DELIGNIFICATION AND FRACTIONATION OF SUGARCANE BAGASSE WITH IONIC LIQUIDS

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The pretreatment and fractionation of sugarcane bagasse into carbohydrate-rich (CRM) and lignin-rich material (LRM) was investigated using a series of cholinium-, triethylammonium- and 1-butyl-3-methylimidazolium ionic liquids in conjunction with acetone-water-mixtures as precipitating antisolvent. Compositional analysis of the CRM showed that the pretreatment with carboxylate anion containing ILs allowed high carbohydrate recovery and moderate to high delignification, while hydrogen sulfate based ILs led to a marked reduction in both lignin and hemicellulose content. The recovery of the solubilized lignin, which could serve as a potentially valuable product stream was found highest for ILs comprising hydrogen sulfate anions. Spectroscopic characterization and comparison with alkaline, Formacell and commercial kraft lignin samples revealed differences in purity, functional group composition and syringyl-to-guaiacyl (S/G) ratio between the individual LRM fractions, giving rise to different potential applications for the derived lignins.

Keywords: ionic liquid, sugarcane bagasse, lignin, pretreatment

INTRODUCTION

In light of the global exhaustion of petroleum-based resources, which have been used for more than a century to supply the majority of the steadily increasing energy and chemical demands worldwide, efforts to utilize biomass as a valuable and highly abundant alternative to fossil resources have gained strong momentum in the recent decades.[1] This development is amplified by the environmental burden of greenhouse gas emissions from the combustion of fossil fuels, resulting in 33.4 GT of CO_2 released in 2016 alone and presenting a clear incentive to establish renewable and carbon-neutral alternatives for production and energy generation.[2] Lignocellulosic plant material has been identified as one of the most important feedstocks for a green and sustainable bio-economy, since it not only accounts for the majority of the estimated 170 billion metric tons of biomass produced annually by photosynthesis,[3] but is also composed of approximately 75% carbohydrates in the form of cellulose and hemicellulose, which can be transformed into a variety of value-added compounds and platform chemicals based on C5- and C6-sugars or may be converted into bioethanol and other potential biofuels.[2,4-5] Lignin, the second most abundant biopolymer representing up to 30% of organic carbon in the biosphere, forms the third main constituent of lignocellulosic biomass and is considered the most important renewable resource for functionalized aromatic compounds.[6] However, despite a number of potential applications for lignin, such as in the production of phenolic resins,[7] adhesives,[8] high-performance carbon materials,[9] biodispersants,[10] biosorbents,[11] antimicrobial coatings,[12] or by depolymerization into its constituent monolignols for the production of a broad range of aromatic chemicals and polymers,[5-6,13-15] only a small margin of the technical lignin produced actually receives value addition, while the vast majority is burned for power generation.[16]

The industrial processing of sugarcane, one of the most important industrial crops in Thailand, with an annual production volume of

approximately 99 million tons,[17] generates large quantities of sugarcane bagasse as a byproduct. Although it bears great potential to be exploited as an abundant, low-cost renewable lignocellulosic resource, the valorization of bagasse is greatly obstructed by its inherent recalcitrance against chemical and biological degradation. To overcome this limitation, an efficient pretreatment step is required to disrupt the complex, three-dimensional bonding network between cellulose, hemicellulose and lignin that protects the plant fibers against deconstruction, thus allowing the separation and down-stream processing of the individual biomass constituents.[18] Further goals of pretreatment are the reduction of cellulose crystallinity and an overall increase of surface area to enhance the digestibility of the carbohydrate material, while, at the same time, minimizing the formation of inhibitors detrimental to the activity of enzymes and fermentative microorganisms used in subsequent value-addition steps.[19] In this context, especially the extraction of lignin from the carbohydrate-rich material was identified as a crucial step in the production of lignocellulosic bioethanol due to minimizing the uncompetitive binding of hydrolytic enzymes with lignin and its degradation products.[20]

Traditional pretreatment methods, such as soda or Kraft pulping, focus mainly on the isolation of high-purity cellulose pulp at the expense of discarding potential value addition to other constituents of the biomass. Side products, such as the fractionated lignin, undergo severe chemical modification during the process and serve mainly as combustibles for power generation or need to be disposed of as waste owing to their low economic value.[10] However, novel pretreatment technologies have emerged in the last decades with the aim to utilize all the fractions of the biomass feed and to generate multiple product streams suitable for the production of a broad range of value-added chemicals and fuels – an approach that coined the biorefinery concept in analogy to the efficient resource utilization in traditional petroleum refining.[21] Particular emphasis has also been put on the quality and physicochemical properties of the recovered lignin fraction, which may differ considerably depending on the type and severity of the extraction process and, in turn, lead to different potential applications for industrial lignins.[22] Ionic liquids (IL) – organic salts typically liquid at ambient temperature or with melting points below 100 °C – have been broadly recognized as promising solvents for the pretreatment of lignocellulosic biomass in general,[23-24] as well as sugarcane bagasse in particular,[25] not long after the first report of cellulose dissolution in different imidazolium-based ILs by Swatloski *et al.*[26] Besides their excellent biomass solvation capability, ILs also feature several unique characteristics considered advantageous over conventional organic solvents, including negligible vapor pressure and good thermal stability under typical pretreatment conditions, low flammability, and the ability to fine-tune their physicochemical properties, such as hydrophobicity, polarity and viscosity, by combining select cations and anions according to process requirements.[27]

The effects of pretreatment with 1-ethyl-3-methylimidazolium acetate ([emim][OAc]), arguably one of the most well-studied ILs for biomass pretreatment, on the composition of different types of biomass, as well as the beneficial changes of properties relevant for downstream-processing the derived material, such as the degree of delignification, cellulose crystallinity and susceptibility for enzymatic saccharification and fermentation, have been extensively documented in the literature.[28-34] Ionic liquids based on the butyl-substituted methylimidazolium ([bmim]) cation have also received increasing attention for biomass pretreatment, due to having similar dissolution and solubilization capabilities, as well as offering easier synthetic accessibility compared to their [emim] counterparts, which usually require specialized equipment to handle the low-boiling ethyl halides used in the synthesis.[35] For example, [bmim][Cl] was utilized for the fractionation of bagasse into cellulose, hemicellulose and lignin using a combination of IL pretreatment and alkaline extraction,[36] for the production of sugars from municipal lignocellulosic waste,[37] and was shown to reduce the hemicellulose and lignin content of oil palm frond.[38] However, with the notable exception of [bmim][HSO_4] and aqueous mixtures thereof, which were successfully applied in the pretreatment of *Miscanthus giganteus*[39], wheat straw,[40] as well as hybrid aspen and Norway spruce,[41] reports of other [bmim]-based ILs used in the pretreatment of lignocellulosic biomass, specifically sugarcane bagasse, have remained sparse to date.

This work aimed to investigate and compare the potential of a series [bmim]-based ILs, as well

as choline acetate ([Cho][OAc]) as an example for a bio-derived IL[42] and triethylammonium acetate ([HTEA][HSO_4]), which has been proposed as an exceptionally low-cost alternative to conventional ILs,[43] for the pretreatment and delignification of sugarcane bagasse. The efficiency of the pretreatment process was judged by the changes in composition and structure of the pretreated material, as well as the rate of recovery of the individual biomass fractions. Moreover, emphasis was put on the recovery and characterization of the removed lignin in order to investigate the influence of the pretreatment parameters and the type of ionic liquid on the properties and potential applications of the obtained lignin fractions.

EXPERIMENTAL

Materials

Sugarcane bagasse was kindly provided by Mitr Phol Sugar Factory, Phu Khiao, Thailand. It was ground, sieved to a particle size of 4 mesh and dried in a hot air oven at 60 °C until constant weight prior to use. The chemicals 1-chlorobutane, 1-bromobutane, 1-methylimidazole, acetic acid, formic acid, sulfuric acid (98% w/w), glycine, choline chloride, triethylamine, acetone, anhydrous ethanol, Hydranal™-formamide and ethyl acetate were purchased from Sigma Aldrich, Singapore, and used as received, unless stated otherwise.

Synthesis of 1-butyl-3-methylimidazolium bromide ([bmim][Br])

1-Butyl-3-methylimidazolium bromide was prepared according to Burrell *et al.*,[44] with minor modifications. In brief, a 2-liter 3-necked round bottom flask, fitted with a dropping funnel, thermometer and a reflux condenser with $CaCl_2$-tube, was charged with 123.2 g (1.5 mol) of 1-methyl imidazole. A slight excess of 1-bromobutane (226.1 g, 1.65 mol) was added dropwise under magnetic stirring at a rate slow enough to keep the temperature of the exothermic reaction below 40 °C in order to minimize coloration of the final product. After complete reagent addition, the reaction mixture was stirred at room temperature for 48 h, during which a biphasic system containing a white to yellowish lower phase formed. After addition of 50 mL of ethyl acetate, the upper phase containing the unreacted starting material was decanted, and the remaining suspension was washed 3 times with ethyl acetate (50 mL), after which the crude ionic liquid precipitated. The product was comminuted and washed further with 50 mL portions of ethyl acetate until a colorimetric test of the washing solution with $CuSO_4$ indicated only a trace amount of residual unreacted 1-methyl imidazole to be present.[45] Subsequent removal of the solvent *in vacuo*, followed by drying at 10 mbar and 70 °C for 8 h to remove any remaining volatile contaminants, furnished 311.2 g (1.42 mol) 1-butyl-3-methylimidazolium bromide (Y = 94.7%) as a hygroscopic white crystalline solid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.86 t (3H, N–$(CH_2)_2$–CH_2–CH_3, J = 7.4 Hz); 1.26 m (2H, N–$(CH_2)_2$–CH_2); 1.80 m (2H, N–CH_2–CH_2); 3.85 s (3H, N–CH_3); 4.15 t (2H, N–CH_2, J = 7.1 Hz); 7.42 d (2H, N–C*H*–C*H*–N, J = 20.0 Hz); 8.69 s (1H, N–C*H*–N). Water content: 4305 ppm.

Synthesis of 1-butyl-3-methylimidazolium chloride ([bmim][Cl])

1-Butyl-3-methylimidazolium chloride was prepared as described for [bmim][Br], with minor modifications. Because of the low reactivity of 1-chlorobutane, the reaction mixture was heated to 70 °C for one week, after which the crude ionic liquid precipitated upon cooling to room temperature. Purification of the crude product analog to 1-butyl-3-methylimidazolium bromide gave 510 g (Y = 83.5%) of 1-butyl-3-methylimidazolium chloride as a hygroscopic white crystalline solid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.84 t (3H, N–$(CH_2)_2$–CH_2–CH_3, J = 7.4 Hz); 1.24 m (2H, N–$(CH_2)_2$–CH_2); 1.77 m (2H, N–CH_2–CH_2); 3.81 s (3H, N–CH_3); 4.12 t (2H, N–CH_2, J = 7.1 Hz); 7.37 d (2H, N–C*H*–C*H*–N, J = 20.0 Hz); 8.64 s (1H, N–C*H*–N). Water content: 3784 ppm.

Synthesis of 1-butyl-3-methylimidazolium acetate ([bmim][OAc])

1-Butyl-3-methylimidazolium acetate was prepared according to the procedure described by Pernak *et al.*[46-47] Briefly, 43.7 g (0.25 mol) of 1-butyl-3-methylimidazolium chloride was dissolved in 100 mL anhydrous ethanol in a 250 mL flask with magnetic stirring. To the solution, an equimolar amount of (19.9 g) potassium hydroxide (85%, w/w) dissolved in anhydrous ethanol was added quickly under vigorous stirring and cooling in an ice bath. After stirring the solution for 10 min, the KBr formed as a white precipitate was filtered off through a sintered glass frit. To suppress coloration of the final product, the freshly prepared 1-butyl-3-methylimidazolium hydroxide solution was mixed immediately with an equimolar amount of glacial acetic acid (15.0 g) under vigorous stirring in an ice bath. After stirring for 30 min, the solvent was removed *in vacuo* and the ionic liquid dried at 10 mbar and 70 °C for 8 h. The crude product was diluted with 50 mL of dry acetone and stored overnight at 4 °C, in order to precipitate and remove any remaining KBr. After filtration of the precipitate, the solvent removal and drying steps were repeated to give 45.9 g of 1-butyl-3-methylimidazolium acetate (Y = 92.7%) as a viscous pale amber liquid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.75 t (3H, N–$(CH_2)_2$–CH_2–CH_3, J = 7.4 Hz); 1.14 m (2H, N–$(CH_2)_2$–CH_2); 1.67 m (2H, N–CH_2–CH_2); 1.75 s (3H, CH_3–COO); 3.72 s (3H, N–CH_3), 4.02 t (2H, N–CH_2, J =

7.1 Hz); 7.28 d (2H, N–C*H*–C*H*–N, *J* = 20.0 Hz); 8.57 s (1H, N–C*H*–N). Water content: 12676 ppm.

Synthesis of 1-butyl-3-methylimidazolium formate ([bmim][OFo])

1-Butyl-3-methylimidazolium formate was prepared as described for 1-butyl-3-methylimidazolium acetate to give 5.0 g of 1-butyl-3-methylimidazolium formate (Y = 90.6%) as a viscous pale-yellow liquid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.75 t (3H, N–$(CH_2)_2$–CH_2–CH_3, *J* = 7.3 Hz); 1.14 m (2H, N–$(CH_2)_2$–CH_2); 1.68 m (2H, N–CH_2–CH_2); 3.72 s (3H, N–CH_3); 4.02 t (2H, N–CH_2, *J* = 7.1 Hz); 7.28 d (2H, N–C*H*–C*H*–N, *J* = 19.3 Hz); 8.25 s (1H, N–C*H*–N); 8.55 s (1H, *H*COO). Water content: 16186 ppm.

Synthesis of 1-butyl-3-methylimidazolium glycinate ([bmim][Gly])

1-Butyl-3-methylimidazolium glycinate was prepared as described for 1-butyl-3-methylimidazolium acetate to give 9.6 g of 1-butyl-3-methylimidazolium glycinate (Y = 90.5%) as a viscous yellowish liquid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.76 t (3H, N–$(CH_2)_2$–CH_2–CH_3, *J* = 7.4 Hz); 1.14 m (2H, N–$(CH_2)_2$–CH_2); 1.68 m (2H, N–CH_2–CH_2); 3.00 s (2H, H_2N–CH_2–COO); 3.73 s (3H, N–CH_3), 3.96 t (2H, N–CH_2, *J* = 7.1 Hz); 7.29 d (2H, N–C*H*–C*H*–N, *J* = 19.1 Hz); 8.55 s (0.1H, N–C*H*–N). Water content: 7043 ppm.

Synthesis of 1-butyl-3-methylimidazolium hydrogen sulfate ([bmim][HSO_4])

1-Butyl-3-methylimidazolium hydrogen sulfate was prepared as described for 1-butyl-3-methylimidazolium acetate from 98.6 g 1-butyl-3-methylimidazolium bromide and 46.0 g sulfuric acid (98% w/w) in 100 mL H_2O to give 103.0 g of 1-butyl-3-methylimidazolium hydrogen sulfate (Y = 96.8%) as a viscous pale amber liquid. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 0.79 t (3H, N–$(CH_2)_2$–CH_2–CH_3, *J* = 7.4 Hz); 1.18 m (2H, N–$(CH_2)_2$–CH_2); 1.72 m (2H, N–CH_2–CH_2); 3.72 s (3H, N–CH_3); 4.07 t (2H, N–CH_2, *J* = 7.2 Hz); 7.32 d (2H, N–C*H*–C*H*–N, *J* = 18.6 Hz); 8.57 s (1H, N–C*H*–N). Water content: 1999 ppm.

Synthesis of choline acetate ([Cho][OAc])

Choline acetate was prepared as described for 1-butyl-3-methylimidazolium acetate from 60.6 g choline chloride and 30.0 g glacial acetic acid to give 78.7 g of choline acetate (Y = 96.4%) as a colorless viscous oil, which crystallized as a white solid upon storage. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 1.76 s (3H, CH_3–COO); 3.05 s (9H, N–CH_3); 3.37 m (N–CH_2–CH_2–OH); 3.90 m (N–CH_2–CH_2–OH). Water content: 17774 ppm.

Synthesis of triethylammonium hydrogen sulfate ([HTEA][HSO_4])

Triethylammonium hydrogen sulfate was synthesized according to the procedure described by Brandt-Talbot *et al.*[43] A 250 mL flask with dropping funnel and magnetic stirrer was charged with 50.6 g (0.5 mol) triethylamine and cooled to 0 °C in an ice bath. A solution of 49.0 g (0.5 mol) sulfuric acid in 100 mL deionized water was added slowly to the amine under cooling and the mixture was stirred for 1 h after complete reagent addition. Removal of water under reduced pressure and drying yielded 99.3 g (Y = 99.7%) triethylammonium hydrogen sulfate. ^{1}H NMR spectrum (400 MHz, D_2O), δ, ppm: 1.03 t (9H, N–CH_2–CH_3, *J* = 7.3 Hz); 1.03 q (6H, N–CH_2–CH_3, *J* = 7.3 Hz). Water content: 7925 ppm.

Determination of water content

The water content of the synthesized ionic liquids was determined by coulometric Karl-Fischer titration using a Mitsubishi CA-200 Moisture Meter (Mitsubishi Chemical Corporation, Tokyo, Japan). Defined quantities of the IL were either injected directly into the titration chamber with a dry syringe (for low-viscous [bmim][OAc], [bmim][OFo], [bmim][Gly]), or dissolved in dry formamide to a 50% (w/w) solution (for high-viscous [bmim][HSO_4] and solid [bmim][Cl], [bmim][Br], [HTEA][HSO_4], [Cho][OAc]) before injection. The amount of injected sample was measured by comparing the weight of the syringe after sample aspiration and injection.

Ionic liquid-based pretreatment and fractionation of bagasse

The pretreatment and fractionation of bagasse was performed according to the process scheme displayed in Figure 1. Therefore, a dried ACE pressure tube with magnetic stirring bar was charged with 250 mg oven-dried sugarcane bagasse and 5 g ionic liquid (1:20 bagasse to IL ratio (w/w); ~ 4.8% solid loading) before sealing with a Teflon cap. The reaction vessel was placed in an oil bath preheated to the selected pretreatment temperature (80-120 °C) and kept for the specified time (2-8 h), after which it was quickly cooled to room temperature in a water bath. Subsequently, 45 mL of an acetone/water (1:1 v/v) mixture was added to the reaction vessel, the pressure tube resealed and shaken vigorously to precipitate the carbohydrate-rich residue (CF) and to dissolve the IL and all soluble biomass components. After stirring the solution for 1 h at room temperature, the CF was separated by centrifugation at 20 °C and 5,000 rpm for 15 min (Biofuge Stratos, Heraeus, Germany) and washed 3 times by suspending into 40 mL of deionized water, followed by centrifugation and decantation of the liquid phase. Then, the collected liquors were pooled, the acetone removed under reduced pressure and the remaining solution diluted to 100 mL with deionized water to precipitate the lignin fraction (LF).

The LF was washed 3 times as described above and the combined liquid fractions were concentrated under reduced pressure before drying at 20 mbar and 70 °C for 8 h to recover the ionic liquid. All solid fractions were dried in a drying cabinet at 60 °C for at least 24 h, weighed and kept refrigerated until further analysis.

Compositional analysis of untreated bagasse and recovered carbohydrate-rich material

The structural composition of raw bagasse and recovered solid fractions from pretreatment was performed via a two-step acid-catalyzed hydrolysis protocol according to National Renewable Energy Laboratory (NREL) procedure.[48] Therefore, biomass samples were mixed with 72% (w/w) sulfuric acid for 1 h at 30 °C, followed by dilution to 4% (w/w) with deionized water and heating to 121 °C for 1 h. The precipitated lignin was removed by filtration through a filter crucible and the lignin content was calculated gravimetrically after drying the precipitate for 24 h at 60 °C and correcting for the amount of acid-insoluble ash, which was determined by combustion of the lignin samples in a muffle furnace at 575 °C for 4 h. The remaining hydrolysate was analyzed for the content of monomeric sugars (glucose, xylose and arabinose) using an HPLC unit (Shimadzu Prominence LC20AD, Shimadzu, Japan), equipped with a Shodex sugar SP0810 column and Refractive Index detector (Shimadzu RID-10A, Shimadzu, Japan), applying degassed HPLC grade water at a flow rate of 0.3 mL/min as the mobile phase and a column temperature of 80 °C. Calculation of the resulting cellulose and hemicellulose mass was performed by multiplication of the monomeric sugar content with the respective anhydro conversion factors; specifically, glucose content by 0.90 ($F_{C6\ sugar}$ = 162/180) and the sum of xylose and arabinose content by 0.88 ($F_{C5\ sugar}$ = 132/150).[48]

Fractionation of alkali and formacell lignins

Alkaline lignin was obtained by pretreatment of sugarcane bagasse with 1% (w/w) NaOH at a mass to volume ratio of 1:10 and heating at 80 °C for 3 h. Afterwards, the resulting black liquor was collected by filtration and adjusted to a pH of 2 with H_2SO_4 to precipitate the lignin, which was collected by filtration, washed with deionized water and dried.

Formacell lignin was fractionated from sugarcane bagasse using a 40:40:20 (v/v) mixture of acetic acid, formic acid and deionized water at a 1:5 mass to volume ratio by heating in an ACE pressure tube at 130 °C for 1 h. The resulting pretreatment liquor was removed from the cellulose pulp by vacuum filtration, followed by removal of the pretreatment solvent under reduced pressure. The resulting concentrated liquor containing the solubilized lignin was diluted with an excess of deionized water to induce lignin precipitation, after which the precipitate was collected by vacuum filtration, washed with deionized water until a neutral pH of the washing solution and dried at 60 °C for 24 h.

Fourier transform infrared (FTIR) spectroscopic characterization of untreated bagasse, fractionated carbohydrates and lignin samples

FTIR spectroscopy was used to qualitatively identify the chemical characteristics of untreated bagasse, as well as fractionated bagasse lignin and carbohydrate-rich material. Therefore, biomass powder samples were placed on glass slides and analyzed using a Bruker Alpha-E spectrometer (Bruker Optics Inc., Ettlingen, Germany), with the detector at 4 cm^{-1} resolution, recording 64 scans per sample. Spectral acquisition and instrument control were performed using OPUS 6.5 (Bruker Optics Ltd, Ettlingen, Germany) software, while the analysis was performed using the CytoSpec software (Cytospec Inc., Berlin, Germany).

RESULTS AND DISCUSSION

Recovery and composition of fractionated solids from ionic liquid pretreatment of sugarcane bagasse

The effects of the 8 different ionic liquids on the structural composition of the pretreated sugarcane bagasse, specifically on the variation in cellulose, hemicellulose and lignin content, were investigated (Table 1). Due to its structural analogy to [emim][OAc] and similar delignification performance, as reported in the pretreatment of maple wood flour by Doherty *et al.*,[49] [bmim][OAc] was chosen to evaluate the influence of pretreatment duration and temperature on the recovery and composition of pretreated sugarcane bagasse. As visible from Table 2, Entry 2, a duration of 4 h at 120 °C and a bagasse-to-IL ratio of 1:20 was sufficient to remove 42% of the initial lignin, while 85% of the initial carbohydrate content could be recovered after regeneration of the dissolved bagasse with a 1:1 (v/v) mixture of acetone and deionized water to precipitate the carbohydrate-rich material (CRM), according to the process scheme outlined in Figure 1. The degree of delignification could be enhanced slightly to 49% upon increasing the reaction time to 8 h, which also augmented the removal of hemicelluloses from 15% to 24%. In contrast to pretreatment duration, the variation of the pretreatment temperature displayed a more drastic influence on the overall efficiency of the process. While the pretreatment with [bmim][OAc] at 80 °C led to only 23% delignification, the increase to 100 °C, 120 °C and

140 °C resulted in 33%, 49% and 72% of the available lignin being removed, respectively. The pronounced increase in delignification efficiency, especially at temperatures greater than 120 °C, might be attributed to surpassing the glass transition temperature of lignin of approximately 130-150 °C,[50] which, in conjunction with the lower viscosity of the IL at high temperatures,[51] greatly enhances the diffusion and mass transfer rate of the cleaved lignin fragments from the biomass into the bulk IL.[52] On the other hand, the carbohydrate recovery was concurrently observed to drop from 84% at 120 °C to only 61% at 140 °C. Especially striking is the comparatively high loss of cellulose (40%), which almost doubled at this temperature and is likely traced back to a drastically accelerated rate of cellulose hydrolysis at temperatures higher than 120 °C.[53] As a result, pretreatment conditions of 120 °C and 8 h were chosen as standard conditions for further experiments to maximize delignification while reducing the loss of carbohydrates to a minimum.

In order to compare the influence of the IL anion on the pretreatment performance, four additional halide- and carboxylate-based [bmim] ILs were synthesized and tested for the pretreatment of sugarcane bagasse (Table 2, Entries 7-10). The IL [bmim][Cl] not only serves as an easily accessible precursor in the synthesis of other [bmim]-based ILs, but also has itself been identified as a potent solvent for the dissolution and fractionation of diverse biomass feedstocks.[36-37,41] However, although in the present study both [bmim][Cl] and [bmim][Br] achieved moderate delignification of 26% and 24%, respectively, these results account for only half the amount of lignin removed with [bmim][OAc]. Furthermore, the other carboxylate-based ILs [bmim][OFo] and [bmim][Gly] were found to allow comparatively high lignin removal of 53% and 73%, respectively, while the majority of the carbohydrates could be recovered. A comparable trend was reported by Li *et al.* for the pretreatment and subsequent anaerobic digestion of grass using different [bmim]-based ionic liquids, in which the authors explained the superior results obtained with [bmim][OAc] over [bmim][Cl] with the higher delignification and lower toxicity of [bmim][OAc].[54] Similarly, Li *et al*. reported the extent of delignification for Eucalyptus pretreated with [bmim][OAc] after 30 min at 120 °C to be 16.97%, while only 7.50% lignin removal was reached with [bmim][Cl] under the same conditions (Li *et al.*).[55] This observed trend in pretreatment efficiency is in good agreement with the solvent polarity of the investigated ILs, specifically the Kamlet–Taft β parameter as a measure of hydrogen bond basicity of the anion, which has previously been correlated with lignocellulose dissolution and lignin extraction capability.[49] While the halide-based ILs (β < 0.85) achieved only moderate bagasse dissolution and delignification, the high β values of carboxylate-based ILs (β > 1.10) resulted in almost complete dissolution of bagasse and significantly higher reduction of lignin content in the recovered CRM. In accordance with this interpretation, [bmim][Gly] as the IL with the highest anion basicity, of β = 1.19,[56] was found to give the best pretreatment results.

Despite the moderate to high delignification observed during pretreatment, attempts to recover the removed lignin via evaporation of the acetone portion of the antisolvent mixture, as reported by different groups,[29,32,50,57] provided only miniscule quantities of product and further required lowering the pH of the aqueous IL-lignin mixture to a pH of 1-2. Since the poor lignin yield and the generation of stoichiometric amount of salt from acidification and subsequent neutralization would render the pretreatment process rather unfavorable from an economic and environmental standpoint, three additional ILs that have previously been demonstrated to be effective solvents for biomass fractionation and recovery of lignin, [Cho][OAc],[42] [HTEA][HSO_4][43] and [bmim][HSO_4][39-41] were synthesized and evaluated for the pretreatment of sugarcane bagasse under different conditions.

As evident from Table 2, Entries 12, 15 and 17, the pretreatment under standard conditions (120 °C, 8 h) reduced the lignin content in the CRM by 60%, 80% and 86% for [Cho][OAc], [HTEA][HSO_4] and [bmim][HSO_4], respectively. The performance of [Cho][OAc] was found comparable to that of [bmim][OAc] and slightly superior to the results reported by Ninomiya *et al*., who achieved a reduction in the bagasse lignin content of about 50% after pretreatment with [Cho][OAc] at 110 °C for 11 h.[42] Conversely, the hydrogen sulfate-based ILs exhibited significantly higher delignification and markedly reduced the hemicellulose content in the CRM. This behavior is somewhat surprising, given that the significantly lower β–value of hydrogen sulfate anion, of only 0.67,[39] would predict poor lignocellulose dissolution ability and pretreatment efficiency for these ILs. However,

the high Brønstedt acidity of the HSO_4^--counterion compared to OAc^- has previously been suggested to compensate for the low H-bond basicity by promoting highly efficient acid-catalyzed cleavage of both the ester linkages in the lignin carbohydrate complexes (LCCs) and the arylether bonds connecting the individual monolignol scaffolds in the lignin itself. As a result, the catalytic activity of the anion is believed to greatly benefit the release and solubilization of lignin fragments, as well as the depolymerization of hemicellulose into C5-monomeric sugars.[24] This was corroborated by a study comparing different [bmim]-based ILs for the pretreatment of *Miscanthus* at 120 °C for 11 h by Brandt *et al.*,[39] who could demonstrate that [bmim][HSO_4] removed lignin and hemicellulose most efficiently, followed by [bmim][$MeSO_4$] and [bmim][OAc], whereas [bmim][Cl] and [bmim][OTf] had only negligible effects on the composition of the pretreated material. Similar to the high delignification of 86.2% after [bmim][HSO_4] pretreatment for 8 h in the current study, the authors found the lignin in *Miscanthus* to be almost completely removed after 22 h. However, it has to be taken into consideration that all ILs utilized by Brandt *et al.* contained 20% (v/v) H_2O, which was previously shown to significantly influence the capability of certain ILs to swell and dissolve lignocellulosic biomass and may also negatively affect the ability of some ILs to extract and solubilize lignin.[51] This is especially true for hydrogen-basic ILs, such as the employed [bmim]-carboxylates, for which the presence of small amounts of moisture (above 1% (w/w)) can diminish the ability to disrupt the intra- and intermolecular hydrogen bonding network in the biomass.[51] On the other hand, hydrogen-acidic ILs do not rely on complete biomass solubilization during the pretreatment process, and the presence of water has consequently been reported to impact the delignification efficiency of HSO_4^--containing ILs to a much lesser extent.[24] In order to minimize the effect of adventitious water in the pretreatment stage, the bagasse as well as the ILs employed in the present study had been dried prior to utilization. However, it has to be noted that the drying process was found to be less effective for ILs with strongly hydrogen-basic anions, *i.e.* acetate and other carboxylates, resulting in higher residual moisture content in these ILs (up to 1.7% (w/w)), compared to their halide and hydrogen sulfate analogs (0.2 to 0.8% (w/w)). In contrast to the superior performance of HSO_4^--containing ILs observed in this study, Bernardo *et al.*[59] recently concluded the pretreatment of wheat straw and eucalyptus residues with [emim][HSO_4]-water mixtures for 1.5 h at 140 °C and at IL to H_2O ratios ranging from 1:4 to 3:2 (w/w) to be ineffective in reducing the lignin content in either of the tested biomass sources, albeit being highly suitable to remove and hydrolyze the hemicellulose fraction into its constituent pentoses and furfural.

Table 1
Names and abbreviations of ionic liquids used in the present study

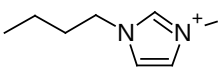
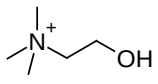
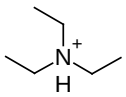
Cation	Anion	Name	Abbreviation
	Br^-	1-butyl-3-methylimidazolium bromide	[bmim][Br]
	Cl^-	1-butyl-3-methylimidazolium chloride	[bmim][Cl]
	$HCOO^-$	1-butyl-3-methylimidazolium formate	[bmim][OFo]
	CH_3COO^-	1-butyl-3-methylimidazolium acetate	[bmim][OAc]
	$H_2NCH_2COO^-$	1-butyl-3-methylimidazolium glycinate	[bmim][Gly]
	HSO_4^-	1-butyl-3-methylimidazolium hydrogen sulfate	[bmim][HSO_4]
	CH_3COO^-	choline acetate	[Cho][OAc]
	HSO_4^-	triethylammonium hydrogen sulfate	[HTEA][HSO_4]

Table 2
Summary of sugarcane bagasse pretreatment results for different ionic liquids and pretreatment conditions

	Conditions			Recovered fractions [a]			Composition of CRM [b]			Recovery [c]				Delignification [d]
#	Ionic liquid	T (°C)	t (h)	%CRM	%LRM	%Loss	%C	%HC	%L	%C	%HC	$\%L_{(CRM)}$	$\%L_{(LRM)}$	(%)
1	Raw bagasse						38.9	25.2	22.0	100.0	100.0	100.0	0.0	0.0
2	[bmim][OAc]	120	4	78.0	-	22.0	42.7	27.5	16.3	85.6	85.3	57.8	n.d.	42.2
3	[bmim][OAc]	120	8	72.8	-	25.4	43.3	26.2	15.5	81.0	75.6	51.3	n.d.	48.7
4	[bmim][OAc]	80	8	88.2	-	11.8	40.1	23.1	19.1	91.0	81.0	76.7	n.d.	23.3
5	[bmim][OAc]	100	8	79.7	-	20.3	39.9	25.1	18.6	81.8	79.3	67.5	n.d.	32.5
6	[bmim][OAc]	140	8	51.4	-	48.6	45.6	31.0	12.0	60.2	63.2	28.0	n.d.	72.0
7	[bmim][Br]	120	8	89.7	-	10.3	40.6	23.9	18.7	93.6	85.1	76.2	n.d.	23.8
8	[bmim][Cl]	120	8	86.5	-	13.5	40.5	22.8	18.8	90.2	78.4	73.8	n.d.	26.2
9	[bmim][Gly]	120	8	65.2	-	34.8	50.2	29.8	9.1	84.1	77.1	26.9	n.d.	73.1
10	[bmim][OFo]	120	8	83.9	-	16.1	42.0	26.8	12.5	90.5	89.1	47.5	n.d.	52.5
11	[bmim][HSO_4]	120	2	32.1	19.1	48.7	76.9	10.9	11.3	63.5	13.9	16.4	87.0	83.6
12	[bmim][HSO_4]	120	8	25.1	18.3	56.6	78.4	10.8	12.1	50.6	10.7	13.8	83.1	86.2
13	[HTEA][HSO_4]	120	2	55.5	7.4	37.1	43.2	18.3	15.2	61.6	40.3	38.5	33.5	61.5
14	[HTEA][HSO_4]	100	8	71.1	2.2	26.7	45.7	25.9	17.0	83.6	73.1	55.1	9.9	44.9
15	[HTEA][HSO_4]	120	8	38.0	20.8	41.2	62.4	22.9	11.8	60.9	34.5	20.3	94.3	79.7
16	[Cho][OAc]	120	4	77.0	2.9	20.1	35.8	29.7	13.8	70.9	90.8	48.1	13.3	51.9
17	[Cho][OAc]	120	8	72.8	5.1	22.1	37.3	32.3	12.3	69.9	93.4	40.7	23.4	59.3

[a] Relative mass of recovered carbohydrate-rich material (%CRM), lignin-rich material (%LRM) and material remaining in solution (%Loss) with respect to the initial weight of bagasse; [b] Percentage of cellulose (%C), hemicelluloses (%HC) and lignin (%L) in the recovered CRM; [c] Percentage of recovered cellulose (%C), hemicelluloses (%HC) and lignin in the CRM ($\%L_{(CRM)}$) and LRM ($\%L_{(CRM)}$) with respect to the available content in raw bagasse; [d] Percentage of removed lignin with respect to the available content in raw bagasse and recovered lignin in the CRM

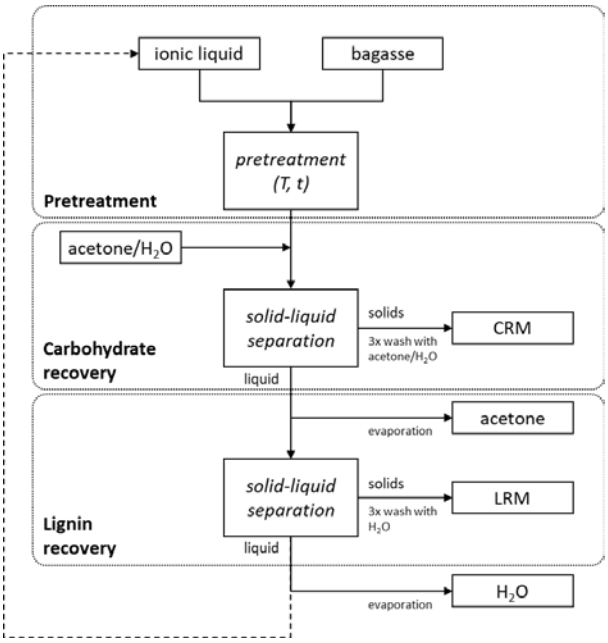


Figure 1: Schematic representation of the ionic liquid-based bagasse fractionation process

However, it is possible that the higher dilution of the IL, as well as the use of water instead of methanol or acetone:water mixtures employed in other studies to precipitate and fractionate the carbohydrate-enriched fraction from the IL-dissolved lignin may have significantly diminished the lignin solubility in the acidic aqueous phase.[30] This may, in turn, lead to redeposition of the solubilized lignin onto the pretreated material and explain the low degree of delignification observed by the authors.

Reduction of pretreatment severity either by shortening the pretreatment duration (Table 2, Entries 11, 14) or by decreasing temperature (Table 2, Entry 14) diminished delignification and hemicellulose removal for [HTEA][HSO_4], while having only a marginal effect on the performance of [bmim][HSO_4]. In the case of [HTEA][HSO_4], similar observations were reported by Brand-Talbot *et al.*, who found the delignification of *Miscanthus* pretreated at 120 °C to peak around 8 h at approximately 88%, whereas only around 71% lignin removal occurred after 2 h.[43] In the present study, only a slightly lower degree of delignification (86.2% *versus* 83.6%) was observed after shortening the pretreatment duration for [bmim][HSO_4] from 8 h to 2 h, albeit being accompanied with a marked increase of cellulose recovery from 51% to 64%. These results suggest a shorter pretreatment duration of only 2 h to be preferable in the case of this IL, which furthermore has the beneficial effect of reducing the energy consumption of the pretreatment process. Notably, both the reduction of hemicellulose content and delignification after 2 h achieved in this work (82.1% and 83.6%, respectively) were found to exceed the values reported for the pretreatment of *Miscanthus* with the same IL for 2 h by Brandt *et al.* (59.5% and 65.7%, respectively).[39] It appears plausible to attribute the difference in performance to the structural variations between the utilized feedstocks, as well as the admixture of 20% (v/v) water to the IL, as described by Brandt *et al.*

Recovery of lignin

Owing to the increasing recognition of lignin as a valuable natural resource with high potential for value addition, we investigated the feasibility of recovering the lignin solubilized during ionic liquid-based pretreatment. In this context, acetone-water mixtures have been successfully applied as antisolvents by several groups[29,32,50,57] to fractionate carbohydrates and lignin, followed by precipitation of the lignin fraction by simple evaporation of the acetone from the pretreatment liquor, as outlined in Figure 1. However, as discussed above, attempts to recover lignin from the pretreatment of bagasse with different [bmim]-halide and [bmim]-carboxylate ILs gave poor results, which prompted the evaluation of alternative ILs that allowed the precipitation of lignin using the aforementioned approach without requiring acidification of the water-IL-lignin

mixture. Figure Figure 2 shows that utilization of [Cho][OAc] as the pretreatment medium facilitated the recovery of 13% of the available lignin after 4 h, which almost doubled to 23% after 8 h. The latter corresponds to 5.1% of the initial bagasse weight and is in good agreement with the value of 5.2% reported by Ninomiya *et al.*, albeit slightly different conditions (110 °C, 22 h) were applied than those in the current study.[42] Notably, the hydrogen sulfate-containing ILs [HTEA][HSO_4] and [bmim][HSO_4] allowed for markedly higher lignin recoveries, totaling 91% and 84% of the initial lignin content after 8 h, respectively. A reduction of the pretreatment duration to 2 h did not have a drastic effect on the lignin recovery for [bmim][HSO_4], for which the percentage of precipitated lignin even increased slightly to 84%. In the case of [HTEA][HSO_4], the lignin yield dropped sharply to only 33% and 10% for 2 h at 120 °C and 8 h at 100 °C, respectively, indicating a pronounced influence of the reaction temperature not only for the delignification of bagasse, but also for the ability to precipitate the solubilized lignin. Furthermore, considering the compositional analysis of the recovered CRM fraction from [HTEA][HSO_4] pretreatment at 120 °C and 8 h revealed 19% of the initial lignin content still to be present, it is assumed the lignin fraction recovered under these conditions contains a non-negligible amount of impurities. It cannot be excluded that a small fraction of [HTEA][HSO_4] remained in the recovered material after the wash cycle, although the lignin precipitate was washed three times with deionized water to remove any residual IL. Another probable explanation for the observed lignin yield greater than 100% is the formation of water-insoluble humins, also referred to as "pseudo-lignin" and proposed to arise from acid-catalyzed decomposition of hemicellulose into furfural and 5-hydroxymethyl furfural, followed by rearrangement into polyaromatic, lignin-like structures.[58]

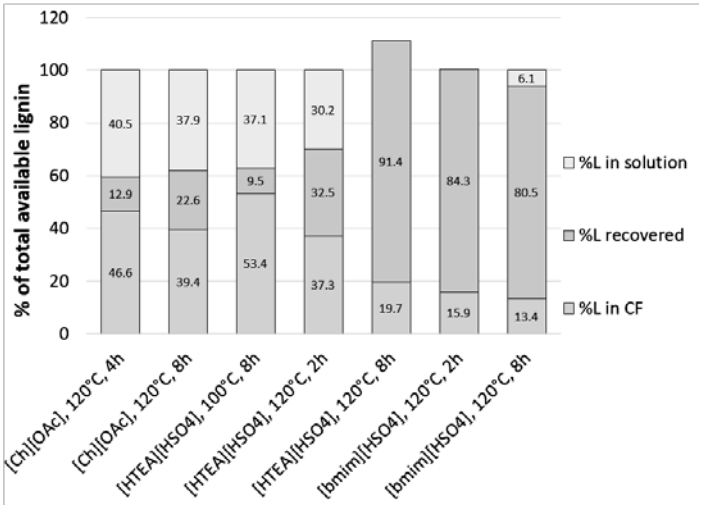


Figure 2: Percentage of recovered lignin precipitate, remaining lignin in CRM and solubilized lignin for different ionic liquids and pretreatment conditions

Similar observations were reported for the pretreatment of sugarcane bagasse at elevated temperature and prolonged pretreatment time with acidic 1-ethyl-3-methylimidazolium alkylbenzenesulfonate, leading to a lignin yield of 118% after heating for 2 h at 190 °C.[60] In addition, pretreatment of *Miscanthus* with [bmim][HSO_4] at 120 °C for 22 h was demonstrated to yield almost double the amount of lignin than theoretically possible. The authors also explained these findings by excessive pseudo-lignin formation under the applied conditions and could remediate the extent of humin contamination by shortening the pretreatment time and diluting the IL with up to 20% (w/w) of water,[39] thereby reducing the yield to approximately 100% of total available lignin. In an earlier study concerning the selective fractionation of hemicelluloses from wheat straw, Carvalho *et al.* concluded convincingly that a higher water content in the IL would disfavor the dehydration and decomposition of hemicellulose-derived monomeric sugars, in particular for high-severity conditions, and thus not only reduce

sugar losses during pretreatment, but also limit the extent of pseudo-lignin formation.[40] Clearly, these findings suggest placing the observed lignin yields from acidic pretreatment media, especially with high severity conditions (low pH, high temperature, extended pretreatment duration), under increased scrutiny and emphasize the necessity to assess the purity of the obtained material to distinguish actual and pseudo-lignin in the extracts.

Characterization of recovered lignin fractions

The precipitated lignin fractions from ionic liquid pretreatment under varying conditions were characterized using different spectroscopic techniques, with the aim of assessing the purity of the derived solids and further to reveal changes of structural characteristics in relation to the pretreatment conditions. To further compound the characterization, lignin samples derived from alkaline and acetic/formic acid pretreatment, as well as a commercial Kraft lignin sample were used as reference material to allow comparison of the observed structural features between different types of pretreatment.

The recorded FTIR spectra provided valuable information on the abundance of functional groups and structural fragments of the investigated lignin samples. As visible from Figure 3, a wide peak between ~3,600 and 3,000 cm^{-1} related to aliphatic and aromatic O−H stretching[61] was observed in all the spectra to a varying extent. Comparison of the peak intensity indicates the total hydroxyl group content is the highest in alkaline and [bmim][HSO_4]-pretreated lignins, and generally increased with pretreatment duration. This correlates well with the assumption that the formation of new aromatic and aliphatic −OH groups by cleaving α-*O*-4 and β-*O*-4 ether linkages is enhanced during pretreatment under more severe conditions,[62] albeit some distortion of the O−H signal by trace amounts of water usually adsorbed during sample preparation cannot be excluded. Asymmetric methyl and methylene C−H stretching vibrations, as well as symmetric C−H stretching vibrations of methoxy groups, were found at wavenumbers of ~ 2,930 and 2,850 cm^{-1}, respectively, confirming the expected abundance of these functional groups in all recovered lignins. However, the intensity of the methyl and methylene signals in the lignin sample from [HTEA][HSO_4] pretreatment for 8 h at 120 °C, together with a shift to higher wavenumbers indicates the presence of non-lignin derived impurities, as suggested earlier from the unusually high lignin recovery in this case. Further evidence can be inferred from the presence of strong absorption bands around 1,160 cm^{-1} and 1,034 cm^{-1}, which have been associated with asymmetric C−O−C and C−O stretching vibrations in carbohydrates.[42] The latter absorption band is also found in other lignins obtained under acidic conditions, albeit with markedly reduced intensity, suggesting that minor impurities may be present in these samples.

The FTIR fingerprint region displayed in Figure 4 features typical absorption bands corresponding to C=O stretching vibrations in conjugated (~1,630 cm^{-1}) and unconjugated carbonyl species (~1,695 cm^{-1}). Especially the latter signal, arising from unconjugated ketones and carboxyl groups, was found to be a prominent feature of Formacell lignin and lignins from pretreatment with acidic ILs, specifically at longer pretreatment times, but is less pronounced or absent in all other lignins. This trend is consistent with the formation of additional carbonyl groups from acid-catalyzed cleavage of α-*O*-4 and β-*O*-4 ether bonds, which is much less dominant during alkaline or Kraft pulping where vinyl ethers and related structures have been proposed to form instead (Fig. 7).[63] At the same time, the intensity of the aromatic O−H signal at 1,371 cm^{-1} attributed to non-etherified phenols was found most pronounced for the lignins derived under acidic conditions, as would be expected assuming the different mechanisms for the cleavage of α-*O*-4 and β-*O*-4 ether linkages outlined in Figure 7. Notably, the increased proportion of reactive carbonyl and phenol groups in these lignins may be of value for applications in which further functionalization, such as epoxidation or alkoxylation of these groups is required to produce lignin-based resins.[10]

In addition, the FTIR fingerprint region contains distinctive spectral features of the aromatic constituents of lignin: specifically, C−C stretching vibrations of the aromatic skeleton at 1,595 and 1,510 cm^{-1}, in-plane C−H deformation of aromatic ring structures at 1,420 cm^{-1} and asymmetric C−H deformation of $-CH_3$ and $-CH_2$ at 1,460 cm^{-1}.[64] The similarity with respect to shape and intensity of these signals among the investigated samples implies the lignin backbone remained largely unaffected by the variation of pretreatment conditions. More importantly, this part of the spectrum contains information about the abundance of the aromatic *p*-hydroxyphenyl

(H), guaiacyl (G) and syringyl (S) units of the lignin (Fig. 8), which exhibit characteristic absorption bands at 1,163 cm^{-1} (ester bonds between H-units), 1,265 cm^{-1} (C−O stretching and ring breathing of G-units), as well as 1,121 cm^{-1} (in-plane C−H deformation in S-units) and 835 cm^{-1} (out-of-plane C−H deformation at 2 and 6 position of S-units), respectively.[61-62]

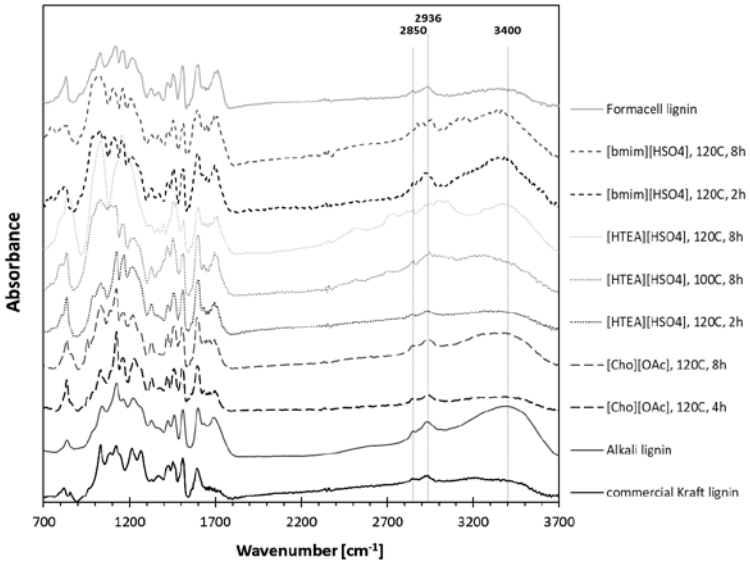


Figure 3: Overlaid FTIR spectra of isolated lignins from IL-based pretreatment of sugarcane bagasse under different conditions, lignins from Formacell and alkaline pretreatment as well as commercial Kraft lignin

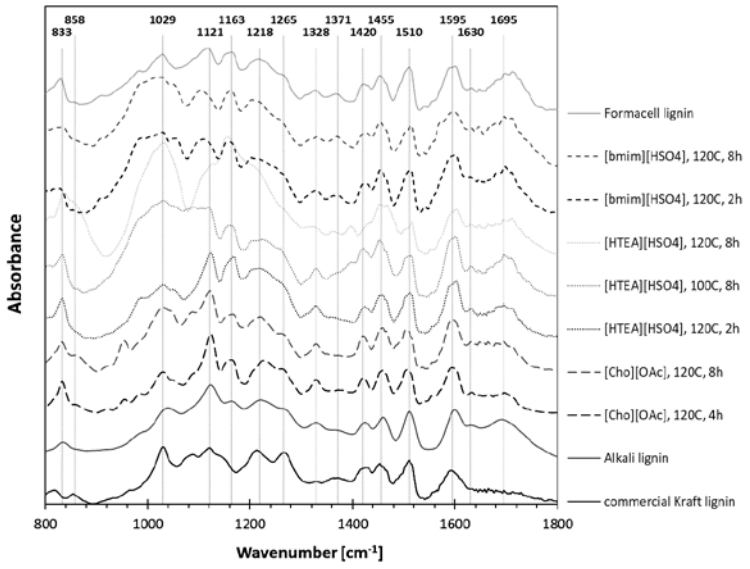


Figure 4: Overlaid spectra of the FTIR fingerprint region for isolated lignins from IL-based pretreatment of sugarcane bagasse under different conditions, lignins from Formacell and alkaline pretreatment as well as commercial Kraft lignin (vertical lines indicate characteristic lignin absorption bands)

Among these aromatic constituents, especially the ratio of H- and G-type to S-type structural content is of particular interest with respect to potential applications for the derived lignins. The production of lignin-based adhesives by reaction with formaldehyde or other aldehydes requires a free position for electrophilic attack at the 5 position (*ortho* with respect to the phenolic OH group) of the lignin aromatic moiety, which is accessible in H and G-type lignin, but blocked by a methoxy group in S-type lignin (Fig. 8).[63] On the other hand, the antioxidant and antibacterial

properties of different lignins have been demonstrated to scale with increasing methoxy content,[12,65] suggesting high S/G ratios and low H-content to be beneficial in cases where the biological activity of lignin is of interest, whereas low S/G values are preferable for the production of lignin-based adhesives. A comparison of bagasse lignins with the commercial Kraft lignin sample suggests a predominantly G-type composition for Kraft lignin, as evidenced by the absence of the 1,163 cm^{-1} signal for H-type lignin, the strong 1,265 cm^{-1} signal of G-type lignin, in conjunction with two absorption bands at 810 and 855 cm^{-1} (out-of-plane C−H deformation at 2, 5 and 6 position of G-units),[22] instead of the single line at 835 cm^{-1} typical for S-type lignins. In contrast, all bagasse lignins display high S- and H-type content with a much smaller contribution from G-type lignin. Due to the fact that Kraft lignin is derived from pine, a softwood species composed mainly of G-type lignin, whereas perennial grasses feature a high abundance of H-, S- and G-type lignins, the observed differences between Kraft and sugarcane bagasse lignins are in line with previous data.[22,63] More subtle variations of the S/G ratio have been estimated among the individual bagasse lignin samples based on the relative absorbances of characteristic peaks for G-type (1,265 cm^{-1}) and S-type lignin (1,121 cm^{-1}), normalized against the absorbance at 1,510 cm^{-1}, as previously described by Shukry *et al.*[62] The S/G ratio of the lignin samples was found to increase in the order alkali (1.25) << $[HTEA][HSO_4]_{2h,120°C}$ (1.58) << Formacell (1.73) < $[bmim][HSO_4]_{2h,120°C}$ (1.77) < $[Cho][OAc]_{4h,120°C}$ (1.81), indicating a relationship between the severity of the pretreatment and the observed structural composition of the lignin residue. High alkalinity, as well as higher acidity of the solvent medium ($[HTEA][HSO_4]$ > $[bmim][HSO_4]$ > Formacell) appeared to decrease the S/G ratio slightly, either caused by demethylation of S-units[14] or due to selective solubilization and precipitation of specific lignin fragments. In this context, Brandt *et al.*[66] concluded a preferential precipitation of G-type lignin, exhibited by C−C bond formation between the G-aromatics at the C6 position to take place during $[bmim][HSO_4]$ pretreatment of *Miscanthus giganteus* as an explanation for the observed enrichment in G-type lignin, which increased with prolonged heating at 120 °C. Notably, a similar decrease of the S/G ratio was observed in the current study when the duration of $[bmim][HSO_4]$-pretreatment was increased from 2 to 8 h. Together with the increase of phenolic hydroxyl group content, this depletion of S-type units in Formacell-, $[bmim][HSO_4]$- and $[HTEA][HSO_4]$-extracted lignins would benefit the use of these materials in the production of lignin-based adhesives. On the other hand, the higher purity and abundance of methoxy groups due to the enrichment in S-type lignin in [Cho][OAc]-derived material may suggest applications in the biomedical field, where the antimicrobial and antioxidant properties of lignin are exploited.

FTIR spectroscopic characterization and effect of ionic liquid pretreatment on the crystallinity of the carbohydrate-rich fraction

Besides the lignin content and the presence of other enzyme-inhibiting compounds, the crystallinity of biomass, specifically that of the cellulose fraction, has been identified as one of the most important factors contributing to the recalcitrance of the lignocellulosic material towards valorization via enzymatic saccharification into its constituent sugars.[67] An important advantage of IL-based pretreatment over other pretreatment and fractionation processes is the ability not only to delignify biomass, but also to dissolve and decrystallize the cellulose content in lignocellulosic substrates, which has been shown to greatly enhance the rate of subsequent hydrolysis reactions. However, the extent of cellulose decrystallisation may vary considerably between different types of ILs, and a good correlation between the Kamlet–Taft β parameter as a measure of the H-bond basicity of the IL and its ability to reduce the crystallinity of cellulose has been established in several studies.[24,49,59] Consequently, we aimed to compare two commonly applied indices for cellulose crystallinity accessible from FTIR spectroscopic characterization, the lateral order index (LOI, defined as the ratio $A_{1,428}/A_{897}$) and the total crystallinity index (TCI, defined as the ratio $A_{1,371}/A_{2,920}$),[29,67-68] to assess the ability of different hydrogen-bond acidic and hydrogen-bond basic ILs to reduce the crystallinity in the pretreated material.

The FTIR spectroscopic data compiled in Figures 5 and 6 show that IL pretreatment generally led to a noticeable decrease in lignin content in the CRM, compared to raw bagasse, as evidenced by the decrease of characteristic bands associated with the aromatic constituents of lignin

(Fig. 6, dashed vertical lines), and hence corroborates the results obtained from the compositional analysis presented above. In turn, typical absorption bands for carbohydrates, specifically the signals at 896 cm^{-1} (C−H vibration of β-(1→4)-glycosidic linkages), 993 cm^{-1} (arabinosyl side chains), 1,032 cm^{-1} (C−O stretching vibration in cellulose), 1,050 cm^{-1} (C−O asymmetric stretching in ester linkages), 1,104 cm^{-1} (C−OH skeletal vibration in pyranosides), 1,159 cm^{-1} (C−O asymmetric vibration), 1,320 cm^{-1} (C−C and C−O skeletal vibrations), 1,428 cm^{-1} ($C-H_2$ symmetric bending vibration) and 2,920 cm^{-1} (C−H stretching vibration),[29,68] appear to be retained or enhanced, consistent with an enrichment in carbohydrates in the recovered material. Out of these bands, the ratio between the so-called "crystalline band" at 1,428 cm^{-1} (associated with cellulose I) and the "amorphous band" at 993 cm^{-1} (associated with cellulose II and amorphous cellulose) has been reported to correlate well with the amount of crystalline and amorphous cellulose in a sample and is commonly referred to as the LOI.[68] In analogy, the ratio between the absorption band at 1,371 cm^{-1}, which is assumed to be unaffected by alterations in crystallinity, and the band at 2,920 cm^{-1} defines the TCI of the material.[68] Lower values for both indices indicate a higher proportion of amorphous regions in a sample and thus lower overall crystallinity.

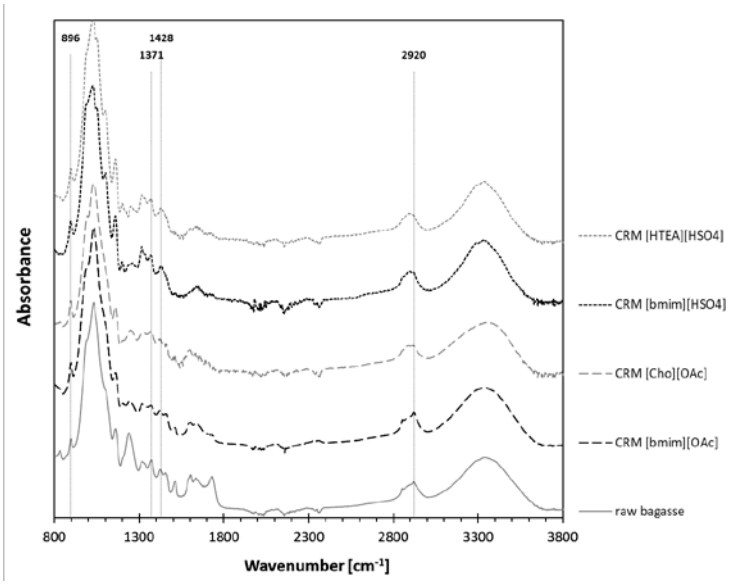


Figure 5: Overlaid FTIR spectra of isolated carbohydrate-rich fractions from IL-based pretreatment of sugarcane bagasse under different conditions (vertical lines indicate the position of absorption bands used to determine the crystallinity of the cellulose fraction)

Table 3
Calculated Total Crystallinity Indices (TCI) and Lateral Order Indices (LOI) for untreated bagasse and recovered carbohydrate-rich material from IL-based pretreatment of sugarcane bagasse

Sample	$A_{1,372/2,900}$ (TCI)	$A_{1,432/897}$ (LOI)
[bmim][OAc], 120 °C, 8 h	1.185	0.489
[Cho][OAc], 120 °C, 8 h	1.371	0.567
[bmim][HSO_4], 120 °C, 2 h	1.422	0.562
[HTEA][HSO_4], 120 °C, 2 h	1.454	0.599
Untreated bagasse	1.580	0.647

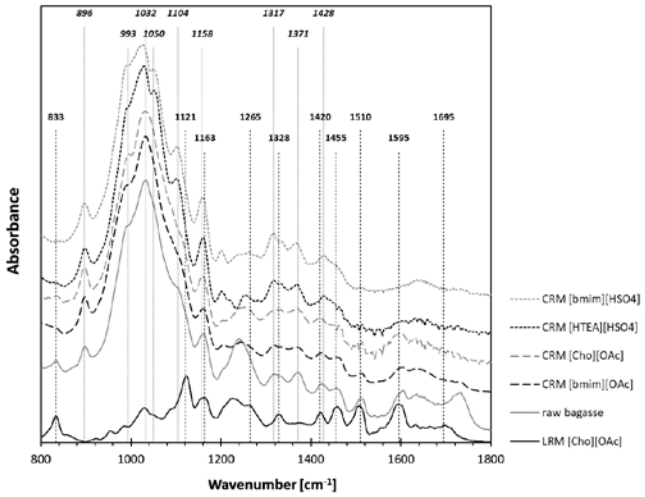


Figure 6: Overlaid spectra of the FTIR fingerprint region for isolated carbohydrate-rich fractions from IL-based pretreatment of sugarcane bagasse under different conditions, as well as for untreated bagasse and IL-extracted lignin (vertical lines indicate the position of characteristic absorption bands associated with the carbohydrate (solid line) and lignin (dashed line) fractions, respectively)

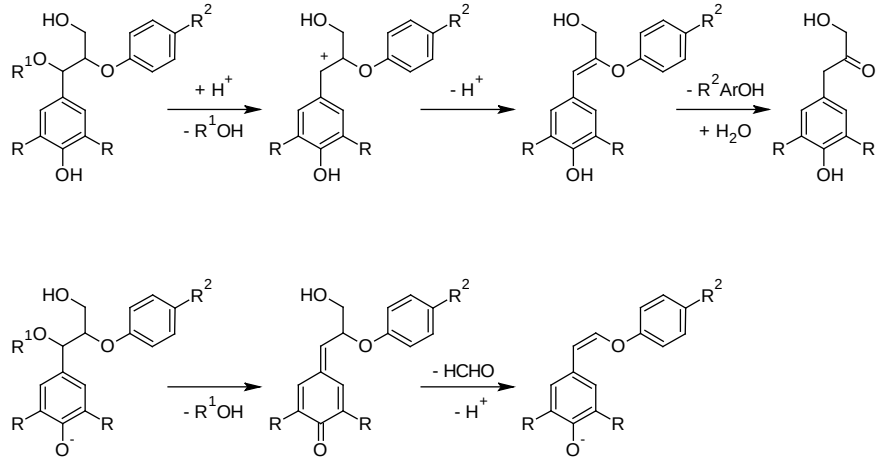


Figure 7: Main structural alterations of lignin during pretreatment under acidic conditions (top panel) and alkaline pretreatment (bottom panel) according to Tejado *et al.*[63]

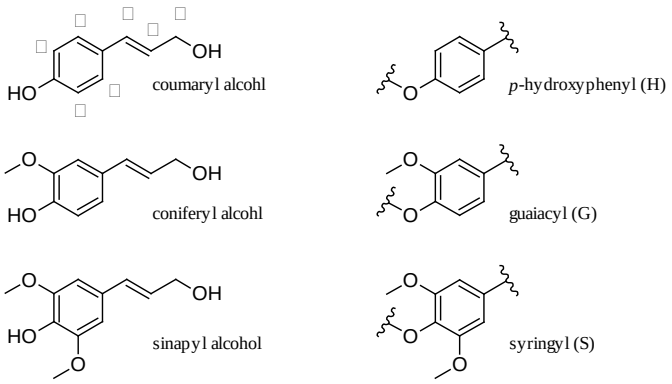


Figure 8: Structures and numbering of C-atoms for the monolignols forming the basic monomers of lignin and the corresponding *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin subunits

As visible from Table 3, both the TCI and LOI were highest for untreated raw bagasse (1.580 and 0.647, respectively) and decreased upon IL pretreatment and fractionation in all studied cases. However, it was found that hydrogen-basic ILs, in particular [bmim][OAc] (TCI and LOI of 1.185 and 0.489, respectively), exhibited the most pronounced reduction in crystallinity, whereas both [bmim][HSO_4] and [HTEA][HSO_4] pretreatment led to only slight decrystallization of the CRM. This behavior is in good agreement with observations reported by Bernardo *et al.*, who compared the impact of H-bond acidic IL [emim][HSO_4] and H-bond basic IL [emim][OAc] on the crystallinity of wheat straw and eucalyptus and found only the latter to produce amorphous pretreated material. Interestingly, despite having similar anion basicity and showing comparable pretreatment performance, [Cho][OAc] was found to result in less distinctive crystallinity reduction than [bmim][OAc] in the present study. A possible explanation could be the slightly higher water content of [Cho][OAc] (~1.7%), which may have diminished the ability to swell and dissolve the cellulose fraction, thus leaving the crystalline regions largely unchanged upon regeneration of the CRM. Nevertheless, while high crystallinity may be disadvantageous for applications that rely on catalytic – in particular enzymatic – deconstruction of the cellulose fraction, other potential avenues for cellulose valorization, such as the production of microcrystalline cellulose or cellulose nanomaterials could benefit from pretreated solids with higher crystallinity.

CONCLUSION

The pretreatment of sugarcane bagasse with 8 different ILs was investigated to assess the changes in structural composition, in particular the reduction of lignin content in the pretreated carbohydrate-rich material. While halide-based ILs achieved only moderate delignification, [bmim] carboxylates allowed high carbohydrate recovery and low residual lignin in the pretreated material, with [bmim][Gly] showing the highest delignification efficiency. [Cho][OAc], a potentially fully bio-derivable alternative to imidazolium-based ILs, showed comparable pretreatment performance to [bmim][OAc]. Notably, acidic ILs, especially [bmim][HSO_4], were shown to exhibit even greater lignin removal at shorter pretreatment duration, while also reducing the hemicellulose content in the pretreated material to a much higher extent. This may prove advantageous for further down-stream processing of the cellulosic material; however, the current inability to recover the solubilized hemicellulose from the pretreatment liquor also means further research would be required to allow adequate valorization of this fraction of the biomass when using acidic ILs.

The precipitation of the solubilized lignin fraction using acetone-water mixtures as an environmentally benign and easily recyclable antisolvent was attempted in order to recover the lignin as a valuable side stream of the pretreatment process. The acidic ILs [HTEA][HSO_4] and [bmim][HSO_4], which were highly effective in the delignification of bagasse also gave the highest lignin yields among all investigated ILs, followed by [Cho][OAc], while the remaining ILs only gave access to minuscule quantities of precipitated lignin.

FTIR spectroscopic characterization of the produced IL-extracted bagasse lignins and comparison with lignin samples from alkaline and Formacell pretreatment provided evidence that the purity and structural composition of sugarcane bagasse derived lignins can be tailored by the applied pretreatment method and conditions, thus leading to different potential applications. Formacell and acidic IL pretreatment gave material with very similar characteristics, comprising elevated unconjugated carbonyl and phenolic OH content and enrichment in G-type lignin, which may be suitable for the production of lignin polymer resins. The pretreatment with [Cho][OAc] provided lignin of higher purity with potential antimicrobial or antioxidant activity, due to the higher proportion of S-type lignin in the derived material.

It is therefore concluded that IL-based pretreatment of sugarcane bagasse constitutes an interesting alternative to existing pretreatment methods for lignocellulosic biomass, which can be tuned to give access to different fractions of the biomass depending on the type and properties of the applied IL. However, further research will be required to improve the process conditions in order to achieve simultaneously high hemicellulose and lignin recovery values, which would increase the economic viability of the process.

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