The Hydrolysis of Cellulosic Materials in Ionic Liquids

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This study's main objective was the dissolution of cellulose from biomass using ionic liquids to obtain saccharides by prehydrolysis. Raw materials were exposed to the ionic liquids (ILs) 1-ethyl-3-methylimidazolium acetate, 1-butyl-3-methylimidazolium chloride, 1-ethyl-2,3-dimethyl-imidazolium chloride, and 3-(2-methoxy-2-oxoethyl)-1-(3-methoxy-3-oxo-propyl)-imidazolium bromide at 105 °C for 6 h. The sugar content of the liquid phase was characterized by high-performance liquid chromatography (HPLC). The vegetal materials after treatments were characterized by Fourier transform infrared spectroscopy (FTIR). Glucose was the main reducing sugar product in each case. Different ILs were found to be most effective, depending on what sample was being dissolved – microcrystalline cellulose or rapeseed stalk.

Keywords: Ionic liquid; Cellulose; Biomass; Lignocellulose

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INTRODUCTION

Lignocellulose is present in all agricultural crop residues and is the most abundant biomass on earth (Ang *et al.* 2011). A potential solution to the world's energy shortage is the utilization of lignocellulosic biomass as an alternative, sustainable energy source used to produce high value-added chemicals and biofuels (Xu *et al.* 2013).

Recently, ionic liquids (ILs), a new class of solvents, have been used for dissolving cellulosic materials (Swatloski *et al.* 2002). ILs have the advantages of low volatility and unique solvent properties, and they are non-flammable, easily recyclable, and have nearly infinite structural variations, providing the possibility for improving lignocellulosic ethanol production (Gremos *et al.* 2011; Wang *et al.* 2011). One of the main advantages of using ILs in the conversion of vegetal materials is their ability to dissolve large amounts of cellulose and lignin; these liquids have low volatility, which enables the dissolution reactions to be carried out at high temperatures (Mäki-Arvela *et al.* 2010).

ILs have been recognized as efficient solvents, and are also sometimes considered to be green solvents (Brosse *et al.* 2010). Pretreatment of lignocellulose with ILs can reduce cellulose crystallinity and partially remove hemicelluloses and lignin without forming degradation products that are inhibitory to enzymes or fermenting microorganisms (Dadi *et al.* 2007; Lee *et al.* 2009).

IL pretreatment methods require less energy and the solvents are easier to handle and more environmentally compatible than other pretreatment methods, such as mechanical milling, steam explosion, or acid, base, or organic solvent processes (Rogers and Seddon 2003; Zhao *et al.* 2009). Consequently, from both the economic and the environmental points of view, the magnitude of the paper and allied products manufacturing imposes serious challenges in the development of green processing technologies for wood and other lignocellulosic materials (Kilpeläinen *et al.* 2007).

Studies have shown that ionic liquids can dissolve various types of agricultural biomass, such as rice husk (Ang *et al.* 2011), corn stover (Kaar and Holtzapple 2000), switchgrass (Li *et al.* 2010), and *Eucalyptus* species (Xu *et al.* 2013).

The objective of this study was to investigate the dissolution of cellulose from vegetal materials and lignocellulose using ILs to obtain saccharides. The sugar contents of the hydrolysates were characterized by high-performance liquid chromatography (HPLC). The vegetal materials after treatment were characterized by Fourier transform infrared spectroscopy (FTIR).

EXPERIMENTAL

Materials

Cellulosic material samples were ground into powder using an electric mill, then dried in an oven at 105 °C. Glucose (Fluka), xylose (Fluka), 3,5-dinitrosalicylic acid (Merck), sodium hydroxide (Lachner), Rochelle salt (Merck), phenol (Fluka), sodium metabisulfite (Reactivul Bucuresti), toluene (Merck), ethanol (Chemical Company from Iasi, Romania), sulfuric acid (98%) (Chemical Company from Iasi, Romania), calcium carbonate (Magis International), hydrochloric acid (Chemical Company from Iasi, Romania), and *Avicel* microcrystalline cellulose (Merck) were of analytical grade and used as purchased. The following ILs were used: 1-ethyl-3-methylimidazolium acetate - IL1 (Sigma-Aldrich), 1-butyl-3-methylimidazolium chloride - IL2 (Merck), 1-ethyl-2,3-dimethylimidazolium chloride - IL3 (Merck), and 3-(2-methoxy-2-oxoethyl)-1-(3-methoxy-3-oxopropyl) imidazolium bromide - IL4 (University Alexandru Ioan Cuza, Iasi, Romania). The structures of these ILs are illustrated in Fig. 1.

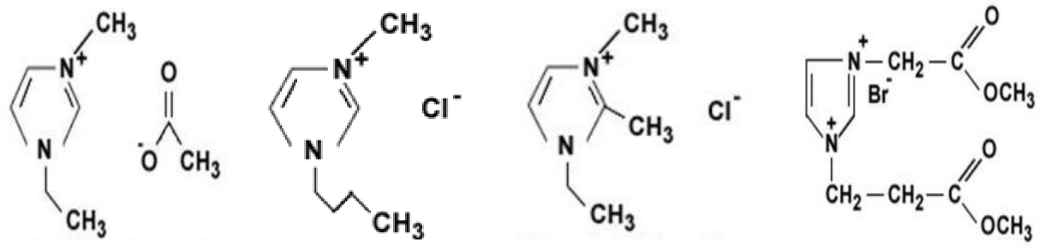

(a) (b) (c) (d)

Fig. 1. Chemical structures of (a) 1-ethyl-3-methylimidazolium acetate, (b) 1-butyl-3-methylimidazolium chloride, (c) 1-ethyl-2,3-dimethylimidazolium chloride, and (d) 3-(2-methoxy-2-oxoethyl)-1-(3-methoxy-3-oxopropyl) imidazolium bromide

Methods

IL pretreatment of the rapeseed stalks or Avicel cellulose

Dissolution experiments with ILs were performed as follows. Rapeseed stalks (0.06 g) or *Avicel* cellulose (0.06 g) and IL (1 g) were put into a 25-mL glass vial and heated to 105 °C for 6 h. After the 6-h incubation time, aqueous HCl (1.66 M, 60 μL) was added and the mixture was stirred vigorously at 130 °C. After 10 min, deionized water (200 μL) was added while stirring, followed by additional aliquots at 20 min (100 μL), 30 min (150 μL), and 60 min (250 μL). After a total reaction time of 2.5 h, the collected precipitate on the filter paper was washed with an additional 1.5 mL of deionized water. Material residue was removed by centrifugation, rinsed twice with water (400 μL), and dried at 60 ºC overnight. The pretreated samples and wash solution were separated by filtration (Binder and Raines 2010). The sugars content of the liquid product was analyzed using high-performance liquid chromatography (HPLC). The lignocellulose was characterized by Fourier transform infrared spectroscopy (FTIR).

Dissolving the lignocellulose from the rapeseed stalks in the ILs

The lignocellulose obtained from 0.5% H_2SO_4 hydrolysis of rapeseed stalks (0.06 g) was then heated with ILs (1 g) at 105 °C for 4.5 h. To this mixture was added aqueous HCl (1.66 M, 45 μL), and the mixture was vigorously stirred at 130 °C. After 10 min, deionized water (150 μL) was added with stirring, followed by an additional 75 μL of water at 20 min, 205 μL of water at 30 min, and 210 μL of water at 60 min. After the 3-h total reaction time, the solution was diluted with water (900 μL) and centrifuged to separate insoluble materials (Binder and Raines 2010). The sugars content of the liquid product was analyzed using high-performance liquid chromatography (HPLC). The residues were characterized by Fourier transform infrared spectroscopy (FTIR).

Figure 2 illustrates the destructuration process of the cellulosic material with ILs.

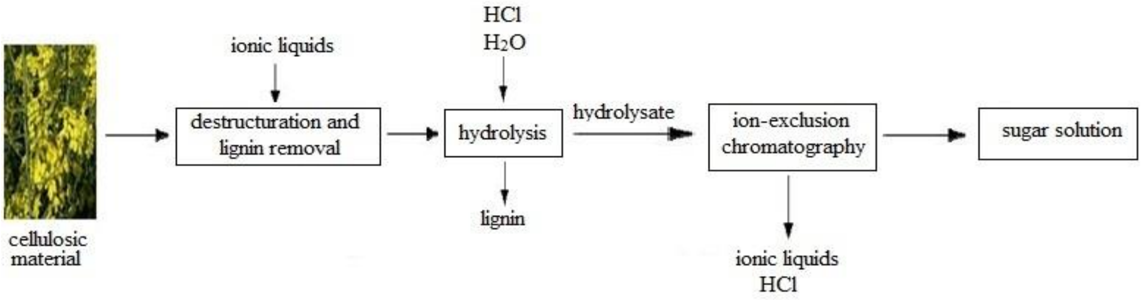


Fig. 2. Cellulosic hydrolysis using ILs

Analytical Methods

Analysis of total reducing sugars

The total reducing sugars were quantified by 3,5-dinitrosalicylic acid (DNS) reduction. Absorbance at 540 nm was measured with a JASCO model V-550 UV-Vis spectrophotometer. Glucose was used as a standard for reducing sugar measurement (Adney and Baker 1996). Prior to determining the total reducing sugars, liquid hydrolysate samples were autoclaved in 4% sulfuric acid at 121 °C for 60 min (Ruiz and Ehrman 1996).

HPLC analysis

The sugars content in the hydrolysates was determined by an HPLC system equipped with a Varian 9010 pump, Waters 717 autosampler, HPLC column oven, a Shimadzu RID-10A refractive index detector (RID), and a Waters 486 UV detector (calibrated). Both detectors were utilized simultaneously in series. A 300 x 7.8 mm Supelcogel C611 column was used, and the mobile phase consisted of ultrapure water at a flow rate of 0.5 mL/min.

Spectroscopy FTIR

Fourier transform infrared spectroscopy (FTIR) of the sample was performed using a Digilab Scimitar FTS 2000 spectrometer. The spectra (4000 to 400 cm^{-1}) were recorded with 4-cm^{-1} resolution and 32 scans per sample. Lignocellulose samples were prepared by mixing the samples with KBr and forming a pellet from the mixture. The sample spectra were processed using the Digilab Merlin series application.

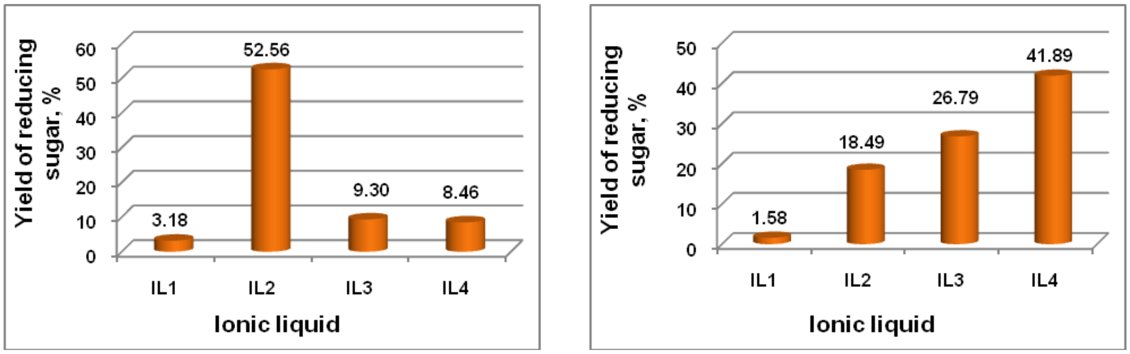
RESULTS AND DISCUSSION

Analysis of Reducing Sugars from IL Pretreatment of Cellulosic Materials

Overall, the ability of ILs to dissolve cellulose depends on the nature of the native cellulose (*e.g.*, degree of polymerization and crystallinity), the operating conditions (*e.g.*, temperature, reaction time, and initial cellulose concentration in the ionic liquid), and the presence of impurities (*e.g.*, water) (Vitz *et al.* 2009).

The catalytic activity of the mineral acids used in hydrolysis of the vegetal materials by 1-butyl-3-methylimidazolium chloride using acids has been determined to decrease according to the following sequence: hydrochloric acid > nitric acid > sulfuric acid > maleic acid > phosphoric acid (Li *et al.* 2008).

Figures 3 through 5 show sugar yields obtained during treatment with ionic liquids. The hydrolysate samples obtained were analyzed to determine the total reducing sugars by the DNS assay. To assess the hydrolytic yield of reducing sugars, *Avicel* cellulose was solubilized using ILs (Fig. 3). The aim of the pretreatment was to increase the yields of sugar and cellulose destructuration.



(a) (b)

Fig. 3. Yields of reducing sugars released during the pretreatment with ILs: (a) *Avicel* cellulose and (b) rapeseed stalks

Of the four ionic liquids tested, 1-butyl-3-methylimidazoliu chloride (IL2) seemed to be the most efficient in the cellulosic material pretreatment. In the case of *Avicel* cellulose, the yield of reducing sugars reached a maximum of 52%, and the use of 3-(2-methoxy-2-oxoethyl)-1-(3-methoxy-3-oxopropyl)-imidazolium bromide (IL4) in the case of rapeseed stalks proved to be the most efficient, the yield of reducing sugars being 42%. The pH of the aqueous phase was 6.56 for the treatment using IL1, 5.82 using IL2, 4.78 using IL3, and 1.10 using IL4.

Moreover, pretreatment with ILs was performed on rapeseed stalks and on prehydrolyzed rapeseed stalk lignocelluloses obtained by a process using static and dynamic systems at 170 °C.

Table 1 shows the reducing substance yields obtained before and after pretreatment with ionic liquids. In the case of prehydrolysis lignocelluloses, supplementary saccharide yields of up to 26% (reported relative to dry weight) with 1-butyl-3-methylimidazolium chloride (IL2) can be seen.

Table 1. Reducing Substance Yields Obtained Before and After Pretreatment with Ionic Liquids

Pretreatment	Yield of TRS * by prehydrolysis with 0.5% H_2SO_4 solution, %	Yield of TRS by pretreatment with IL2 **, %	Yield of TRS by pretreatment with IL3 ***, %
IL and lignocellulose obtained by prehydrolysis of rapeseed stalks with 0.5% H_2SO_4, 170 °C, dynamic conditions	14.86	24.04	11.35
IL and lignocellulose obtained by prehydrolysis of rapeseed stalks with 0.5% H_2SO_4, 170 °C, static conditions	15.69	24.06	9.53
IL and lignocellulose obtained by prehydrolysis of rapeseed stalks with 3% $Al_2(SO_4)_3$ solution, 170 °C, dynamic conditions	3.21	26.01	6.98
IL and lignocellulose obtained by prehydrolysis of rapeseed stalks with 3% $Al_2(SO_4)_3$ solution, 170 °C, static conditions	17.96	19.37	7.74
IL and lignocellulose obtained by prehydrolysis of rapeseed stalks with H_2O, 170 °C, dynamic conditions	4.41	-	9.86
IL and lignocellulose obtained of prehydrolysis rapeseed stalks with H_2O, 170 °C, static conditions	1.67	25.61	18.82
Rapeseed stalks	-	26.79	18.49
Avicel cellulose	-	9.30	52.56

** Total reducing sugars*
***1-butyl-3-methylimidazolium chloride*
****1-ethyl-2,3-dimethylimidazolium chloride*

Spiridon *et al.* 2010 obtained the hydrolysis rate of *Asclepias syriaca* seed floss (As) pretreated with 1-*n*-butyl-3-methylimidazolium chloride ([BMIM]Cl); the sugars yield was only 8.8% after 50 h of enzymatic hydrolysis, greater than that obtained for As treated with 1-ethyl-3-methylimidazolium tetrachloroaluminate ([EMIM]Cl-$AlCl_4$). Moreover, the sugars yield had a value of 17.35% for poplar seed floss when it was pretreated with [BMIM]Cl and 12.55% for poplar seed floss treated with [EMIM]Cl-$AlCl_4$. Pezoa *et al.* 2010 obtained the best experimental conditions using 1-ethyl-3-methylimidazolium chloride on wheat, corn, and *Eucalyptus* residues at 150 °C for 60 min, thus attaining total sugar yields of 46, 48, and 30%, respectively. Xu *et al.* 2013 using raw materials (*Eucalyptus* wood) pretreated with [BMIM]Ace/1,4-dioxane, [BMIM]Ace / DMSO, [BMIM]Ace / methanol, [BMIM]Ace / acetone, [BMIM]Ace / DMF, and fresh [BMIM]Ace, obtaining the following yields of hemicellulosic fractions

(%, dry matter): 16.8, 17.5, 20.0, 19.8, 16.1, and 17.3%, respectively, based on the initial *Eucalyptus.*

Analysis of Sugars from Cellulose Materials by High-Performance Liquid Chromatography (HPLC)

For sample analysis, a reversed phase column (Supelcogel C611) was used at 60 °C with a refractive index (RI) detector and a UV detector with an eluent flow rate of 0.5 mL/min. Only ultrapure water was applied as mobile phase (eluent), and all samples were filtered through a 0.45-µm PVDF membrane syringe filter.

HPLC analysis of the liquid phase, resulting from the pretreatment with ILs, was carried out to determine the amount of dissolved sugar in the sample. Analyses focused on glucose, which is the monomer for cellulose. As illustrated in Fig. 4, the sample included monosaccharides and oligosaccharides, respectively.

Due to the high concentrations of ILs, the samples were diluted with distilled water prior to HPLC analysis.

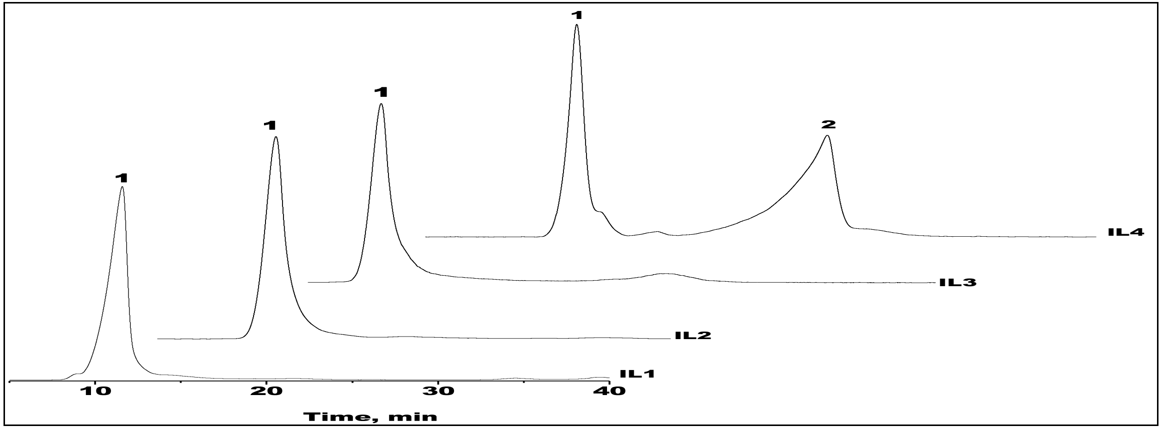


Fig. 4. HPLC analysis of sugar from *Avicel* cellulose prehydrolysis using ILs (column Supelcogel C611): (1) glucose and (2) cellobiose

Figure 5 illustrates the results of the HPLC analysis of dissolved sugars after rapeseed stalk pretreatment with ILs.

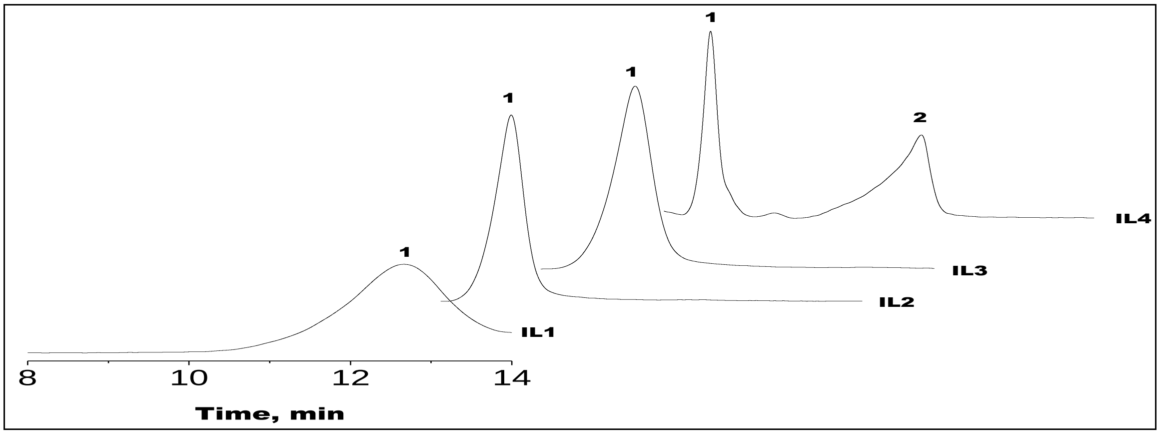


Fig. 5. HPLC chromatograms of samples obtained from prehydrolysis of rapeseed stalks using ILs (column Supelcogel C611): (1) glucose and (2) xylose

Figure 6 shows the chromatograms of prehydrolysates from treatment of lignocellulose with IL2.

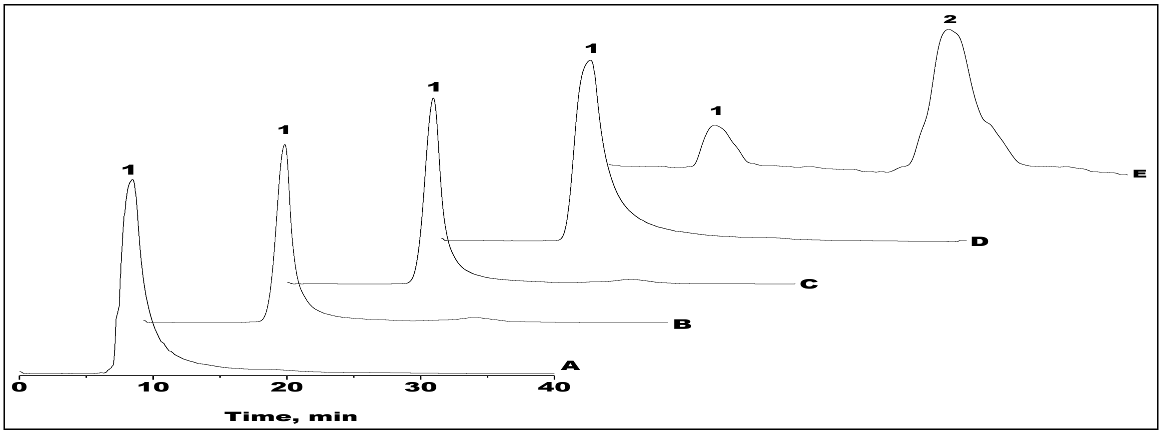


Fig. 6. HPLC chromatograms of samples from hydrolytic treatment of lignocellulose with IL2 (column Supelcogel C611): (1) glucose and (2) xylose. A-Treatment of IL2 and rapeseed stalk lignocellulose resulting from prehydrolysis in the presence of water under static conditions at 170 °C; B-Treatment of IL2 and rapeseed stalk lignocellulose resulting from prehydrolysis in the presence of 3% $Al_2(SO_4)_3$, dynamic conditions at 170 °C; C-Treatment of IL2 and rapeseed stalk lignocellulose resulting from prehydrolysis in the presence of 3% $Al_2(SO_4)_3$, static conditions at 170 °C; D-Treatment of IL2 and rapeseed stalk lignocellulose resulting from prehydrolysis in the presence of 0.5% H_2SO_4, dynamic conditions at 170 °C; E-Treatment of IL2 and rapeseed stalk lignocellulose resulting from prehydrolysis in the presence of 0.5% H_2SO_4, static conditions at 170 °C.

Chromatograms obtained by the treatment with ionic liquids in all cases emphasize that glucose is the main component of the saccharides, which demonstrates that the ionic liquids act on the cellulose in rapeseed stalks or lignocelluloses, as demonstrated by the analysis of *Avicel* cellulose in prehydrolysates.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Cellulose Materials

FTIR analysis was conducted to examine the structures of *Avicel* cellulose, rapeseed stalks, and the samples treated with ILs (Fig. 7).

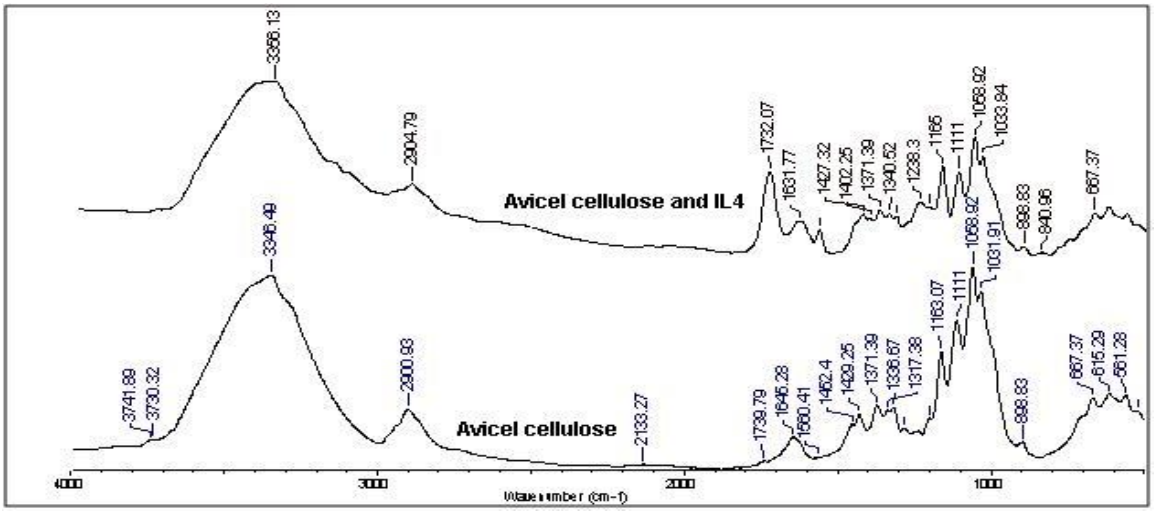


Fig. 7. FTIR spectra for *Avicel* cellulose and IL-treated *Avicel* cellulose

As shown in Fig. 7, *Avicel* cellulose presented an intense band in the 1160-cm^{-1} and 960-cm^{-1} regions, which is identified by specific vibration absorption of polysaccharides (Xu *et al.* 2013).

Considering the FTIR spectra of the lignocelluloses from rapeseed stalk prehydrolyzed by H_2SO_4 0.5% or aluminum sulfate 3% or deionized water, their crystallinity index was obtained as the ratio of peak areas at 1370-cm^{-1} / 2900-cm^{-1} (CR 1) and the ratio of peak areas at 1430-cm^{-1} / 899-cm^{-1} (CR 2) (O'Connor *et al.* 1958). These ratios and the ratio of polysaccharide (Pz) / lignin (L) (1060-cm^{-1} / 1510-cm^{-1}) are shown in Table 2.

Table 2. Absorbance Ratio (Crystallinity index) Determined by FTIR Spectroscopy

Pretreatment	Pz / L A(1060) / A(1510)cm^{-1}	CR 1 * A(1370) / A(2900)cm^{-1}	CR 2 ** A(1430) / A(899)cm^{-1}
Avicel cellulose		0.11	1.45
Rapeseed stalks	2.10	0.07	1.61
Rapeseed stalks and IL4		0.04	0.83
IL2 and lignocellulose obtained by prehydrolysis of rapeseed stalks with 3% $Al_2(SO_4)_3$ solution, 170 °C, dynamic conditions	2.63	1.83	0.16
IL2 and lignocellulose obtained by prehydrolysis of rapeseed stalks with 3% $Al_2(SO_4)_3$ solution, 170 °C, static conditions	3.21	1.70	0.14
IL2 and lignocellulose obtained by prehydrolysis of rapeseed stalks with 0.5% H_2SO_4 solution, 170 °C, dynamic conditions	4.00	4.22	0.26
IL2 and lignocellulose obtained by prehydrolysis of rapeseed stalks with 0.5% H_2SO_4 solution, 170 °C, static conditions	8.33	1.32	0.04
IL2 and lignocellulose obtained by prehydrolysis of rapeseed stalks with H_2O, 170 °C, static conditions	1.44	1.39	0.09

** Crystallinity index*
*** Crystallinity index*

Based on the index of crystallinity, CR 2, of the rapeseed stalks extracted by organic solvent (1.61) and comparing the crystallinity index of the lignocellulose obtained by pretreating lignocellulose obtained from rapeseed stalks prehydrolysis by 0.5% H_2SO_4, both in dynamic and static conditions, the crystallinity index decreases to 0.04, which implies a strong reduction of the percentage of crystalline areas in cellulose. Probably, during the pretreatment with IL's, the cellulose materials lost their crystalline structure and, instead, restructured themselves into mostly amorphous forms. This decrease in cellulose crystallinity index (CR 2) implies that the treated cellulose material has an increase in cellulose surface accessibility (Spiridon *et al.* 2010).

The results indicated that the highly crystalline cellulose lignocellulose was transformed to an amorphous state after pretreatment with 1-butyl-3-methylimidazolium chloride.

Figure 8 presents the FTIR spectra of hydrolytic lignocellulose after pretreatment with ionic liquid (1-butyl-3-methylimidazolium chloride).

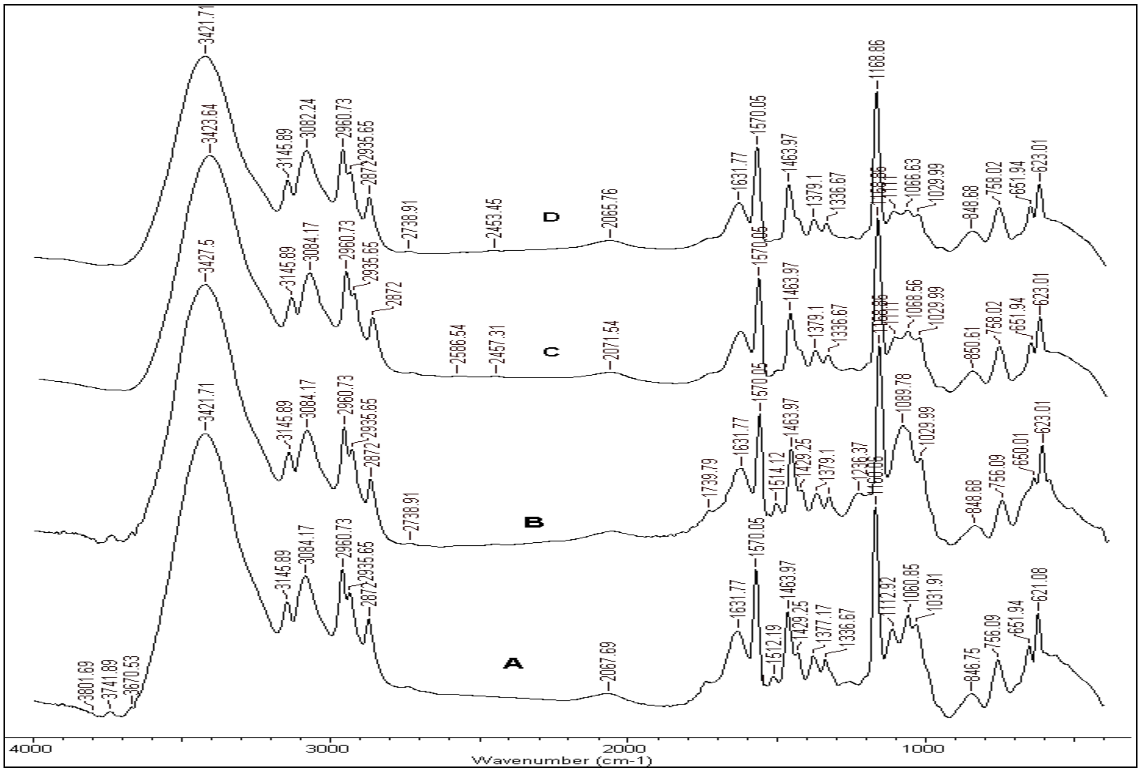


Fig. 8. IR spectra of hydrolytic lignocellulose treated with IL2. A-Lignocellulose obtained by prehydrolysis of rapeseed stalks in the presence of 3% $Al_2(SO_4)_3$, dynamic conditions at 170 ºC; B-Lignocellulose obtained by prehydrolysis of rapeseed stalks in the presence of 3% $Al_2(SO_4)_3$, static conditions at 170 °C; C-Lignocellulose obtained by prehydrolysis of rapeseed stalks in the presence of 0.5% H_2SO_4, dynamic conditions at 170 °C; D-Lignocellulose obtained by prehydrolysis of rapeseed stalks in the presence of 0.5% H_2SO_4, static conditions at 170 °C.

The absorption band at 1420-cm^{-1} represents CH_2 scissoring motion in cellulose, the 1373-cm^{-1} band is the C–H bending mode, and the 2917-cm^{-1} band represents C–H and CH_2 stretching, which is unaffected by changes in crystallinity (Nelson and O'Connor 1964). The band at 1167-cm^{-1} is for C-O stretching, while that at 1030-cm^{-1} is due to the C-O-C vibration (Liu *et al.* 2007).

CONCLUSIONS

1. The potential of several cellulosic materials for sugars production was investigated. Of the ionic liquids tested, the most efficient ionic liquid used for the pre-treatment of *Avicel* cellulose was 1-butyl-3-methylimidazolium chloride. In the case of rapeseed stalks, the most efficient was 3-(2-methoxy-2-oxoethyl)-1-(3-methoxy-3-oxopropyl)-imidazolium bromide (IL4). Thus, in the case of prehydrolysis lignocelluloses, up to 26% reducing substance yields may be obtained when using 1-butyl-3-methylimidazolium chloride (IL2).
2. Analysis by FTIR showed that the cellulose and lignin do not undergo structural degradation during the process of dissolving the mixture in lignocellulose / 1-butyl-3-methylimidazolium chloride. The small differences observed between the FTIR spectra of 1-butyl-3-methylimidazolium chloride and lignocelluloses were due to a lower crystallinity of the cellulose when dissolving.

ACKNOWLEDGMENTS

This work was carried out with financial support from the POSDRU CUANTUMDOC "Doctoral studies for European performances in research and innovation" ID79407 project funded by the European Social Fund and the Romanian Government.

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Article submitted: September 17, 2013; Peer review completed: November 2, 2013; Revised version received: November 6, 2013; Accepted: November 7, 2013; Published: November 18, 2013.