Green Chemistry



View Article Online

COMMUNICATION

Check for updates

Cite this: Green Chem., 2018, 20, 809

Received 7th October 2017, Accepted 9th January 2018 DOI: 10.1039/c7gc03029k

rsc.li/greenchem

Biomass pretreatment using deep eutectic solvents from lignin derived phenols[†]

Kwang Ho Kim, ^{(b) a,b} Tanmoy Dutta, ^{(b) a,b} Jian Sun, ^{(b) a,b} Blake Simmons ^{(b) a,c} and Seema Singh ^{(b) *a,b}

In future biorefineries, the development of inexpensive and renewable reagents is highly desirable. In this work, we report a renewable and new class of deep eutectic solvent (DES) synthesized from lignin-derived phenolic compounds. Novel DESs tested in this work showed their potential as solvents for biomass pretreatment in terms of lignin removal and sugar release after enzymatic saccharification. The recycling and reusability of DESs were also evaluated. The results from this study prove that DESs synthesized from renewable resources are promising solvents for future biorefineries.

In current biorefineries, biomass pretreatment to remove lignin and increase enzyme accessibility to sugars still remains an essential step.¹ Recently, room temperature ionic liquids (ILs) have gained considerable attention as a new solvent for biomass pretreatment due to their greener solvent properties.^{2–5} Nearly universal solvating capabilities, negligible vapor pressures and high thermal stability have made ILs more versatile solvents.² After the pretreatment of biomass using ILs, cellulose and hemicellulose are readily recovered for further hydrolysis to glucose and xylose, respectively.^{6,7} Pretreatments employing ILs can occur under milder conditions, and ILs have been proved to be recycled and reused after biomass processing.⁸

Due to their potential for large-scale application, ILs prepared from cheap and renewable resources are highly desirable. Recently, renewable ILs have been prepared and studied using biomass derivatives and tested for biomass pretreatment. Socha *et al.* reported a new process for synthesizing ILs from materials derived from hemicellulose and lignin, including vanillin, anisole and *p*-anisaldehyde.² They newly synthesized ILs by the reductive amination of three monomeric aldehydes followed by reacting with phosphoric acid, and investigated the pretreatment efficacy of renewable ILs. Enzymatic saccharification of pretreated switchgrass yielded 90% and 96% of total possible glucose from ILs made of furfural and anisole, respectively. ILs with high sugar yield showed the high net basicity, implying high capability of removing lignin during the pretreatment step. It clearly showed that biomass-derived ILs perform comparably to traditional ILs in biomass pretreatment although the method reported was limited to monomers with certain functionality (aldehyde) and requires multiple steps to synthesize ILs.

More recently, DESs have been gaining attention as suitable alternative solvents compared to conventional ILs.9 DESs typically form by complexion of quaternary ammonium salts (e.g. choline chloride, ChCl), hydrogen bond acceptors, with hydrogen bond donors (HBDs) such as alcohols or carboxylic acids.^{10,11} It is believed that the interaction of the HBD with the quaternary salt reduces the anion-cation electrostatic force forming hydrogen bonds, which results in a significant decrease in the freezing point of the mixture.^{10,12} DESs share the promising solvent properties of ILs, typically including low volatility, wide liquid range, non-toxicity and biocompatibility.⁹ Additionally, DESs have several advantages over traditional ILs such as their ease of synthesis and wide availability from relatively inexpensive components, which allow large scale application.13 Recent studies on biomass processing using DESs have been focusing on the extraction of biomass component¹⁴ and biomass solvation.9 Although a few studies on biomass pretreatment using DESs have been published,^{15,16} most of the reported DESs were prepared using acids (oxalic acid, levulinic acid, malonic acid, etc.), alcohols (glycerol, ethylene glycol, etc.), and amines (urea). There are very few DESs reported utilizing lignin-derived phenolics and their application to biomass pretreatment is not well reported yet.

In this work, we design a new class of renewable DESs with lignin-derived phenols. As lignin accounts for 20–30% of biomass depending on the type of feedstock, large production of lignin is expected in lignocellulosic biorefineries. Thus,

^aDeconstruction Division, Joint BioEnergy Institute, Emeryville, CA, USA ^bBiological and Engineering Sciences Center, Sandia National Laboratories, Livermore, CA, USA. E-mail: seesing@sandia.gov; Tel: +1 510 486 7315 ^cBiological and Engineering Sciences Division, Lawrence Berkeley National Laboratory. Berkeley. CA, USA

[†]Electronic supplementary information (ESI) available. See DOI: 10.1039/ c7gc03029k

Communication

View Article Online

lignin valorization is a very important topic for future bioeconomy. With this in mind, we focused on the use of ligninderived compounds as potential raw materials for DES preparation. We believe that biomass processes utilizing DESs directly from biomass could lower costs by achieving the closed-loop biorefinery proposed in Fig. 1.² In addition to the introduction of renewable DESs, we also report their application to biomass pretreatment.

As listed in Table S1,† ten phenolic compounds were tested as a HBD to synthesize novel DESs at varying molar ratios with ChCl. An initial screening test showed that four monophenolic HBDs formed DESs with ChCl, which are 4-hydroxybenzyl alcohol (ChCl-HBA), catechol (ChCl-CAT), vanillin (ChCl-VAN) and p-coumaric acid (ChCl-PCA) under the synthesis conditions tested in this work. As summarized in Table S1,† both HBA and CAT formed DESs with ChCl at three different molar ratios (1:0.5, 1:1 and 1:2). However, ChCl-VAN was observed to form at the 1:2 molar ratio and ChCl-PCA DES was not formed at the 1:2 molar ratio. It is noted that two DESs, ChCl-VAN and ChCl-PCA, turned to the solid phase upon storing at room temperature after 2-3 days. A strong hydrogen bond between ChCl, a hydrogen bond acceptor, and a HBD was formed during the synthesis at 100 °C, the strength of which decreased at room temperature resulting in the phase transition to solid slowly. Thus, ChCl-VAN and ChCl-PCA resulted in a new eutectic with a melting point between room temperature and 100 °C, respectively. Table S1[†] also shows that 4-hydroxylbenzoic acid, ferulic acid and vanillic acid partially formed DES under given synthesis conditions. Based on the screening test, it is believed that the phenolic hydroxyl group (Ar-OH) plays an important role to form a strong hydrogen bond with ChCl for DES formation. Interestingly, the HBDs with methoxyl groups in the ortho position (guaiacyl unit and syringyl unit) did not form DES compared to the HBDs without methoxyl groups. For example, syringyl acid and sinapic acid did not show any physical changes such as melting point depression during the synthesis. It is therefore likely that steric hindrance by the methoxyl group adjacent to

the hydroxyl group is a major cause that interrupts formation of a DES. However, it should be noted that the fundamental principles behind DES formation are difficult to be established. Hydrogen bonds present different contact distances and binding energies that are not dependent only on the chemical nature of the donor and acceptor, which is different from normal chemical bonds.^{9,17}

Table 1 shows the list of four DESs chosen after the screening test and they were used for biomass pretreatment. Pretreatment of switchgrass with four DESs was conducted at 160 °C for 3 hours, a condition previously determined to be optimal for switchgrass.³ After pretreatment, the residual DESs were washed out to remove potential inhibitory effects on enzy-

Entry	Compound	Structure	<i>T</i> _m of HBD (°C)	Molar ratio of ChCl : HBD
1	4-Hydroxybenzyl alcohol (HBA)	CH ₂ OH	114-122	1:1
2	Catechol (CAT)	ОН	100-103	1:1
3	Vanillin (VAN)	ОНСНО	81-83	1:2 ^{<i>a</i>}
4	<i>p</i> -Coumaric acid (PCA)	OH COOH OH	214	1:1 ^{<i>a</i>}

Table 1 List of DESs used for biomass pretreatment

^a Changed to solid after 2-3 days.



Fig. 1 Future biorefinery concept using renewable DESs derived from lignocellulosic biomass (modified from ref. 2).

matic hydrolysis and subsequent fermentation steps by phenolic compounds. It has been reported that phenolic compounds, especially the low molecular weight phenols, exert a considerable inhibitory effect on the fermentation of lignocellulosic hydrolysates.¹⁸⁻²⁰ The recovered solid after the pretreatment was subjected to compositional analysis to evaluate the pretreatment efficacy of DESs. Table 2 shows the compositional analysis of cellulose, hemicellulose and lignin obtained from raw and pretreated switchgrass. As shown in this table, the amount of glucan increased regardless of the type of DES tested for the pretreatment. Among the four DESs, ChCl-HBA provided the highest solid recovery (83.3%) but showed the poorest pretreatment performance with respect to lignin removal, whereas other DESs showed a relatively high lignin removal. The order of lignin removal is: ChCl-PCA (60.8%) > ChCl-VAN (52.5%) > ChCl-CAT (49.0%) >> ChCl-HBA (0.4%). Lignin removal of lignocellulosic biomass after pretreatment highly depends on reagents, reaction conditions, etc. For example, ionic liquid pretreatment of switchgrass using cholinium lysinate or 1-ethyl-3-methylimidazolium acetate can remove lignin up to 69% and 87%, respectively.²¹ Dilute acid pretreatment, however, has been reported to remove only a small amount of lignin (~8%) due to lignin accumulation.²² In this respect, a considerable amount of lignin was removed after pretreatment using DESs, showing its potential as the pretreatment reagent. It is believed that DESs with high lignin removal are capable of solubilizing lignin during the pretreatment, resulting in the high glucan yield^{2,14} although they show lower solid recovery (57-67%). Additionally, xylan was also removed during the pretreatment step. As shown in Table 2, a substantial amount of xylan was undesirably removed when switchgrass was pretreated with ChCl-PCA (70.7%), followed by ChCl-VAN (49.6%) and ChCl-CAT (43.2%). From the technoeconomical perspective, after pretreatment, hemicellulose-derived sugars need to be fully recovered along with glucan for the total utilization of biomass-derived sugars. The DESs tested in this work, however, showed poor xylan recovery during the pretreatment step, which requires a more in-depth study to develop new DESs that can recover both glucan and xylan.

Clearly, pretreatment using renewable DESs prepared in this work (except for ChCl–HBA) removed substantial amounts of lignin. To analyze the digestibility of sugars from the pretreated switchgrass, enzymatic hydrolysis was conducted using commercial enzyme cocktails, Cellic® CTec2 and HTec2 (Novozyme). Glucose and xylose yields after 72 h are shown in



Fig. 2 Saccharification yield from the DES pretreated switchgrass (expressed as the percentage of the theoretical maximum based on the initial glucan and xylan contents in the starting biomass).

Fig. 2. As shown in this figure, the highest glucose yield, 85.7% of the maximum possible, was observed from ChCl–PCA pretreated switchgrass, which is comparable with that from other IL pretreatments.² The glucose yields from switch-grass pretreated with ChCl–VAN and ChCl–CAT were 79.8 and 77.0%, respectively. Undoubtedly, the ChCl–HBA pretreated switchgrass yielded the lowest glucose yield (32.0%) as most of the lignin remained after the pretreatment, which significantly decreased the enzyme accessibility.²³⁻²⁶

As expected, the xylose yields were relatively low due to the high xylan loss during the pretreatment step. Among four DESs, the ChCl–CAT pretreated switchgrass yielded the highest xylose (42.4%), followed by ChCl–VAN (35.3%), ChCl–PCA (28.8%) and ChCl–HBA (19.2%). As observed from the compositional analysis of the pretreated switchgrass, a significant amount of xylan, approximately 71%, was removed during the ChCl–PCA pretreatment, resulting in a lower xylose yield after saccharification compared with the switchgrass pretreated with ChCl–CAT and ChCl–VAN. Although the ChCl–HBA pretreatment showed the lowest xylose removal rate (28.6%) during the pretreatment process, the xylose yield after saccharification was only 19.2%, which is likely due to the lower enzyme accessibility as discussed above.

2D NMR analysis of saccharification residues obtained from four DES pretreated switchgrass was conducted to elucidate the effect of DES pretreatment on cell wall structure. 2D $^{1}H^{-13}C$ heteronuclear single-quantum coherence (HSQC) spectra are shown in Fig. 3. As shown, no distinct differences in the relative content of representative lignin units and interunit linkages were found. In other words, major linkages in lignin remained intact during pretreatment/saccharification.

Table 2	Compositional	analysis	of	pretreated	switchgrass	using	DESs	(wt%)

DES	Solid recovery	Glucan	Xylan	Lignin	Lignin removal	Glucan loss	Xylan loss
Raw material	N/A	36.9 ± 1.5	25.3 ± 1.0	25.5 ± 0.8	N/A	N/A	N/A
ChCl-HBA	83.3 ± 2.8	41.4 ± 1.8	21.7 ± 1.3	30.5 ± 0.4	0.4	6.5	28.6
ChCl-CAT	66.5 ± 0.7	52.9 ± 0.5	21.6 ± 0.4	19.6 ± 0.2	49.0	4.7	43.2
ChCl-VAN ChCl-PCA	63.4 ± 2.8 57.4 ± 1.2	50.9 ± 1.7 47.9 ± 1.6	$\begin{array}{c} 20.1 \pm 0.3 \\ 12.9 \pm 0.0 \end{array}$	19.1 ± 0.8 17.5 ± 1.3	52.5 60.8	12.5 25.5	49.6 70.7



Fig. 3 2D HSQC NMR spectra of saccharification residues obtained from (a) ChCl–PCA, (b) ChCl–VAN, (c) ChCl–CAT, and (d) ChCl–HBA pretreated switchgrass. A, β -O-4' unit; B, β -5' unit; C, β - β ' unit; PB, *p*-hydroxybenzoate unit; G, guaiacyl unit; S, syringyl unit; X, xylan unit; X', acetylated xylan unit; Glu, glucose unit.

All HSQC spectra of saccharification residue, mainly the lignin-rich fraction, showed correlations corresponding to the side chain C/H pairs for β -O-4' (substructure A), β -5' (substructure B), and β - β ' (substructure C) units. The aromatic regions are dominated by the signals from the syringyl (S) units and guaiacyl (G) units. In addition to S and G units, signals from *p*-hydroxybenzoate (PB) were observed.

Although there were no distinct differences in the signals assigned to the lignin structure, the HSQC spectra showed some differences in the carbohydrate signals. Clearly, the signals associated with xylans (X_1-X_5) from the solid residue pretreated with ChCl-HBA are dense and dominant. As the pretreatment efficiency of the ChCl-HBA DES was poor and enzyme accessibility was very low due to the existing lignins,

most of the carbohydrates remained unchanged after saccharification. For example, *O*-acetylated xylans, namely, 2-*O*-acetyl- β -D-xylopyranoside (X'₂) and 3-*O*-acetyl- β -D-xylopyranoside (X'₃) were more predominant from the ChCl–HBA pretreated sample as compared with other DES pretreated switchgrass. Additionally, the anomeric C/H correlation for β -D-glucosyl (Glu) residues, mainly due to cellulose, appears at δ_C/δ_H 101.7/4.24 ppm ²⁷ and intensity of which is apparently higher than the ChCl–HBA pretreated sample.

It is clear that DESs examined in this study have a potential as a reagent for biomass pretreatment. Besides pretreatment efficacy, recovery and recycling of DESs are required for commercial use in biomass pretreatment considering sustainable bioeconomy. Thus, it was hypothesized that DESs can be recovered, reused and would maintain pretreatment efficacy in terms of sugar release. Fig. 4 shows the process flow for the potential DES-based biorefinery and DES recycling. To test this hypothesis, switchgrass was pretreated with ChCl-PCA, the best overall performing DES among the four DESs tested in this work, at 160 °C for 3 h. The recovered solids can be used for fuel and chemical production, while the remaining liquid (liquid 1) was further tested. Residual lignin in liquid 1 was removed by ultrafiltration under 20 bar nitrogen, and the resulting solvents (liquid 2) were further evaporated using a rotary evaporator to recycle EtOH and H₂O. And then, the recovered DES was reused in the next biomass pretreatment. Mass balance analysis revealed that ~95% of DES was recovered in each recycle. With the recovered DES, the next round of biomass pretreatment was conducted. Pretreated switchgrass with the recycled ChCl-PCA DES was enzymatically hydrolyzed and the results are shown in Fig. 5.

As shown, a slight decrease in the glucose yield was observed with the increasing number of DES recycles. A fresh ChCl–PCA resulted in approximately 87% glucose yield, and the yield decreased to 78% and 70% for 2nd and 3rd runs, respectively. The decrease in DES pretreatment efficacy can be attributed to the increase of impurities in the DES after each recycle.²⁸ It has been reported that carboxylic acids derived from the decomposition of carbohydrates (cellulose and hemicellulose) and phenolic compounds originated from the lignin





are present,²⁹ resulting in the decreased pretreatment efficacy of DES. Although it requires more in-depth studies on DES recovery and chemistry beyond recycling, the result of this study clearly demonstrates that renewable DESs can be recycled and reused in biomass pretreatment, and it shows the potential to reduce the cost and chemical uses for efficient biomass pretreatment.

Conclusion

In conclusion, we demonstrate that novel DESs entirely formed from a renewable-derived resource, lignin. DESs were prepared by mixing ChCl and HBD combination at an appropriate molar ratio. The resulting DESs can be utilized for multiple applications including biomass pretreatment. They were proven to be effective pretreatment solvents, which is comparable with the conventional IL pretreatment process. ChCl–CAT, ChCl–VAN and ChCl–PCA showed their high performance during the pretreatment step in terms of lignin removal resulting in high sugar release. Also, we successfully demonstrated



Fig. 4 Simplified process flow diagram for the potential DES-based biorefinery and DES recycling

Communication

that DESs could be recycled and reused for biomass pretreatment without any significant loss of pretreatment performance. In-depth understanding of chemistries such as hydrogen bond interactions and melting point depression of a eutectic mixture beyond DES formation is necessary to establish an important foundation in other industrial applications. Finally, the ease of synthesis, availability and economic viability of the components make DESs more versatile and it would be good alternative to ILs.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was conducted at the DOE Joint BioEnergy Institute (http://www.jbei.org) and supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy. The publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.

References

- K. H. Kim, T. Dutta, J. Ralph, S. D. Mansfield, B. A. Simmons and S. Singh, Impact of lignin polymer backbone esters on ionic liquid pretreatment of poplar, *Biotechnol. Biofuels*, 2017, 10, 101.
- A. M. Socha, R. Parthasarathi, J. Shi, S. Pattathil, D. Whyte, M. Bergeron, A. George, K. Tran, V. Stavila, S. Venkatachalam, M. G. Hahn, B. A. Simmons and S. Singh, Efficient biomass pretreatment using ionic liquids derived from lignin and hemicellulose, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, 111, E3587–E3595.
- 3 C. Li, B. Knierim, C. Manisseri, R. Arora, H. V. Scheller, M. Auer, K. P. Vogel, B. A. Simmons and S. Singh, Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification, *Bioresour. Technol.*, 2010, 101, 4900–4906.
- 4 A. P. Dadi, S. Varanasi and C. A. Schall, Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step, *Biotechnol. Bioeng.*, 2006, **95**, 904–910.
- 5 K. Shill, S. Padmanabhan, Q. Xin, J. M. Prausnitz, D. S. Clark and H. W. Blanch, Ionic liquid pretreatment of cellulosic biomass: enzymatic hydrolysis and ionic liquid recycle, *Biotechnol. Bioeng.*, 2011, **108**, 511–520.

- 6 S. Singh, B. A. Simmons and K. P. Vogel, Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass, *Biotechnol. Bioeng.*, 2009, **104**, 68–75.
- 7 G. Cheng, P. Varanasi, R. Arora, V. Stavila, B. A. Simmons, M. S. Kent and S. Singh, Impact of ionic liquid pretreatment conditions on cellulose crystalline structure using 1-ethyl-3-methylimidazolium acetate, *J. Phys. Chem. B*, 2012, **116**, 10049–10054.
- 8 J. Sun, J. Shi, N. M. Konda, D. Campos, D. Liu, S. Nemser, J. Shamshina, T. Dutta, P. Berton and G. Gurau, Efficient dehydration and recovery of ionic liquid after lignocellulosic processing using pervaporation, *Biotechnol. Biofuels*, 2017, **10**, 154.
- 9 M. Francisco, A. van den Bruinhorst and M. C. Kroon, New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing, *Green Chem.*, 2012, **14**, 2153–2157.
- 10 Z. Maugeri and P. D. de Maria, Novel choline-chloridebased deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols, *RSC Adv.*, 2012, 2, 421–425.
- 11 A. P. Abbott, D. Boothby, G. Capper, D. L. Davies and R. K. Rasheed, Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, *J. Am. Chem. Soc.*, 2004, **126**, 9142– 9147.
- 12 C. Ruß and B. König, Low melting mixtures in organic synthesis-an alternative to ionic liquids?, *Green Chem.*, 2012, 14, 2969–2982.
- 13 E. L. Smith, A. P. Abbott and K. S. Ryder, Deep eutectic solvents (DESs) and their applications, *Chem. Rev.*, 2014, 114, 11060–11082.
- 14 C. Alvarez-Vasco, R. S. Ma, M. Quintero, M. Guo, S. Geleynse, K. K. Ramasamy, M. Wolcott and X. Zhang, Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization, *Green Chem.*, 2016, 18, 5133–5141.
- 15 A. Procentese, E. Johnson, V. Orr, A. G. Campanile, J. A. Wood, A. Marzocchella and L. Rehmann, Deep eutectic solvent pretreatment and subsequent saccharification of corncob, *Bioresour. Technol.*, 2015, **192**, 31–36.
- 16 A. Procentese, F. Raganati, G. Olivieri, M. E. Russo, L. Rehmann and A. Marzocchella, Low-energy biomass pretreatment with deep eutectic solvents for bio-butanol production, *Bioresour. Technol.*, 2017, 243, 464–473.
- 17 G. R. Desiraju and T. Steiner, *The weak hydrogen bond: in structural chemistry and biology*, International Union of Crystal, 2001.
- 18 E. Ximenes, Y. Kim, N. Mosier, B. Dien and M. Ladisch, Inhibition of cellulases by phenols, *Enzyme Microb. Technol.*, 2010, 46, 170–176.
- E. Palmqvist and B. Hahn-Hagerdal, Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification, *Bioresour. Technol.*, 2000, 74, 17–24.

- 20 E. Palmqvist and B. Hahn-Hagerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, *Bioresour. Technol.*, 2000, 74, 25–33.
- 21 N. Sun, R. Parthasarathi, A. M. Socha, J. Shi, S. Zhang, V. Stavila, K. L. Sale, B. A. Simmons and S. Singh, Understanding pretreatment efficacy of four cholinium and imidazolium ionic liquids by chemistry and computation, *Green Chem.*, 2014, **16**, 2546–2557.
- 22 Y. Yang, R. Sharma-Shivappa, J. C. Burns and J. J. Cheng, Dilute acid pretreatment of oven-dried switchgrass germplasms for bioethanol production, *Energy Fuel*, 2009, 23, 3759–3766.
- 23 C. A. Mooney, S. D. Mansfield, M. G. Touhy and J. N. Saddler, The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods, *Bioresour. Technol.*, 1998, 64, 113–119.
- 24 L. Kumar, V. Arantes, R. Chandra and J. Saddler, The lignin present in steam pretreated softwood binds enzymes and

limits cellulose accessibility, *Bioresour. Technol.*, 2012, **103**, 201–208.

- 25 S. H. Lee, T. V. Doherty, R. J. Linhardt and J. S. Dordick, Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis, *Biotechnol. Bioeng.*, 2009, **102**, 1368–1376.
- 26 P. Alvira, E. Tomás-Pejó, M. Ballesteros and M. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review, *Bioresour. Technol.*, 2010, 101, 4851–4861.
- 27 H. Kim, J. Ralph and T. Akiyama, Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6, *Bioenergy Res.*, 2008, 1, 56–66.
- 28 Z. H. Qiu and G. M. Aita, Pretreatment of energy cane bagasse with recycled ionic liquid for enzymatic hydrolysis, *Bioresour. Technol.*, 2013, **129**, 532–537.
- 29 B. Li, J. Asikkala, I. Filpponen and D. S. Argyropoulos, Factors Affecting Wood Dissolution and Regeneration of Ionic Liquids, *Ind. Eng. Chem. Res.*, 2010, **49**, 2477–2484.