h; (◆) 8 h; (◇) 24 h. Reaction conditions: 10 mg of extracted triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm.

Table 5. Effect of Extraction Time on Composition and Enzymatic Hydrolysis of the Triticale Straw Residues

lignin extraction[a]			composition of the residue[b] (%)				enzymatic hydrolysis of the residue[c]	
time (h)	extracted lignin[d] (%)	residue recovery[e] (%)	cellulose	xylan	AIL	ASL	released glucose (mg)	digestibility (%)
untreated	0.0	100	32.20 (0.26)[f]	19.29 (0.06)	13.97 (0.09)	1.05 (0.01)	0.59 (0.02)	16.5 (0.5)
0.5	13.9	76.9	30.08	16.71	10.20	0.96	3.49 (0.04)	80.4 (0.9)
1.5	20.0 (1.9)	76.6 (2.4)	30.85 (0.97)	16.66 (0.28)	10.15 (0.56)	1.00 (0.03)	3.76 (0.03)	84.0 (0.6)
5	21.1	77.2	31.61	16.97	10.06	1.08	4.07 (0.12)	89.4 (2.6)
8	26.7	74.6	31.33	15.94	9.11	1.07	4.34 (0.01)	93.1 (0.3)
24	30.3 (1.3)	66.0 (2.9)	29.91 (0.70)	13.36 (0.34)	7.18 (0.20)	1.00 (0.03)	4.91 (0.09)	97.6 (1.8)

[a] 500 mg of triticale straw (0.5 mm screen) was incubated in 10 g of the ionic liquid [emim]Ac (1-ethyl-3-methylimidazolium acetate) at 90 °C. [b] Determined by NREL protocol (LAP version 2007). Results are expressed as a percentage of the native, oven-dried basis. AIL, acid insoluble lignin; ASL, acid soluble lignin. [c] Reaction conditions: 10 mg of recovered triticale straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm, 11 h. [d] Acid insoluble lignin extracted was determined by UV−vis spectrometry at 280 nm after acid precipitation (pH 2) and redissolving in alkali solution, with the Indulin AT standard. Results are expressed as a percentage of extracted lignin relative to the original AIL in the straw. [e] Percent of recovered triticale straw (residue) relative to untreated triticale straw, oven-dried basis. [f] Values in parentheses are standard deviations.

Table 6. Effect of the Kind of Straw on Composition and Enzymatic Hydrolysis of the Cellulosic Residues

lignin extraction[a]			composition of the residue[b] (%)				enzymatic hydrolysis of the residue[c]	
kind of straw[d]	extracted lignin[e] (%)	residue recovery[f] (%)	cellulose	xylan	AIL	ASL	released glucose (mg)	digestibility (%)
untreated TS	0.0	100	32.20 (0.26)[g]	19.29 (0.06)	13.97 (0.09)	1.05 (0.01)	0.59 (0.02)	16.5 (0.5)
TS	30.3 (1.3)	66.0 (2.9)	29.91 (0.70)	13.36 (0.34)	7.18 (0.20)	1.00 (0.03)	4.91 (0.09)	97.6 (1.8)
untreated FS	0.0	100	26.14 (0.22)	11.77 (0.18)	22.39 (0.09)	0.83 (0.00)	0.49 (0.04)	16.9 (1.5)
FS	14.0 (1.4)	65.3 (1.5)	23.64 (0.03)	6.67 (0.09)	17.55 (0.01)	0.53 (0.03)	3.50 (0.06)	86.9 (1.6)
untreated WS	0.0	100	34.48 (0.10)	17.85 (0.13)	16.44 (0.03)	1.02 (0.01)	0.43 (0.01)	11.1 (0.3)
WS	29.6 (0.7)	68.1 (0.1)	31.48 (0.63)	12.81 (0.60)	8.43 (0.01)	0.98 (0.03)	4.74 (0.07)	92.2 (1.3)

[a] 500 mg of straw (0.5 mm screen) was incubated in 10 g of the ionic liquid 1-ethyl-3-methylimidazolium acetate at 90 °C for 24 h. [b] Determined by NREL protocol (LAP version 2007). Results are expressed as a percentage of the native, oven-dried basis. AIL, acid insoluble lignin; ASL, acid soluble lignin. [c] Reaction conditions: 10 mg of recovered straw, 3.5 mL of 50 mM citrate buffer (pH 4.8), 35 U/mL cellulase from *Trichoderma reesei*, 50 °C, 100 rpm, 11 h. [d] Abbreviations of straws: TS, triticale straw; FS, flax shives; WS, wheat straw. [e] Acid insoluble lignin extracted was determined by UV−vis spectrometry at 280 nm after acid precipitation (pH 2) and redissolving in alkali solution, with the Indulin AT standard. Results are expressed as a percentage of extracted lignin relative to the original AIL in the straw. [f] Percent of recovered straw (residue) relative to untreated straw, oven-dried basis. [g] Values in parentheses are standard deviations.

literature (*35*, *38*). More than 95% of cellulose in triticale straw extracted at 130 and 150 °C was hydrolyzed after 11 h; comparatively, only 16.5% was hydrolyzed for the native triticale straw. Both initial rate (first 30 min) of cellulose hydrolysis and extent of conversion after 11 h increased with increasing extraction temperature, with an exception that the initial hydrolysis rate of triticale straw extracted at 150 °C (1.72 $gL^{-1} \cdot h^{-1}$) was a little bit lower than that at 130 °C (1.76 $g \cdot L^{-1} \cdot h^{-1}$). We speculate that the phase transition of lignin might make the residue more rigid, resulting in a lower initial rate of cellulose hydrolysis, because the glass transition temperature of lignin is around 150 °C (*36*). Besides, the difference between the initial rates at 130 and 150 °C was magnified in **Figure 4**, because the cellulose contents were different for the residues extracted at different temperatures and the value in the figure was cellulose hydrolyzed (%) instead of glucose released (mg/mL). Lignin is a major obstacle to enzymatic hydrolysis of cellulose in lignocellulosic materials because it prevents enzyme accessibility (*10*). Confirmatively, our results demonstrated the strong correlation between lignin extracted and cellulose digestibility, as shown in **Figure 5**. With more lignin extracted, cellulose in triticale straw became more accessible, and the crystallinity of cellulose declined (*32*, *35*, *37*, *38*) during the dynamic precipitation−dissolution equilibrium. Therefore, the enzymatic hydrolysis of cellulose was significantly enhanced following the ionic liquid extraction.

Influence of Extraction Time. Various samples of [emim]Ac-extracted triticale straw were prepared by changing the incubation time. The incubation time of triticale straw in [emim]Ac was varied from 0.5 to 24 h at 90 °C (**Table 5**). Increased extraction time gradually led to increased lignin extraction, the same as reported by Lee et al. (*35*) and Li et al. (*38*); 21.1% of lignin was extracted from triticale straw within 5 h and 30.3% was extracted after 24 h. Lignin content in the residues was correspondingly decreased after the extraction. There was little change in cellulose content up to 24 h, whereas the degradation of hemicellulose was obvious. Quantitatively, 7% of cellulose and 31% of hemicellulose were lost after 24 h of extraction at 90 °C, in which the hemicellulose loss was calculated on the basis of xylan contents. **Figure 6** shows the time course of glucose released during the enzymatic hydrolysis. Both initial rate of hydrolysis and cellulose conversion after 11 h increased significantly with increasing IL extraction time up to 24 h, yielding >95% cellulose hydrolyzed. These results show that a longer time of contact makes the extraction more effective; however, additional research is required to optimize the extraction temperature, time, and biomass/IL ratio.

Influence of the Variety of Straw. To further understand the lignin extraction and enhanced cellulose hydrolysis, flax shives and wheat straw were also investigated in this work. Both flax shives and wheat straw were incubated in [emim]Ac at 90 °C for 24 h and analyzed by using the same procedures. The results are listed in **Table 6**, with the data of triticale straw under the same condition for comparison. As seen, lignin in all three straws was substantially extracted by [emim]Ac, and cellulose digestibility was largely enhanced for all of the recovered residues. In addition, little cellulose and considerable hemicellulose were removed during the extraction. Comparatively, wheat straw is more similar to triticale straw in lignin extraction than flax shives; 30.3 and 29.6% of acid insoluble lignin were extracted from triticale straw and wheat straw, whereas only 14.0% was extracted from flax shive. The cellulose digestibility of recovered flax shives (86.9%) was also lower than that of triticale straw (97.6%) and wheat straw (92.2%). This was not surprising because triticale is a hybrid of wheat and rye, but flax is quite different. Flax is significantly different from other herbaceous crops in terms of the chemical structure and spatial organization of cell wall polymers (*53*), and lignin in flax shives is more similar to hardwood lignin (*48*).

CONCLUSION

Ionic liquid [emim]Ac can effectively extract lignin from triticale straw, flax shives, and wheat straw, and cellulose digestibility of the recovered residues is significantly enhanced. The ionic liquid [bmim]Cl is less efficient than [emim]Ac for delignification of straw. DMEAF, DMEAA, DMEAG, and DMEAS are not suitable for this purpose. Within the range of extraction temperatures (70−150 °C) and extraction times (0.5−24 h) investigated, higher temperatures and longer extraction times are beneficial for improved lignin extraction and cellulose hydrolysis of the residues. Specifically, 52.7% of acid insoluble lignin in triticale straw was extracted by [emim]Ac at 150 °C after 90 min, yielding >95% cellulose digestibility of the residue. In addition, little cellulose was removed during the extraction, but loss of hemicellulose was not negligible. Delignification of straw by ILs is potentially an efficient technique for pretreatment of straw destined for biofuel production. Complete lignin removal is not necessary to achieve good cellulose degradability (>90%). Lignin from straw, a good raw material for use as a binder, dispersant, and emulsifier (*54*), was easily recovered by acid precipitation. Further studies are required to optimize the extraction conditions and to develop an effective and efficient process flow, especially for the recycling of ILs. Screening and design of ILs with improved lignin extraction capability are also necessary.

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