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Fractionation of Lignin from Eucalyptus Bark Using Amine-Sulfonate Functionalized Ionic Liquids

Peifang Yan, Zhanwei Xu, Chao Zhang, Xiumei Liu, Wenjuan Xu, Z. Conrad Zhang*

Dalian Institute of Chemical Physics, CAS, Dalian, 116023, China

Abstract

A series of amine-sulfonate functionalized ionic liquids (ASF-ILs) were synthesized, characterized, and evaluated for the dissolution of model biopolymers (cellulose, xylan, kraft lignin and lignosulfonate). The ASF-ILs were prepared in high atomic efficiency. Most of the ASF-ILs dissolve kraft lignin and lignosulfonate efficiently at 373 K, with solubilities of 0.220–0.385g for kraft lignin and of 0.150-0.290 g for lignosulfonate per gram of ASF-ILs. In contrast, xylan and cellulose are scarcely soluble (<5mg g^{-1}) in the ASF-ILs. Based on the results of the model lignin dissolution, four most promising ASF-ILs were selected and applied for the pretreatment of eucalyptus bark. The lignin was selectively fractionated by the ASF-ILs (393 K for 10h, lignin removal is more than 40%).The fractionated lignin and the eucalyptus bark residues were then characterized by infrared spectroscopy and thermo gravimetric analysis. Importantly, we demonstrate that enzymatic hydrolysis of the polysaccharides components of the eucalyptus bark to sugars was substantially enhanced after the pretreatment, most pronouncedly with the ASF-IL[Et_4N][$Me_2NC_4SO_3$].

KEYWORDS: Lignin; Amine-sulfonate functionalized ionic liquids; Biomass pretreatment; Enzymatic hydrolysis; Lignin removal.

State Key Laboratory of Catalysis, Dalian National Lab for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian, 116023, China.

E-mail: zczhang@yahoo.com; Fax (+)86 411 8437 9462

Introduction

Lignin is one of the three major components of lignocellulosic biomass.[1,2] The lignin content in plants varies widely, typically in the range of 15 to 25% in the weight (wt%) of dry biomass depending on its source; lignin content in tree barks is typically higher, e.g. up to 40%. As a three dimensional amorphous polymer containing dominantly phenylpropane units, lignin is potentially an abundant renewable resource for producing aromatics and cyclic alkanes.[3-5] Lignin can be isolated in large quantity from lignocellulosic materials by strong dilute acid treatment, [6] alkaline treatment,[7] organosolve isolation[8] or sulphite pulping process.[9] Lignin fractionation from biomass is a critical step for biomass utilization, not only in the potential value of the isolated lignin, but also in enabling the utilization of the remaining polysaccharides. In order to access the carbohydrates in the biomass for biological conversion, an additional deconstruction step, also commonly called pretreatment, is typically required to bring the sugar polymers accessible to enzymes and yeasts for hydrolysis and subsequent fermentation. The lignin crust has been identified as one of the major obstacles for an energy-efficient biomass deconstruction process.[10] A successful biomass pretreatment should result in high cellulose and hemicellulose recovery with tolerable lignin content by the subsequent bio-processing. The above mentioned traditional pretreatment methods, have been mostly developed for biomass utilization, but these methods in general carry serious environmental consequences, strong corrosion, and some involve energy intensive processes.[11]

Recently, dissolution of biomass in some ionic liquids has been demonstrated as a promising alternative pretreatment method. Ionic liquids (ILs) are regarded as ‘‘green solvents’’ due to their unique properties such as nearly total non-volatility, high oxidative and thermal stabilities, wide liquid temperature range, and tunable properties.[12,13] There are numerous reports in the literature on using ionic liquids for cellulose dissolution[14-16] and lignin dissolution, isolation, and depolymerization.[17-24] The properties of ionic liquids, as determined by the chemical functionality of the cation and anion pairs, are important factors on the pretreatment specificity and

efficiency in deconstructing the biomass. Some ionic liquids have been reported to swell, decrystallize, and dissolve lignocellulosic biomass and microcrystalline cellulose (MCC),[14-16] while others have been shown to selectively remove lignin from lignocellulosic biomass.[17,22-24] For example, 1-ethyl-3-methylimidazolium acetate (EmimAc),[25] 1-butyl-3-methylimidazolium chloride (BmimCl),[26] or broadly 1-allyl-3-methylimidazolium chloride[27] have been shown to dissolve MCC and wood, while 1,3-dimethylimidazolium methylsulfate (MMim $MeSO_4$),[17] 1-butyl-3-methylimidazolium methylsulfate (Bmim $MeSO_4$),[17] 1-butyl-3-methylimidazolium acesulfamate (Bmim Ace),[24] 1-ethyl-3-methylimidazolium acesulfamate (Emim Ace)[24] and 1-ethyl-3-methylimidazolium xylenesulfonate (Emim ABS)[18] were reported to dissolve and effectively extract lignin from biomass. Of the ILs studied, sulfate based ionic liquids were most effective in terms of lignin dissolution and isolation from biomass.

Although the above mentioned sulfate based ionic liquids are effective in terms of lignin dissolution and isolation from biomass, there are also some drawbacks, such as the required high temperature for high lignin extraction by the ABS IL,[18] considerable carbohydrate losses,[17,18] and highly toxic, poorly biodegradable and a relatively high cost of the imidazolium-sulfate ionic liquids.[28] Ionic liquids contain the sulfate group readily absorb moisture to become acidic, so the above sulfate based ionic liquids show acidity in the case of a small amount of water at biomass pretreatment temperature.[29] Importantly, cellulose or hemicellulose is depolymerized in the presence of acid media. We hypothesize that ILs containing an alkaline group linked to the sulfate group may offer the potential ability for the ILs to protect cellulose or hemicellulose from destruction. There are reports of the alkaline ionic liquids used in the biomass pretreatment process, such as cholinium amino acid-based ILs.[30-32] But the thermal stability of these ILs are relatively poor, as the decomposition temperatures of the ILs which show good lignin solubility is only about 150 ℃.[30] To overcome these limitations, a set of ionic liquids which incorporates the amine-sulfonate functionalized anion were designed in our laboratories for lignin

dissolution and fractionation from biomass. Here we call them amine-sulfonate functionalized ionic liquids (ASF-ILs), which are mainly referred to the anion compositions, with cations consisting of tetraethylammonium or cholinium. The ASF-ILs (Fig. S1) used in this work are prepared using an atom-efficient and high-yield synthetic route.[33] To the best of the authors' knowledge, no reports on studies with regard to the interaction of ASF-ILs with either cellulose, lignin, or full lignocellulose biomass are available at present. In addition to the introduction of the alkalinity to the otherwise acid-forming sulfate based anions, the use of both more stable cations and anions compared with the reported cholinium amino acid-based ILs is expected to improve the thermal stability of the synthesized ASF-ILs.

In this work, we report the design and synthesis of a series of ASF-ILs. These ILs are further characterized in great details. To assess their performance in biomass fractionation, we first determined the dissolution capacity of model biopolymers including cellulose, xylan, kraft lignin and lignosulfonate in the ASF-ILs. Encouraged by the excellent selectivity and efficiency in lignin dissolution based on the model biopolymer study, we further studied lignin removal efficiency from eucalyptus bark using selected ASF-ILs. The fractionated lignin and the eucalyptus bark residues were well characterized by infrared spectroscopy and thermo gravimetric analysis. To further verify the effect of lignin fractionation on the saccharification of the polysaccharides, sugar yields from enzymatic hydrolysis of the polysaccharides components of the raw and ASF-ILs pretreated eucalyptus bark also were compared.

Results and discussions

The ASF-ILs were synthesized by methods analogous to the reported literature procedures (Fig. S1).[33] The two-step synthesis method offers the advantages of high-yield (> 98%) at room temperature, and water as the only byproduct. The synthesis requires minimal amounts of inexpensive and benign solvents. All the ASF-ILs were characterized using NMR spectroscopy and FT-IR spectroscopy (see experimental section).

High thermal stability is an important fundamental requirement for the ILs to be

suited for biomass pretreatment process. As shown in Table 1, the decomposition temperatures (T_d), calculated from the intersection of the baseline and the tangent line in the TGA curves of the ASF-ILs (Fig. S2 and S3), are in the range of 193–268□, indicating that all these ASF-IL shave far superior thermal stability over that of the reported amino-acid ILs.[30] The T_d values showed a clear dependence on the cation structure. The ASF-ILs with tetraethylammonium cation have higher thermal stability (Table 1, entries 1-4，239-268□) than the ILs with cholinium cation (Table 1，entries 5-12，193-232□). But the anion structure also affects the T_d values. For the ASF-ILs with tetraethylammonium cation, elongation of the side chain resulted in higher T_d values (Table 1, entry 1-3), and the branched anion has a lower T_d value than the linear anion (Table 1, entry 1 and 2). But there is an exception as the ASF-IL [Ch][n-$HeNHC_3SO_3$] gave the lowest T_d value.

Viscosity is a measure of the internal friction or resistance to flow caused by intermolecular interactions and is therefore a very important property in all physical processes that involve fluid movement of dissolved solutes in fluids. Viscosity is therefore one of the most important factors affecting the applications of ILs, especially as solvents. Viscosities were measured at 100 ℃ with a GB/T 265 Ubbelohde viscometer. The temperature was controlled by a glycerol bath. The viscosities of the ASF-ILs in Table 1 are essentially determined by the flexibility of the chain and their tendency to form hydrogen bonds. Mainly because the OH functional group in the cholinium cation tends to form hydrogen bonds with an anion, the ASF-ILs with a cholinium cation generally have higher viscosities (Table 1, entries 5-12, > 100mm^2/s) than ASF-ILs with a tetraethylammonium cation (Table 1, entries 1-4, 35.4-51.0 mm^2/s). Also, the structures of anion have a strong effect on the viscosities of the ASF-ILs. An increase of the chain length in the anion decreases the mobility of the ILs, leading to an increase in the viscosities of the ASF-ILs such as [Ch][n-$BuNHC_3SO_3$](145.1mm^2/s)<[Ch][n-$HeNHC_3SO_3$](162.3mm^2/s). Anions with branched chain structure have a higher viscosity than straight ones, as evidenced by the viscosity order of the following ASF-ILs:[Et_4N][n-$BuNHC_3SO_3$] (51.0mm^2/s)<[Et_4N][i-$BuNHC_3SO_3$](59.1mm^2/s),

and[Ch][n-BuNHC$_3$SO$_3$](145.1mm^2/s)<[Ch][i-BuNHC$_3$SO$_3$](156.8 mm^2/s).

The pH values of ASF-ILs (5mM solution in water) were also measured. As shown in Table 1, the pH values of the ASF-ILs are very close, all in the range of 10-11, showing that the ASF-ILs are base ionic liquids. The ASF-ILs with the anion [Me$_2$NC$_4$SO$_3$]$^-$, [Et$_2$NC$_3$SO$_3$]$^-$ or [Et$_2$NC$_4$SO$_3$]$^-$ show a relatively lower value (around 10.6)compared with the others (in the range of 10.7-11.03). The alkaline nature of the ASF-ILs comes from the amine group of the anion, but the basicity of amines is determined by the combination of electronic effects, solvation effects and spatial effects.

Table 1Properties of the amine-sulfonate functionalized ionic liquids

Entry	ASF-ILs	T_d/℃[a]	Viscosity/mm^2/s[b]	pH/5mM
1	[Et$_4$N][i-BuNHC$_3$SO$_3$]	249	59.1	10.80
2	[Et$_4$N][n-BuNHC$_3$SO$_3$]	268	51.0	10.79
3	[Et$_4$N][i-PrNHC$_3$SO$_3$]	239	37.9	10.93
4	[Et$_4$N][Me$_2$NC$_4$SO$_3$]	239	35.4	10.67
5	[Ch][n-HeNHC$_3$SO$_3$]	193	162.3	10.90
6	[Ch][n-BuNHC$_3$SO$_3$]	194	145.1	10.84
7	[Ch][i-BuNHC$_3$SO$_3$]	231	156.8	10.76
8	[Ch][i-PrNHC$_3$SO$_3$]	205	144.0	10.81
9	[Ch][i-PrNHC$_4$SO$_3$]	208	127.1	11.03
10	[Ch][Et$_2$NC$_3$SO$_3$]	232	133.8	10.68
11	[Ch][Et$_2$NC$_4$SO$_3$]	226	153.1	10.63
12	[Ch][Me$_2$NC$_4$SO$_3$]	198	108.1	10.69

[a]Decomposition temperatures (T_d) were measured by TGA with a heating rate of 10℃min^{-1} under nitrogen. [b] Kinematic viscosity were measured at 100 ℃.

The structure and properties of lignin from different sources show big difference because of the difference in plant type, environmental factors, and even genotype. The method of deconstruction and fractionation of the plant biomass further alters the lignin structure and properties. We selected two lignin samples, kraft lignin and ligninsulfonate, as models to establish the basis of the lignocellusic biomass

dissolution study. Kraft lignin and ligninsulfonate were both purchased from Sigma–Aldrich. Kraft lignin and lignosulfonate are two important sources of industrial lignin. Kraft lignin is produced in the sulfate cooking process during which most of the lignin contained in the wood is degraded into small fragments and dissolved in an aqueous solution of NaOH and Na_2S.[34] Water soluble lignosulfonates are obtained as the byproduct of sulfite cooking, in which delignification of wood is performed by means of $HSO_3^-$and SO_3^{2-}ions.[35] As shown in Table S1, elemental analysis results of kraft lignin and ligninsulfonate are consistent with previously reported results.[34,35] The content of sulfur in kraft lignin (1.74%) is lower than that in ligninsulfonate (4.60%).The oxygen content of ligninsulfonate is 10% higher than that of kraft lignin and appears also related to the sulfonate content. The water soluble property of lignosulfonate is caused by a higher content of sulfonate groups in its structure. Both the kraft lignin and ligninsulfonate contain about 1% nitrogen, this was attributed to the formation of protein–lignin complexes during delignification process.[36] Different treatment process resulted in different lignin constitutions.

A series of ASF-ILs were screened for their abilities to dissolve model biopolymers. First, the solubility of kraft lignin was assessed, because the kraft pulping process is presently the most widely applied chemical wood pulping technology. The dissolution of lignin in ASF-ILs was monitored *ex situ* via polarizing optical microscopy. Fig.1(a) shows undissolved lignin particles suspended in the $[Et_4N][Me_2NC_4SO_3]$ IL. Fig.1 (b) shows the clear solution obtained after the lignin was fully dissolved in the $[Et_4N][Me_2NC_4SO_3]$ IL. After each addition of lignin to the IL (5 mg each), the mixture was stirred at 100°C and a drop of the lignin–IL mixture was examined under the microscope to evaluate the lignin solubility. This was very useful to assess the difference between a suspension and a homogeneous solution at high lignin concentrations, since high content of lignin resulted in very dark, viscous solutions.

As can be seen in Table 2, kraft lignin dissolved well in most of the ASF-ILs. At 100□, with the solubility was more than 220 mg g^{-1}. Lignin is a biomaterial known to dissolve in alkali solution. Because the alkalinity of the ASF-ILs in this work is very

close, the difference in the solubility of lignin may be mainly ascribed to the difference in the viscosity of the ILs. For example, the solubility of kraft lignin at 100 □ in the ASF-ILs with tetraethylammonium cation was in the range of 320-385 mg $g^{-1}$and in those with cholinium cation was in the range of 220-265 mg g^{-1}. The corresponding viscosity values are 35.4-59.1 mm^2/s and 108.1-162.3 mm^2/s for the ASF-ILs with tetraethylammonium cation and with cholinium cation, respectively. For the ASF-ILs with a same cation, the solubility of the lignin also follows the viscosity trend. For example, the highest dissolution of kraft lignin was observed in the least viscous ASF-ILs of each cation group, i.e. $[Ch][Me_2NC_4SO_3]$ and $[Et_4N][Me_2NC_4SO_3]$. In the next step, the solubility of lignosulfonate was measured. Although the ASF-ILs dissolved less lignosulfonate compared with kraft lignin, the amount of dissolved lignosulfonate was also substantial (150-290 mg g^{-1}).

The solubility of cellulose and xylan in the ASF-ILs were also assessed. As shown in Table 2, cellulose and xylan showed little or no solubility in all the ASF-ILs (<5 mg g^{-1}).These results provide verification that ASF-ILs are able to dissolve large amounts of lignin, but dissolve little to no cellulose. Therefore, these ASF-ILs are highly promising solvent for selective lignin fractionation from biomass.

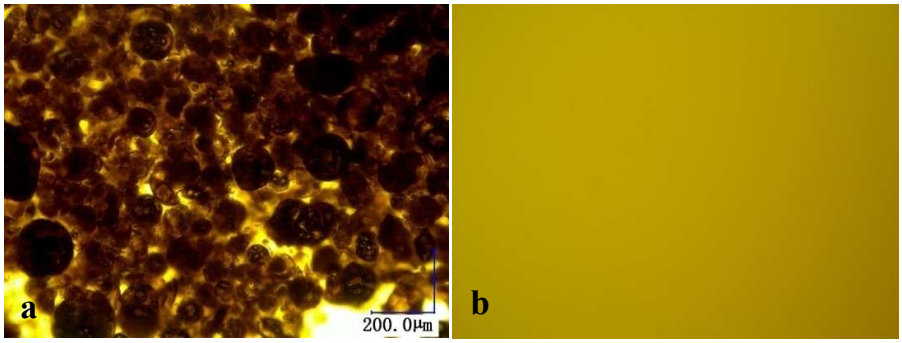

Figure 1 Observation of lignin solubility via polarization microscopy of the lignin–ASF-IL mixture: (A) before dissolution, lignin particles are visible in the ASF-ILs; (B) after dissolution, a clear solution is obtained.

Table 2 Solubility of kraft lignin (KL)，ligninsulfonate（LS）, cellulose and xylan in amine-sulfonate functionalized ionic liquids at 100 ℃ (ASF-IL: 1g)

Entry	ILs	Kraft Lignin(mg g^{-1})	Ligninsulfonate (mg g^{-1})	Cellulose (mg g^{-1})	Xylan (mg g^{-1})
1	$[Et_4N][i\text{-}BuNHC_3SO_3]$	320	250	< 5	< 5
2	$[Et_4N][n\text{-}BuNHC_3SO_3]$	320	250	<5	<5
3	$[Et_4N][i\text{-}PrNHC_3SO_3]$	345	265	<5	<5
4	$[Et_4N][Me_2NC_4SO_3]$	385	290	<5	<5
5	$[Ch][n\text{-}HeNHC_3SO_3]$	230	150	<5	<5
6	$[Ch][n\text{-}BuNHC_3SO_3]$	220	170	<5	<5
7	$[Ch][i\text{-}BuNHC_3SO_3]$	225	160	<5	<5
8	$[Ch][i\text{-}PrNHC_3SO_3]$	230	185	<5	<5
9	$[Ch][i\text{-}PrNHC_4SO_3]$	230	170	<5	<5
10	$[Ch][Et_2NC_3SO_3]$	240	180	<5	<5
11	$[Ch][Et_2NC_4SO_3]$	230	170	<5	<5
12	$[Ch][Me_2NC_4SO_3]$	265	195	<5	<5

After solubilizing in the ASF-ILs, the lignin was recovered from the ASF-ILs by precipitation with an excess amount of ethanol. After the lignin was precipitated, the ASF-ILs were recovered by evacuation under vacuum, which helped to remove ethanol and possibly residual water. To check the stability of ASF-ILs, ^{1}H NMR and ATR-FTIR spectra were recorded of both the recovered and original ILs. The IL ($[Et_4N][Me_2NC_4SO_3]$) is discussed here as an example (Fig. S4). Both the^{1}H NMR and ATR-FTIR spectra are identical, indicating that the molecular structure of the ASF-ILs was well preserved throughout the process of lignin dissolution and precipitation.

Based on the lignin solubility results, four representative ASF-ILs, $[Et_4N][i\text{-}PrNHC_3SO_3]$, $[Et_4N][Me_2NC_4SO_3]$, $[Ch][Et_2NC_4SO_3]$ and $[Ch][n\text{-}BuNHC_3SO_3]$, were selected for eucalyptus bark pretreatment. The total pretreatment time was 10 h at 120 ℃.

Table 3 shows compositional analysis of the eucalyptus bark before and after pretreatment with the four ASF-ILs. Solid recovery refers to the mass percentage of

biomass (dry weight) recovered from the original biomass load. After washing, between 71 and 79 % of the eucalyptus bark was recovered, which are higher than those obtained in other alkaline pretreatment methods.[37,38] Three of the major plant cell wall components of eucalyptus bark, cellulose, hemicellulose, and acid insoluble lignin were analyzed before and after pretreatment. Untreated eucalyptus bark contained 32.68 wt% cellulose, 12.50 wt% hemicellulose and 19.64 wt% acid insoluble lignin. After pretreatment by the four selected ASF-ILs, the relative content of cellulose and hemicellulose was increased and the lignin content was decreased in pretreated eucalyptus bark. The lignin removal followed the order of $[Et_4N][Me_2NC_4SO_3]$>$[Et_4N][i\text{-}PrNHC_3SO_3]$>$[Ch][n\text{-}BuNHC_3SO_3]$>$[Ch][Et_2NC_4SO_3]$; the corresponding values were 61.84%, 55.66%, 43.56% and 41.80%, respectively. From the lignin removal data, the ASF-ILs with $[Et_4N]$ cation were evidently more effective than the ASF-ILs with [Ch] cation. The amounts of hemicellulose removal from pretreatment by the four ILs were close, ranging from 9.02% to 11.51%. Cellulose removal in the solid fraction was nearly 0 % and was not affected by different ILs. Thus, the cellulose content increased to about 44 wt% in the solid phase after pretreatment from 32 wt% in the raw eucalyptus bark, mainly because of the partial degradation of the lignin and the hemicellulose. In addition, in this study we choose the raw eucalyptus bark (with moisture content of 7.72 wt%, in reference to oven dried substrate) as the substrate for the ASF-ILs pretreatment in order to reduce the energy consumption by the drying process.

The eucalyptus bark pretreatment results are consistent with the dissolution results obtained on the model biopolymers. The ASF-ILs, especially those with $[Et_4N]$ cation, removed the lignin effectively from the biomass without affecting the cellulose content and kept the majority of the hemicellulose. The results are therefore of high significance in biomass pretreatment for subsequent saccharification and fermentation processes. The recent reported protic ILs[39] or amino acid ILs[40] also show good ability in lignin extraction from biomass without affecting the cellulose, but for hemicellulose, both the protic ILs and amino acid ILs show 30-50% removal. Evidently, the hemicellulose removal is much higher than ASF-ILs results.

Table 3 Chemical composition, solid recovery, and component removal of eucalyptus bark before and after pretreatment with amine-sulfonate functionalized ionic liquids[a]

	Pretreatment	Solid	Cell-wall composition (%)			Chemical composition removal (%)		
Entry		yield[a](%)	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
1	Untreated	100.0	32.68	12.50	19.64			
2	$[Et_4N][i\text{-}PrNHC_3SO_3]$	72.51	44.33	15.76	12.01	0.72	9.02	55.66
3	$[Et_4N][Me_2NC_4SO_3]$	71.66	45.58	15.53	10.46	0.00	11.40	61.84
4	$[Ch][Et_2NC_4SO_3]$	77.96	44.20	14.20	13.50	0.00	11.51	43.56
5	$[Ch][n\text{-}BuNHC_3SO_3]$	78.99	44.64	14.28	13.86	0.00	9.56	41.80

[a] Compositions and solid recoveries reported are based on the dry weight of untreated or pretreated biomass; percentage removal of each component, cellulose, hemicellulose or lignin during pretreatment are based on the original amount in the untreated biomass.

The thermo gravimetric analysis (TGA) was employed to determine the impact of different ASF-ILs pretreatments on eucalyptus bark. TGA analysis was conducted for eucalyptus bark and model biopolymers (avicel, kraft lignin and xylan) in this study first. The weight loss and derivative (DTG) curves of different samples are shown in Fig.S5. Kraft lignin decomposes in a wide temperature range (200–500 ℃) due to its heterogeneity and the lack of a defined primary structure. The final residual mass of the kraft lignin was 44% at 800°C due to the formation of highly condensed aromatic structures, [34] and it was the highest among the analyzed samples. The avicel sample was nearly fully decomposed at 363°C with high decomposition rate (the derivative of weight loss).[41] The xylan had two obvious weight loss peaks in the temperature range 200-350 ℃. It has the lowest DTG_{max} (239 °C) among the analyzed samples. The TGA curve of eucalyptus bark showed two main mass losses: the first mass loss in the temperature range 200-300°C, which is ascribed to the decomposition of hemicellulose present in the biomass and the other at higher temperature (300-400°C) to that of the cellulose.[42] A residual mass of eucalyptus bark at the end of the TGA experiment is between that of the kraft lignin and that of cellulose. This residue may be attributed to the intermediate lignin content of the eucalyptus bark between the pure kraft lignin and pure cellulose.

In order to further understand the impact of ASF-ILs pretreatment on the destruction of biomass, the thermal stability of raw and different ionic liquids pretreated eucalyptus bark was also measured by TGA (Fig. 2). As shown in Fig.2, the residual mass of pretreated eucalyptus barks are considerably reduced compared to that of raw material. The four pretreated eucalyptus bark samples display even higher decomposition temperatures than the raw eucalyptus barks. This observation can be explained by considering the good thermal stability of avicel (363□). Gentle removal of lignin increases the overall degree of crystalline components in the residues, resulting in an increased thermal stability. In addition, the decomposition rate of pretreated samples increased obviously compared to the raw eucalyptus bark. After lignin removal, the cellulose rich samples showed largely increased decomposition, as evidenced by the TGA analysis conducted from 50-500°C (Fig.S5).

Above 400°C for all the samples, the decomposition follows a slow and continuous weight loss. The higher decomposition rate of the $[Et_4N][Me_2NC_4SO_3]$ pretreated sample maybe caused by the relative lower lignin content (Table 3, entry 3). The results suggest that $[Et_4N][Me_2NC_4SO_3]$ show better lignin removal compared to that pretreated by other three ILs, by judging from the reduced impact of lignin on the DTG characteristics.

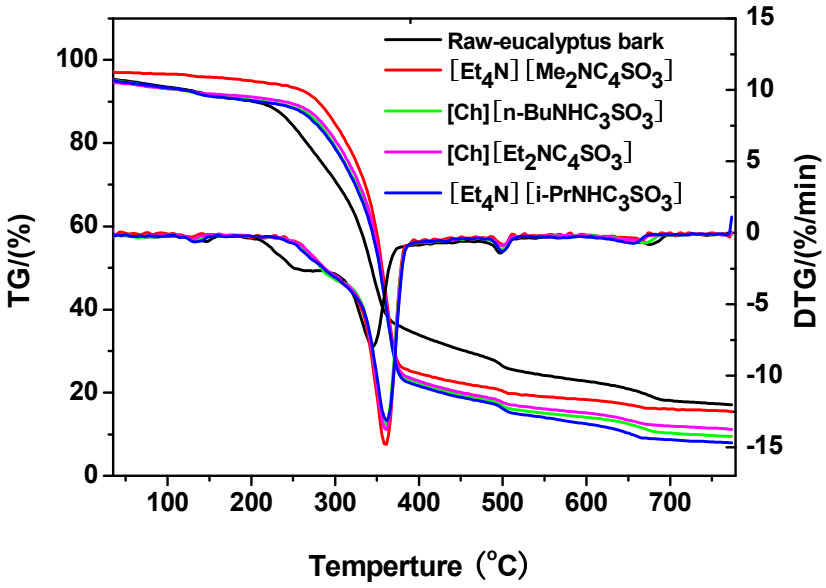

Figure 2 TGA thermo grams of raw and pretreated eucalyptus bark

FTIR spectroscopy is a useful method for monitoring the changes in the composition of lignocellulosic biomass after various pretreatments. The ATR-FTIR spectra of raw and different ASF-ILs pretreated eucalyptus bark are shown in Fig. 3. Compared with the raw eucalyptus bark, lignin removal by the pretreatment with the ASF-ILs resulted in reduced intensities for a number of bands that are associated with aromatic- and ether-containing structures: aliphatic and phenolic OH-groups (3500 to 3100 cm^{-1}),[43] C-H stretching in methyl and methylene groups (2940, 2870cm^{-1}),[44] aromatic skeletal vibrations (1610, 1510, and 1460cm^{-1}) which are characteristic peaks of lignin. In addition, the obvious peaks at 828, 1160, 1240, and 1320 cm^{-1} represent C–H, O–H, or CH_2 bending frequencies[45] and are the indicators of lignin. All above peaks are also present in the recovered lignin after the fractionation (Fig. 4).

The ATR-FTIR spectra of cellulose (Avicel), xylan (from beech wood) and kraft lignin are shown in Fig. S6. For the three model biopolymers, the bands in the range of 650-1500 cm^{-1} are very similar. There are no characteristic bands in the range 1500-1800 cm^{-1} for crystalline cellulose due to the absence of unsaturation bond in its structure. But for both kraft lignin and xylan from beech wood, there are three similar bands (1510, 1610 and 1720 cm^{-1}), and the band at 1510 cm^{-1} for kraft lignin is more intense than that of the xylan. Because the hemicellulose removal for all the samples are very close (Table 3, 9%-11%), we attribute the difference in the FTIR to the difference in lignin removal. The FTIR intensity of all pretreated eucalyptus bark samples follows the order of $[Et_4N][Me_2NC_4SO_3]$ <$[Et_4N][i\text{-}PrNHC_3SO_3]$ <$[Ch][Et_2NC_4SO_3]$< $[Ch][n\text{-}BuNHC_3SO_3]$< raw eucalyptus bark. This trend agrees well with the lignin removal order (Table 3, entry 2-5). In addition, the pretreated eucalyptus bark by ASF-ILs still shows bands in the region from 1740 to 1500cm^{-1}, characteristic for the presence of C=O and C=C bonds which indicating the presence of residual lignin.

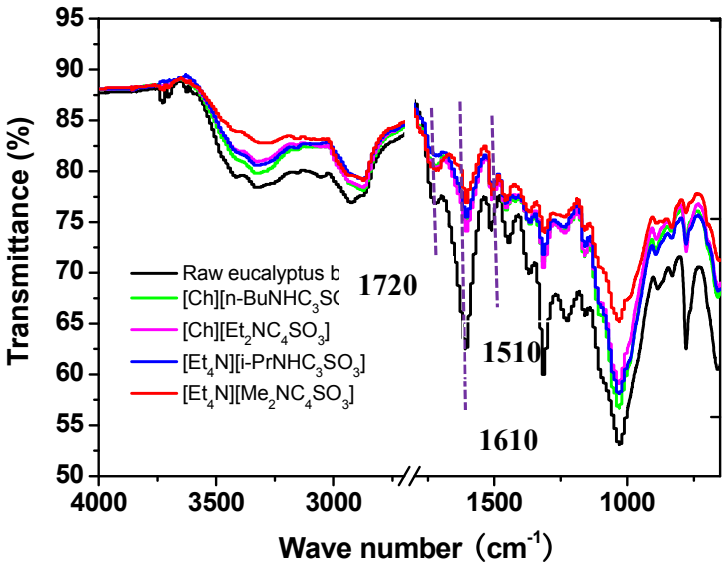

Figure 3 FTIR spectra of raw and pretreated eucalyptus bark

All the isolated lignin samples from ASF-ILs at 393K show similar brown color. The ATR-FTIR spectra of the extracts obtained at 393 K from eucalyptus bark are

compared with that of kraft lignin (Fig. 4). In comparison with other published data,[18] All extracts are confirmed as lignin, but the FTIR spectra of the lignin obtained in this work also suggest that it has differences from the kraft lignin. This difference may be attributed to the different lignin sources and the different pretreatment methods.[4] All of the four spectra show a broad absorption band at 3500 to 3200 cm^{-1} due to aliphatic and phenolic OH–groups. Other peaks are assigned to C=C stretching (1610, 1510, and 1460 cm^{-1}), C=O stretching (1720) cm^{-1} and alkane C–H stretching (2940, 2870) cm^{-1}. A more detailed assignment of IR spectral bands for lignin can be found in the literature.[46] The presence of both C=C (1610, 1510, and 1460 cm^{-1}) and C=O (1720 cm^{-1}) stretching bands in the extracts can only arise from aromatic lignin fractions, but the potential presence of hemicellulosic material, due to reactive hemicellulose breakdown products, may be expected. Further studies are required to verify this possibility.

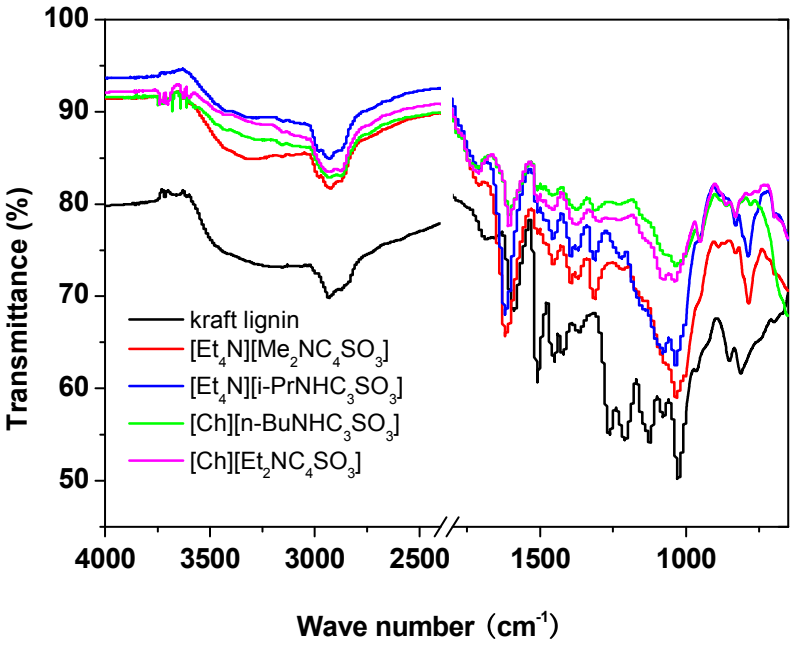

Figure 4 FTIR spectra of kraft lignin and lignin extracts obtained from eucalyptus bark after pretreatment with different ASF-ILs

The chemical structure of the extract part was also studied by ^{1}H NMR spectrometry to further verify the existence of lignin in the extracts. Fig. S7 shows the ^{1}H NMR spectra of the lignin extract part. The signal at around 2.5 ppm is indicative of protons in DMSO, and the signal at around 3.35 ppm is indicative of a small amount of water in DMSO. As shown in Fig. S7, the integral of signals between 6.0 to 9.0 ppm may be attributed to aromatic protons in the aromatic units of extracted lignin while the signals between 0.8 and 1.5 ppm may be attributed to the aliphatic units. The methoxy proton which is linked to aromatic unit shows an intense signal at around 3.67 ppm. The ^{1}H NMR data are also consistent with the reported.[46]

The results of enzymatic digestion of the raw and pretreated eucalyptus bark are shown in Fig.5. It was found that the initial saccharification rates as well as the cellulose conversion increased markedly after pretreatment by $[Et_4N][Me_2NC_4SO_3]$ and $[Et_4N][i\text{-}PrNHC_3SO_3]$, due to the extensive removal of lignin (Table 3, entries 2 and 3, 61.84% and 55.86%). And the cellulose conversion was increased to 80.33% and 48.39%, respectively (Fig. 5). In reference, the cellulose conversion of raw eucalyptus bark was only 8.83% after enzymatic hydrolysis for 96 h. The cellulose conversion of $[Ch][Et_2NC_4SO_3]$ and $[Ch][n\text{-}BuNHC_3SO_3]$ pretreated eucalyptus bark was 37.73% and 23.37% respectively (Fig. 5). The difference may be attributed to the presence of high contents of lignin in these two [Ch]-ASF-ILs pretreated eucalyptus bark (Table 3, entries 4 and 5). It is known that lignin is not only a physical barrier, but also inhibits the hydrolysis by adsorbing the enzymes.[47,48] In addition, all of the ASF-ILs pretreated eucalyptus bark did not gave a high cellulose conversion in short time (24h). The cellulose crystallinity of eucalyptus bark before and after pretreatment shown in Table S2 may be an important reason. All of the samples kept native cellulose I structure; the higher cellulose crystallinity than that of untreated eucalyptus bark is due to the increased mass fraction of cellulose in the pretreated samples. The ASF-ILs can extract the lignin from eucalyptus bark effectively but do not destruct the cellulose crystallinity.

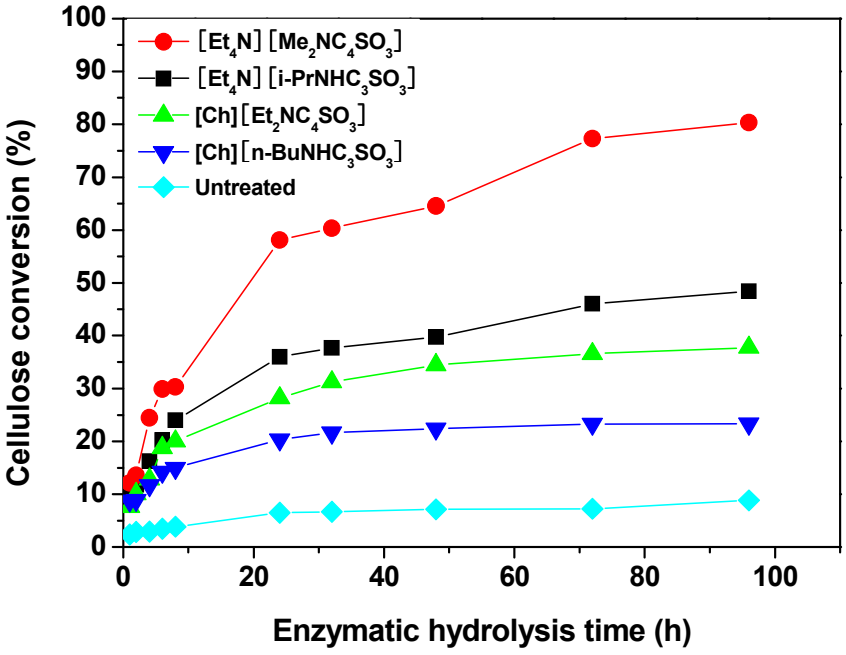

Figure 5 The conversion of cellulose for eucalyptus bark before and after pretreatment.

Conclusion

Amine-sulfonate functionalized ionic liquids are prepared using an atom-efficient and high-yield synthetic method. These ASF-ILs have good thermal stabilities. They not only dissolve industrial lignin materials like kaft lignin and lignosulfonate, but also offer unique selectivity and efficiency in fractionating lignin from eucalyptus bark. Remarkably, the ASF-ILs can remove lignin selectively from the biomass without affecting the cellulose, and with only a small amount of hemicellulose removal. This efficient and selective lignin fractionation process offers significant advantages in obtaining lignin in high purity and in the retention of cellulose and majority of hemicellulose in the biomass from the pretreatment. It is demonstrated that the pretreatment by the ASF-ILs is highly effective to achieve high sugar yield from a downstream enzymatic saccharification, which is essential for a co-fermentation process. In addition, the TGA and FTIR are two useful methods for monitoring the changes in the composition of lignocellulosic biomass after various

pretreatments and for monitoring the isolated lignin. Overall, the results of this work reveal that ASF-ILs are uniquely selective solvent with high dissolution capacity to fractionate lignin from lignocellulose. The change of both cation or anion structures can cause large differences in the properties of ILs and impact the results of biomass pretreatment. Therefore, rational design the IL's structure is essential for application of IL in the biomass pretreatment process.

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Fractionation of Lignin from Eucalyptus Bark Using Amine-Sulfonate Functionalized Ionic Liquids

Peifang Yan, Zhanwei Xu, Chao Zhang, Xiumei Liu, Wenjuan Xu, Z. Conrad Zhang*

Dalian Institute of Chemical Physics, CAS, Dalian, 116023, China

Amine-sulfonate functionalized ionic liquids not only dissolve industrial lignin materials like kaft lignin and lignosulfonate, but also offer unique selectivity and efficiency in fractionating lignin from eucalyptus bark.

Eucalyptus bark

120℃, 10h

pretreated by ASF-ILs