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Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans

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This work contributes to the development of integrated lignocellulosic-based biorefineries by the pioneering exploitation of hardwood xylans by solubilization and extraction in deep eutectic solvents (DES). DES formed by choline chloride and urea or acetic acid were initially evaluated as solvents for commercial xylan as a model compound. The effects of temperature, molar ratio, and concentration of the DES aqueous solutions were evaluated and optimized by using a response surface methodology. The results obtained demonstrated the potential of these solvents, with 328.23 g L^{-1} of xylan solubilization using 66.7 wt% DES in water at 80 °C. Furthermore, xylans could be recovered by precipitation from the DES aqueous media in yields above 90%. The detailed characterization of the xylans recovered after solubilization in aqueous DES demonstrated that 4-*O*-methyl groups were eliminated from the 4-*O*-methylglucuronic acids moieties and uronic acids (15%) were cleaved from the xylan backbone during this process. The similar M_w values of both pristine and recovered xylans confirmed the success of the reported procedure. DES recovery in four additional extraction cycles was also demonstrated. Finally, the successful extraction of xylans from *Eucalyptus globulus* wood by using aqueous solutions of DES was demonstrated.

Introduction

The depletion of fossil resources and the environmental concerns associated with their massive consumption has led to the search for alternatives, with the aim to supply society with sustainable energy/fuels, chemicals, and materials. The biorefinery concept, as an integrated approach for the conversion of biomass into commodities and fine chemicals,[1–3] has emerged as a promising solution, yet it requires a paradigm shift given both the intrinsic nature of biomass and the requirement for more sustainable conversion processes. The use of green solvents, both for extraction/fractionation and conversion of biomass, are amongst the main challenges for the development of new sustainable processes based on biomass. The so-called deep eutectic solvents (DES), first reported by Abbot et al. in 2004,[4] are composed of at least a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) species, which, when mixed, establish strong hydrogen-bonding interactions and form eutectic mixtures with a melting point lower than the starting compounds alone, often becoming liquid at conditions close to room temperature.[5–8] The eutectic mixture composed of choline chloride and urea has attracted significant attention as a reference DES owing to its unique properties.[9] The properties of DESs, along with their straightforward preparation, make them an ideal medium for a variety of applications, such as catalysis, organic synthesis, electrochemistry, materials chemistry, and extraction processes,[5,8,10,11] including biomass fractionation and processing.[7]

Their potential as green solvents has attracted growing interest owing to their resemblance to ionic liquids (ILs),[12] which are known for their potential in biomass pretreatment and fractionation.[12,13] Furthermore, some limitations of ILs (e.g., complexity of preparation, cost, and poor biodegradability) can be overcome by DESs,[2] and particularly by the so-called natural deep eutectic solvents (NADES), in which both the HBA and the HBD are of natural origin (e.g., sugars, choline chloride, glycols, and natural organic acids).[10,14] NADES have been considered by the Confederation of European Paper Industries (CEPI),[15] as "*…the most promising platform for the future fractionation of biomass…aiming at improving added value and reduce the CO_2 emissions as the main objective of this sector to achieve a European low-carbon bio-economy by 2050*".

In particular, the potential of DES and NADES for the extraction of bioactive components from biomass has been demonstrated by the extraction of low molecular bioactive phenolic compounds from different biomass sources.[16–20] However, the main challenge is to use these solvents for the fractionation of the major components of lignocellulosic biomass, namely lignin and polysaccharides. A few studies have reported the

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Supporting Information and the ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/cssc.201702007. The Supporting information contains DES information, experimental details of the solubility assays, NMR analysis, MALDI-TOF-MS analysis, experimental points used in the factorial planning, model equations, xylan solubility results obtained experimentally and the respective calculated values and statistical analysis connected to the response surface methodology, and further experimental details.

potential of NADES for lignin dissolution[21] and depolymerization,[22–24] as well as for polysaccharides dissolution.[25,26] However, considering that hydrogen bonding is an essential aspect in DES formation, as well as in the solubility and processing behavior of polysaccharides, it might be expected that DES will have the ability to efficiently disrupt the intermolecular hydrogen bonding of polysaccharides, and as a result, promoting their efficient solubilization/processing. Moreover, pure DES often present high viscosities, which can be considered as an obstacle to their application. The possibility of using them in aqueous solutions is a promising alternative, and it has already been demonstrated that DES aqueous solutions may perform better than pure DES.[14,21]

Considering the potential of DES and NADES as promising alternative solvents and the importance of the wood-based pulping industry, it is of high relevance to study their application in the fractionation of these woods components, namely as alternatives to the harsh conditions used in current pulping processes. In this context, and following our interest in the solubilization of wood and particularly hardwood macromolecular components using greener solvents,[21] in this work, we study the solubility of the most abundant hemicelluloses present in hardwoods, namely xylans, in DES and their aqueous solutions. Several conditions were tested, namely the HBD and HBA molar ratio, temperature, and DES concentration in aqueous solution, which were optimized using a response surface methodology approach. This study was performed using commercially available beechwood xylan. Finally, the best conditions were applied to evaluate the potential of DES for the extraction of xylans from *E. globulus* wood, as this is currently one of the most important hardwoods for pulp production.[27]

Results and Discussion

Xylan solubility assays

Xylan from beechwood was used (structure in Figure 1) for these assays. The different DES used in the xylan solubility assays were prepared according to the experimental section (the details and NMR spectra can be found in Table S1 and Figure S1). The first solubility assays were performed with choline chloride/acetic acid (ChCl/AA), at a molar ratio of 1:2, at a fixed temperature (90 °C). This combination of HBA/HBD was chosen owing to its similarity with choline acetate, an IL with good performance in xylan dissolution.[13] However, the solubility results obtained with pure ChCl/AA and its aqueous solutions (maximum $\approx$62 mg g^{-1} with 35 wt% of DES in water) were significantly lower than those obtained with the analogue IL (206 $\pm$ 5.7 mg g^{-1}).[13] This suggested that beyond the structural similarity between the selected DES and the IL, the different donor/acceptor nature of the components may strongly influence the solubility, although the pH effect cannot be discarded because the choline acetate solution has a pH of 8 whereas the DES solution displayed pH values of 1–3 (Table S2).

In the next step, the solubility of xylans in ChCl and urea (ChCl/U)-based DESs was tested for molar ratios of 1:2, 1:1, and 2:1 at 90 °C. The results obtained, shown in Figure 2 and Tables S3–S4, were also compared with those obtained with choline acetate and a 1.67 M aqueous NaOH solution (the solvent used in the conventional extraction of hemicelluloses).

The combination with a 1:2 HBA/HBD molar ratio displayed the best performance for the dissolution of xylans. The highest xylan solubility (304 $\pm$ 8.7 mg g^{-1}) was obtained with a 50 wt% of ChCl/U (1:2), surpassing the 40% the solubility in 83.3 wt% choline acetate aqueous solution. Most importantly, the solubility of xylan in 50 wt% ChCl/U (1:2) was similar to that obtained in conventional media (316 $\pm$ 1.9 mg g^{-1}; in 1.67 M aqueous NaOH solution), which further highlights the potential of DES as an alternative solvent media for the solubilization of xylans and for their extraction from biomass sources. This is particularly important when considering the milder pH conditions (pH $\approx$ 8), which can be a determining factor for the integrity of both xylan and the remaining wood components, as well as equipment maintenance. Moreover, the milder conditions used with DES aqueous solutions for wood fractionation may decrease the occurrence of side reactions during the wood pre-treatment, which in some cases might be inhibitory for further biochemical downstream processing.[28] As for the effect of water, the results did not allow for a conclusion.

After the promising results obtained with aqueous ChCl/U (1:2), the effect of temperature was investigated at 70, 80, and 90 °C. The temperatures were kept high to decrease the viscosity of the DES solutions as this is important for wood treatment.[29] However, the temperatures used in conventional processes are usually considerably higher (e.g., about 160 °C[30]) when compared to those proposed herein. Therefore, it is expected that a decrease in the extraction temperature can be achieved by using DES.

Interesting xylan solubility results were obtained by combining different aqueous solutions with variable ratios of ChCl/U

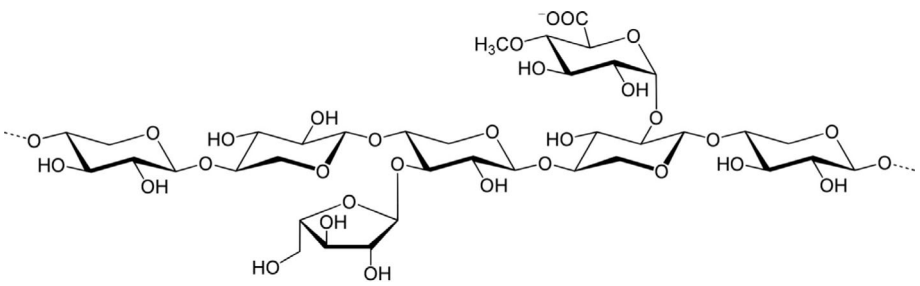

Figure 1. Basic structure of the repeating unit of xylan.

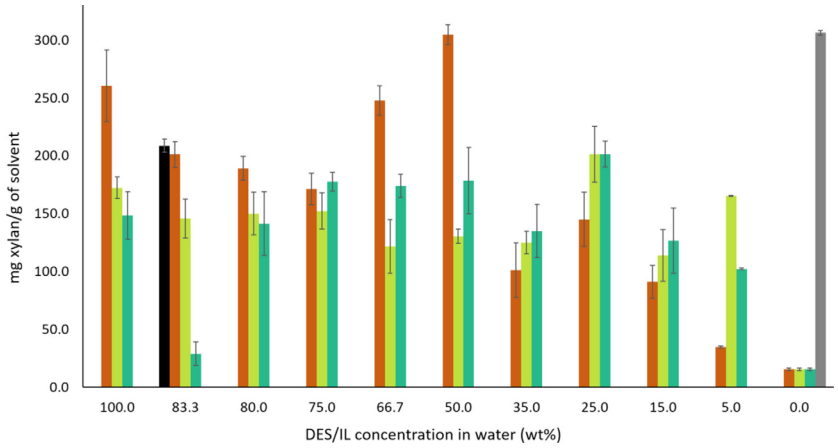

Figure 2. Solubility of xylan in ChCl/U (1:2 ■, 1:1 ■, 2:1 ■), 1.67 M aqueous NaOH ■ and choline acetate 83.3 (wt%) ■ at 90 °C.

(1:2) and different temperatures (Figure 3 and Table S5). As shown in Figure 3, 301 ± 14 mg mL^{-1} of xylan could be dissolved using 80 wt% of DES aqueous solutions at 70 °C, 328 ± 29 mg mL^{-1} with 66.7 wt% DES aqueous solutions at 80 °C, and 321 ± 18 mg mL^{-1} with 50 wt% DES aqueous solutions at 90 °C. These results further show that there is an inverse relationship between the temperature and DES concentration, which means that at higher temperatures we can use less concentrated DES solutions, whereas lower temperatures require higher percentages of DES in the solutions to maximize the solubility of xylans. This interesting relationship is important for the optimization of efficiency, sustainability, and versatility of a process for the extraction of xylans.

To understand the influence of the individual HBD and HBA aqueous solutions on the solubility of xylan, an assay using only 50 wt% aqueous solutions of urea or of ChCl was performed at 90 °C to compare with the results obtained with the corresponding DES aqueous solution (Figure 4).

Overall, the aqueous solutions of ChCl or urea do not lead to the same xylan solubility as that obtained with ChCl/U aqueous solutions. Although we could not confirm if the DES was maintained in such aqueous media, there was a synergistic effect resulting from the presence of both components; however, the solubility enhancement seems to mainly result from a hydrotropic mechanism. Hydrotropes are a class of amphiphilic compounds capable of increasing the solubility of solutes in solution, with recent studies proposing the co-aggregation of solutes with hydrotropes.[31] Hydrotropy has already been proposed in other studies to explain the use of DESs for biomass fractionation.[21]

Xylan solubilization optimization by a response surface methodology (RSM)

To optimize the solubility of xylans in ChCl/U so that it can be successfully used for the extraction of xylans from wood, a RSM approach was used. This methodology allows the exploitation of the relationship between the response (mg g^{-1} of xylan solubilized) and the independent variables/conditions that influence the xylan solubility. A 2^3 (3 factors and 2 levels) factorial planning was executed. The parameters studied were the DES concentration in water (*C*, wt%), temperature (*T*, °C) and HBA/HBD ratio (*R*, wt HBA/wt DES). The influence of these three variables on the xylan solubilization is illustrated in Figure 5. Variance analysis (ANOVA) was used to estimate the statistical significance of the variables and their interactions.

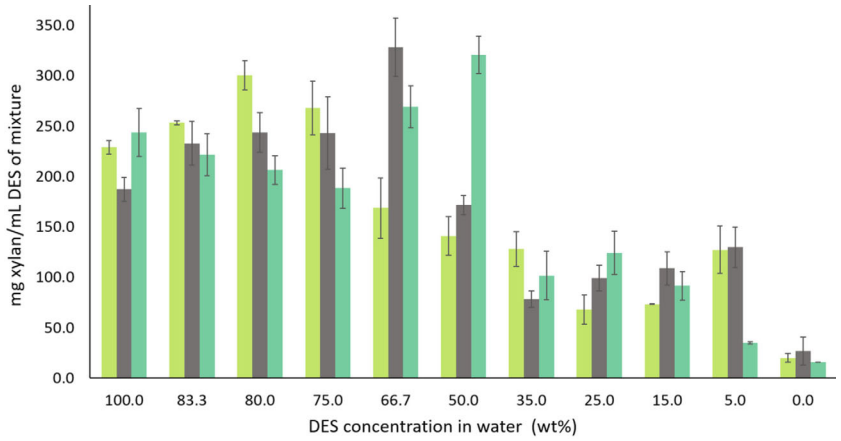

Figure 3. Solubility of xylan in aqueous ChCl/U (1:2) at different percentages and different temperatures (70 ■, 80 ■ and 90 °C■).

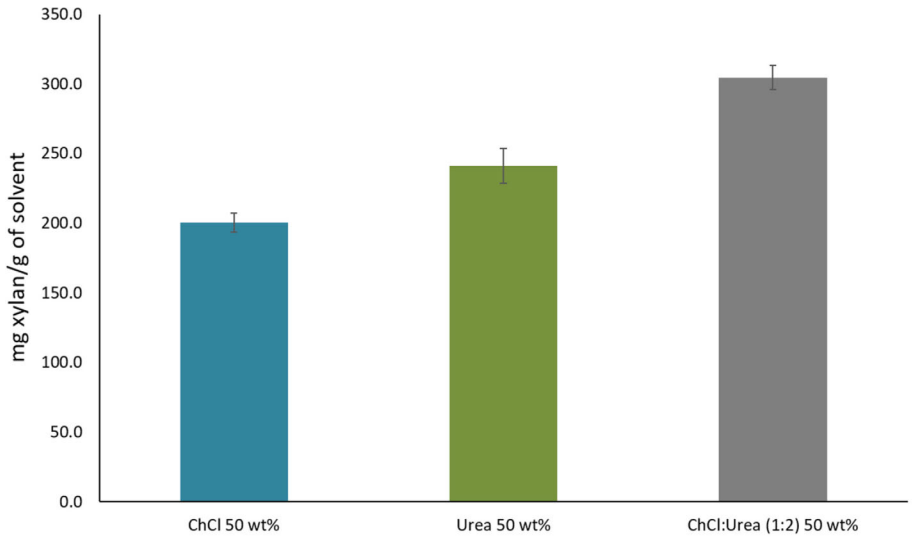

Figure 4. Xylan solubility in 50% aqueous solutions of ChCl, urea, and ChCl/urea (1:2) at 90 °C.

The experimental points used in the factorial planning, the model equation, the extraction yield of HMR obtained experimentally and the respective calculated values, and the correlation coefficients obtained, as well as all the statistical analyses, are given in Tables S6–S10.

As shown in Figure 5, and as previously demonstrated in the solubility assays, by adjusting the different variables we can obtain similar extraction efficiencies. This was demonstrated in the case of the DES concentration and temperature; by increasing the value of one condition the value of the other could be decreased while maintaining the xylan solubility. The same applies to the HBA/HBD ratio and temperature; however, in this case, higher HBA/HBD ratios required higher temperatures. However, for high DES concentrations, which result in higher xylan solubility, lower HBA/HBD ratios are preferred. Thus, the response surface design suggests solvents composed of lower HBA/HBD ratios and high temperatures/lower DES concentrations or lower temperatures/higher DES concentrations as more appropriate. This trend and dependence further demonstrates the process flexibility and its extensive tailoring possibilities. The Pareto chart in Figure S2 confirms the importance of the combined conditions.

In summary, from the combination of the results reported in Figure 5, and those obtained in the initial solubility assays shown in Figures 1 and 2, the conditions corresponding to a 1:2 HBA/HBD ratio and 90 °C/50 wt% of DES, 80 °C/66.7 wt%

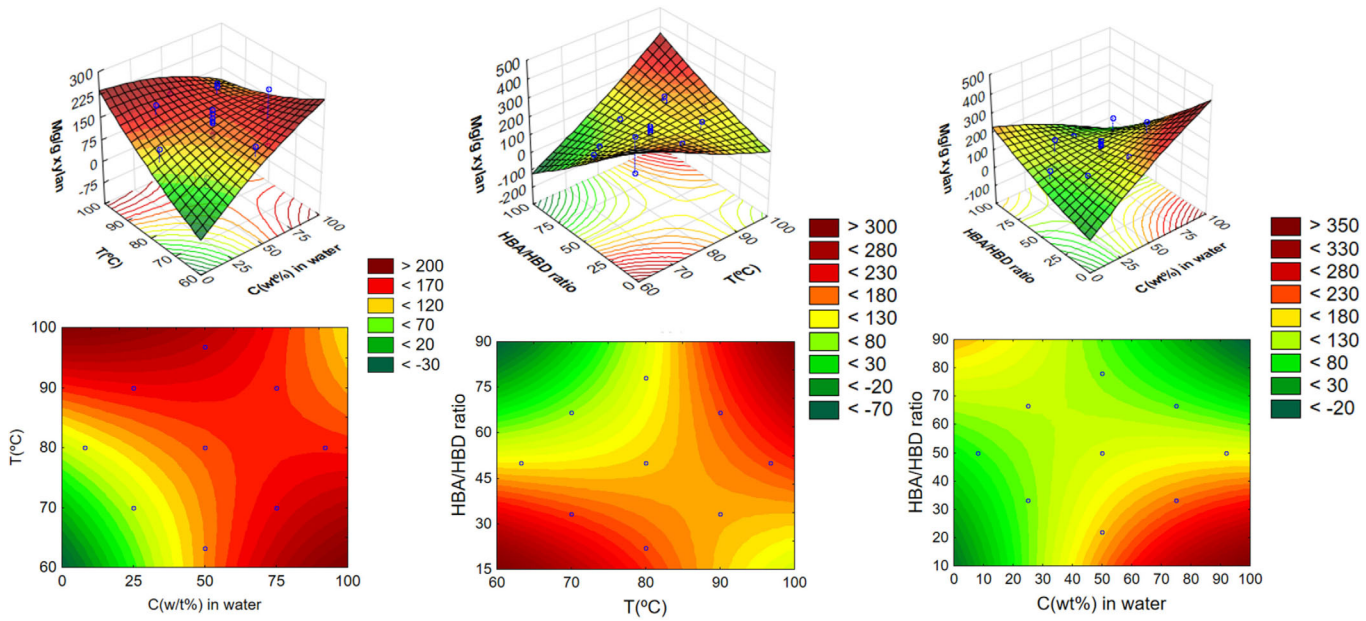

Figure 5. Response surface (top) and contour plots (bottom) of the xylan solubility using ChCl/U with the combined effects of: (i) DES concentration in water (*C*) and temperature (*T*) (ii) HBA/HBD ratio and DES concentration in water (*C*); and (iii) HBA/HBD ratio and temperature (*T*).

of DES, and 70 °C/80 wt% of DES appear to be the optimal conditions and solvents that maximize the xylan solubility. These conditions were further used for the extraction studies from *E. globulus* biomass described below.

Xylan recovery from DES aqueous solutions

The recovery of xylan from the DES aqueous solutions was studied and the recovered material was compared with the pristine material. To this end, 1 g of xylan was dissolved in 4 g of 50 wt% aqueous ChCl/U (1:2), at 90 °C. At these conditions, the assay was far from the saturation point, which meant that all of the xylan was properly solubilized. After the complete dissolution of xylan, 5 g of absolute ethanol was added and an immediate precipitation of xylan was observed (Figure S3). The samples were then filtered and washed with ethanol and acetone, and once again with ethanol to assure that the DES was completely removed from the xylan sample. The samples were then dried in a ventilated oven at 40 °C overnight and weighed to obtain recovery yields of 92 ± 4.6%.

Characterization of the xylan recovered from the DES aqueous solutions

To confirm the integrity and purity of the isolated xylan, the precipitated material was first analyzed by FTIR-attenuated total reflectance (ATR) (Figure 5), which showed a spectroscopic profile similar to the pristine xylan and in agreement with published data for this polysaccharide,[32] namely, an absorption band between 897–890 cm^{-1} attributed to the glyosidic bond β-(1→4) between the xylopiranose units of the main xylan chain, intense absorption bands at 1200–1000 cm^{-1} corresponding to the C–OH elongation vibrations, and a band of maximum absorption at 1041–1035 cm^{-1}, which corresponded to the C–O–C stretching of pyranoid-ring xylans. The bands observed between 1600–1500 cm^{-1} corresponded to the C–C bond and the bands present at 1450–1400 cm^{-1} corresponded to the C–H bond. The bands in the 3700–3408 cm^{-1} region correspond to the CO–H elongation vibrations. Furthermore, as shown in Figure 6, the band position in the IR spectra and their width indicates that the OH groups are involved in inter- and intramolecular OH bonds.[33] Furthermore, the FTIR-ATR of the recovered material did not reveal contamination with the DES used in the extraction process (Figure 6). The absence of DES contamination was further confirmed through elemental analysis of the isolated xylan, which revealed a nitrogen content below 0.02 wt%.

The recovered xylan was further analyzed and compared to pristine xylan by both ^{1}H and ^{13}C NMR and MALDI-TOF-MS. In the case of ^{1}H NMR, the pristine xylan displayed typical resonances of the 4-OCH_3 and H-1 in the 4-*O*-methyl-D-glucuronic acid moiety (4-*O*-methy-α-D-GlcpA), at approximately δ 3.23 and 5.18 ppm, respectively, and all the different protons of the nonsubstituted β-D-xylopyranose [(1→4)-β-D-Xylp] residues between δ 4.36 and δ 2.62 ppm.[34] In the case of the recovered xylan, the resonance assigned to the 4-OCH_3 was absent, which suggested that this group was eliminated with the formation of an hexenuronic moiety or the complete removal of 4-*O*-methyl-α-D-GlcpA. The same was observed in the ^{13}C NMR spectra (Figure S4 and Table S11), in which all the typical peaks of xylan were present for both samples, and all the carbons present in (1→4)-β-D-Xylp and 4-*O*-methy-α-D-GlcpA units, with the exception of the signal corresponding to 4-OCH_3, in agreement with the observations in the ^{1}H NMR spectra.[35]

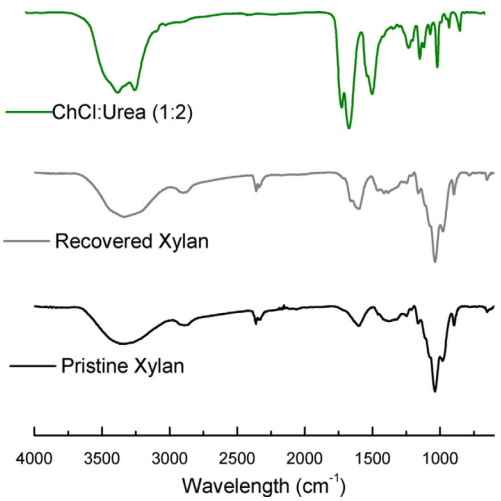

Figure 6. FTIR-ATR spectra of commercial (pristine) xylan and the xylan recovered from the DES solutions and the DES [ChCl/U (1:2)].

The MALDI-TOF-MS spectra (Figure S5) were essentially the same for both the pristine and recovered xylans, which was also in agreement with published data for this type of hemicelluloses.[36] Both spectra showed peaks with regular intervals of $m/z = 132$, corresponding to the loss of xylose units. Additionally, peaks with intervals of $m/z = 44$ and $m/z = 190$ corresponded to the loss of CO_2 from 4-*O*-methylglucuronic acid and the loss of 4-*O*-methylglucuronic acid residues, respectively. The absence of the $m/z = 190$ difference in the recovered xylan was in agreement with the NMR data and showed that 4-*O*-methylglucuronic acid groups were lost during the solubilization/precipitation process.[36]

The uronic acids content of the pristine and recovered xylans showed a decrease from 13 to 11 wt%. Although we have no data for the commercial xylan used, these values are in the same range as those reported for other commercial sources.[37,38] Despite the elimination of 4-OCH_3 from 4-*O*-methyl-α-D-GlcpA, the NMR and MS results show that only 15% of the uronic acids are being removed from the xylan backbone, whereas the ^{1}H NMR data did not reveal any acetyl groups in both the pristine and recovered xylans.

To confirm the preservation of the molecular weight of the recovered and pristine xylans, the samples were subjected to SEC analysis using a 0.1 M sodium acetate aqueous solution as the eluent. The results show that both xylans have molecular weight values in the same range (Figure 7) and SEC traces that are similar to the ones reported in the literature for birch wood xylan,[39] with a bimodal molecular weight distribution. The SEC traces also showed that high molecular weight xylan fraction was preserved (same M_w values), whereas a small frac-

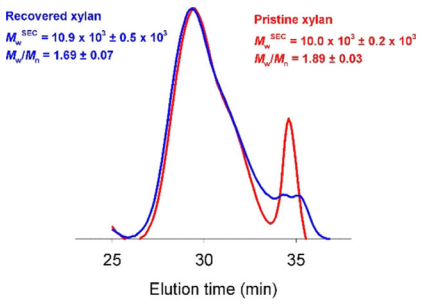

Figure 7. SEC profiles of the pristine and recovered xylans.

tion of low molecular weight population was lost during the solubilization/recovery process.

In conclusion, the various characterization techniques clearly showed that apart from the removal of 4-*O*-Methy-α-D-GlcpA units, the xylan structure was largely preserved during the DES solubilization/recovery process.

NADES recyclability

The last step to achieve a sustainable xylan extraction process based on DES was to evaluate the possibility of recovering and recycling the solvent. Thus, after the xylan precipitation, ethanol and water were removed with a rotary evaporator and the resulting DES residue was analyzed by ^{1}H and ^{13}C NMR (Figure S6), showing that the DES was successfully recovered from the assays and could be reused for the extraction of xylans. This was demonstrated by using the same DES in four extraction cycles (Figure 8). During this cyclic process, the DES aqueous solution gained a slightly brownish color owing to the dissolution of low molecular weight compounds that were not fully precipitated using ethanol (e.g., phenolic compounds, furfural, or other furanic compounds). However, this did not have a significant effect on the xylan solubilization and recovery. At least two recycling cycles could be performed without significantly decreasing the xylan solubility (less than 5% decrease).

Proof of concept: Extraction of xylan from *E. globulus* wood

Extraction assays were conducted using pretreated *E. globulus* wood sawdust preextracted with both ethanol/toluene to remove extractives.[40] The best conditions identified above for xylan solubilization (90 °C, 1:2 HBA/HBD ratio and 50 wt% of DES in water) and the fixed extraction time of 24 h and two different solid/liquid (S/L) ratios (r = 0.1 and r = 0.04) were used. Extraction with an aqueous 1.67 M NaOH solution was used for comparative purposes. After the extraction, the xylans were precipitated, washed, and dried, as reported in the experimental section. The extraction yields are shown in Table 2. The *E. globulus* biomass typically has approximately 14 wt% of hemicellulose.[41] The assays corresponding to a S/L ratio of 0.1 led to extraction yields far below those expected for this wood. As shown in Table 1, a S/L ratio of 0.04 resulted in an extraction yield of 15%, which performed even better than aqueous NaOH and water (12 and 7 wt%, respectively), as expected from the solubility assays.

Finally, the isolated xylans were characterized by FTIR (Figure 9), showing that in the case of the samples extracted with aqueous DES, there was some contamination with other polysaccharides, most probably pectin and starch (1112 cm^{-1}

Table 1. Xylan extraction yields (wt xylan/wt of dry wood) with ChCl/U, aqueous NaOH, and water at 90 °C, extraction time of 24 h, at two solid/liquid (S/L) ratios.

Solvent	S/L ratio = 0.1		S/L ratio = 0.04	
	yield [%]	± deviation	yield [%]	± deviation
ChCl/U (1:2)	3.1	0.83	14.8	0.61
NaOH 1.67 M	4.2	1.27	12.3	2.00
Water	1.7	0.09	7.9	1.39

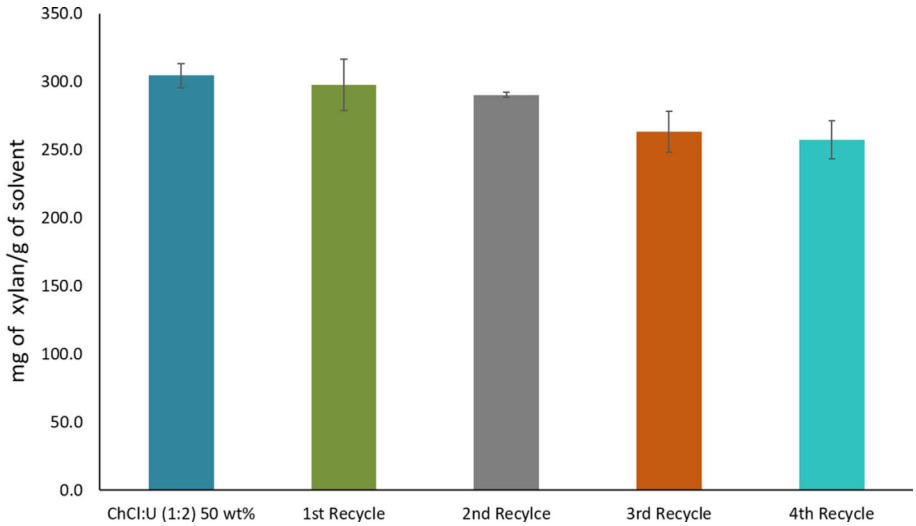

Figure 8. Xylan dissolution and recovery yields using recycled ChCl/U (1:2) aqueous solutions under optimized conditions.

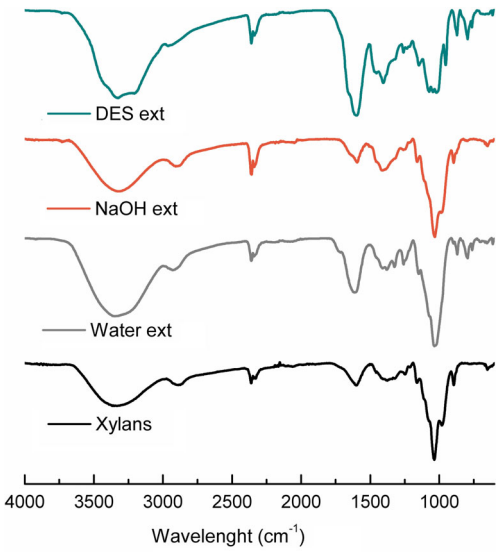

Figure 9. FTIR-ATR spectra of the xylan samples extracted from *E. globulus* wood.

and 998 cm^{-1}), both known to be present in *E. globulus* wood,[42,43] and prone to solubilization in DES.[25,44]

These final results serve to demonstrate the potential of ChCl/U aqueous solutions for biomass pre-treatment and xylans extraction. Although further process optimization and refinement is still needed, we showed that aqueous solutions of DES at a milder temperature and pH than those used currently with alkaline aqueous solutions, are promising alternative solvents and processes for xylan extraction.

Conclusions

We demonstrated the possibility of using aqueous solutions of DES for the solubilization/extraction of xylans. The results obtained with ChCl/U were far superior to those obtained in previous works using ILs and are comparable to those obtained with harsh treatments using aqueous alkaline solutions. A DES molar ratio of 1:2 (HBA/HBD) led to the best results. Furthermore, by RSM optimization, we confirmed the presence of relevant relationships between temperature and concentration of DES, which can be tailored to achieve similar solubility results (about 310 mg g^{-1} of xylan). The xylan recovery was also successfully achieved with high yields (above 90%).The structural characterization showed that during the extraction/recovery process, there was elimination of 4-*O*-methyl groups from 4-*O*-methylglucuronic acids moieties, as well as the cleavage of uronic acids (15%) from the pristine xylan structure. Finally, the SEC traces showed that the high molecular weight fraction of the xylan was preserved in terms of its M_w values, whereas a small fraction of low molecular weight population was lost during the solubilization/recovery process.

The DES used could also be successfully recycled, up to four cycles, with a 5% decrease of the xylan solubility after the first two cycles. The application of the optimized aqueous solutions of DES for the extraction of hemicelluloses from *E. globulus* wood was successfully achieved, with a yield of 14.81 ± 0.61%, which was higher than the yields obtained with water or alkali solutions.

This work provides an important contribution to the understanding of the use of DES in the wood fractioning processes, especially with regard to the extraction of xylans from hardwood, and opens up promising prospects for the development of integrated biorefineries in which DES might play a central role. Furthermore, the conditions optimized with the commercial beechwood xylan were successfully applied to the extraction of xylans from *Eucalyptus globulus* hardwood, which showed that the application of the process to other hardwoods can also be easily optimized. Further work will focus on a better understanding of the dissolution process and optimization of the DES and remaining operational conditions for the extraction of hemicelluloses from *E. globulus* wood.

Experimental Section

Chemicals

Choline Chloride (ChCl) was used as HBA and Urea (U) and acetic acid (AA) were used as HBDs (Table S1). Their water content was measured through a Metrohm 831 Karl Fisher coulometer, to guarantee the correct molar proportion in the preparation of DES. Xylan from beechwood, obtained from Sigma (≥90%), was used as a model compound for the solubility assays. Choline acetate, from Iolitec with >99% purity was used as a starting reference for the solubility assays.[13]

DES preparation

The different DESs prepared for this study are presented in Table 2. The humidity of the different DES precursors (HBA and HBD) was taken into account for their preparation and was measured using a Metrohm 831 Karl Fisher coulometer. The precursors were weighed and placed in sealed glass vials with constant stirring and heated until a transparent liquid was formed. After the formation of a liquid, the mixture was kept at this temperature for 1 h before it was allowed to return to room temperature. Their compositions were confirmed by NMR. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance 300 at 300.13 MHz and 75.47 MHz, respectively, using deuterated water as solvent and trimethylsilyl propanoic acid (TMSP) as an internal reference [results illustrated in Figure S2 for ChCl/U (1:2)].

The DES aqueous solutions (0, 5, 15, 25, 35, 50, 66.7, 75, 80, 83.3, and 100 wt%) were prepared by diluting the neat DES in deionized water. The pH of the DES aqueous solutions (Table S2) was measured at 25.0 ± 0.01 °C using a Metrohm 827 pH meter equipment with an uncertainty of ±0.01. The calibration of the pH meter was performed with two buffer solutions (pH of 4.00 and 7.00). Furthermore, the density of the aqueous solutions was also measured at

Table 2. List of DESs prepared for this study.

DES	Molar ratios (HBA/HBD)	Melting point [°C]
ChCl/Acetic Acid	1:2	22.1
	1:2	12.2
ChCl/Urea	1:1	55.7
	2:1	142.5

atmospheric pressure and in the temperature range from 0 to 30 °C using an automated SVM 3000 Anton Paar rotational Stabinger viscometer–densimeter (temperature uncertainty: ±0.02 K; absolute density uncertainty: $\pm 5 \times 10^{-4}$ g cm^{-3}).

Xylan characterization

The pristine beechwood and recovered xylans from the solubility assays and the xylan extracted from *E. globulus* wood were characterized by measuring their acetylation degree and uronic acid content. The acetylation degree was measured through integration of the corresponding ^{1}H NMR resonances (at $\delta = 2.2$ ppm).[45] For the quantification of uronic acids, approximately 1–2 mg of xylan was weighed and added to 0.400 mL of 72% aqueous H_2SO_4 and left to react for 3 h at room temperature. Then, distilled water (2.2 mL) was added and the samples were heated at 100 °C for 2.5 h to promote hydrolysis. The hydrolyzed sample was then diluted using distilled water (3 mL). To quantify the uronic acids in the sample, a calibration curve in the range of 20–200 μg mL^{-1} was prepared with glucuronic acid. For all concentrations and samples, a blank was prepared. To each sample 50 mM sodium borate in 95% H_2SO_4 (3.0 mL) was added and heated to 100 °C for 10 min. Then, the samples were placed in an ice bath and 0.15% *m/v* *m*-phenylphenol in 0.5% *m/v* NaOH (100 μL) was added. The samples and blanks were left in the dark for 30 min and their absorbance was measured at 520 nm.[46,47]

Xylan solubility assays

Xylan from beechwood was added in excess to 2.0 ± 0.1 g of pure DES, DES aqueous solutions, ChCl, urea, choline acetate aqueous solution, 1.67 M aqueous NaOH, and pure water. The solvents and pure xylan were put in sealed glass vials and allowed to equilibrate at constant temperature (70, 80, and 90 °C) with stirring (800 rpm) on a stirring plate with heat control Agimatic-N Sensoterm II from P-selecta and a specific aluminum disk to support the sealed glass vials with a stirring bar. Because the obtained solutions had a high viscosity, it was not possible to separate the different phases so the glass vials were placed in an aired oven at the temperature of the assays overnight to equilibrate and for the undissolved xylan to deposit in the bottom of the vials. Then, the concentration of dissolved xylan in the different solvents was measured by FTIR-ATR, following a similar approach to the one reported by Soares et al.[21,48] for the quantification of dissolved lignin, but in this case using the xylan band at 1045 cm^{-1} typical of C–OH stretching and C–O–C deformation in polysaccharides.[33] A FTIR system Spectrum BX, PerkinElmer, equipped with a single horizontal Golden Gate ATR cell and a diamond crystal was used for the measurements. All data was recorded at room temperature in the range of 4000–600 cm^{-1} by accumulating 32 scans with a resolution of 4 cm^{-1} and intervals of 1 cm^{-1}. At least three individual samples were analyzed for each mixture and temperature. Calibration curves were made for each DES, IL, ChCl, urea, and aqueous solutions tested (Figures S8–S12).

Response surface methodology (RSM)

A RSM was applied to simultaneously analyze various factors (operational conditions) and to identify the most significant parameters, which enhance the xylan dissolution. In a 2^k surface response methodology, there are *k* factors that contribute to a different response, and the data are treated according to a second order polynomial equation [Eq. 1]:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j \quad (1)$$

in which *y* is the response variable and β_0, β_i, β_{ii}, and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic, and interaction terms, respectively, and $X_i$and $X_j$are independent variables. This model allows the drawing of surface response curves and the optimal conditions can be determined through their analysis.[49] The 2^3 factorial planning is defined by the central point (zero level), the factorial points (1 and −1, level one) and the axial points (level α) The axial points are encoded at a distance α from the central point [Eq. 2]:

$$\alpha = \left(2^k\right)^{1/4} \quad (2)$$

ChCl/U was selected to perform a 2^3 factorial planning with the aim of optimizing the future extraction yield of xylans. The 2^3 factorial planning used is provided in the Supporting Information. The obtained results were statistically analyzed with a confidence level of 95%. Student's t-test was used to check the statistical significance of the adjusted data. The adequacy of the model was determined by evaluating the lack of fit, the regression coefficient [and the F-value obtained from the analysis of variance (ANOVA)] that was generated. The Statsoft Statistica 10.0 software was used for all statistical analyses and for representing the response surfaces and contour plots. Furthermore, Matlab 2015b, The MathWorks, was used to confirm the response surfaces and contour plots obtained.

Xylan recovery from aqueous DES solutions

The Xylan recovery from aqueous DES solutions was tested after the solubility assays by adding the same weight (as the DES solution) of ethanol to the DES solution and stirring at 800 rpm for 24 h. All assays were made in triplicate. After that, the precipitated material was washed with ethanol and acetone for analytical purposes. The recovered solid was then vacuum filtered using nylon Whatman 0.45 μm pore filters. The recovered xylan was then put in a 40 °C ventilated oven overnight and weighed. Both the pristine and recovered xylan (after DES dissolution and precipitation) were analyzed using NMR spectroscopy. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance 300 at 300.13 MHz and 75.47 MHz, respectively, using deuterated water as solvent and TMSP as an internal reference.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis were performed using a Bruker Daltonik Autoflex III smartbean MALDI-TOF-MS mass spectrometer from the Centro de Apoio Tecnológico e de Investigação in Vigo (CACTI). Ions were formed upon irradiation by a smartbeam nitrogen laser (337 nm) using an accelerating potential of 20 kV and a frequency of 200 Hz. Each mass spectrum was obtained by averaging 3500 laser shots collected across the whole sample spot surface by rastering in the range *m/z* 700–4000. The laser irradiance was set to 45–60% (relative scale 0–100) arbitrary units according to the corresponding threshold required for the applied matrix system. Low molecular ion gating was set to 650 Da to remove the ions below this value arising from the matrix and their clusters or other unknown contaminants. All spectra were acquired and treated using the FlexControl 3.0 and FlexAnalysis 3.0 software (Bruker Daltonik, Bremen, Germany), respectively. The dried-droplet sample preparation technique was used, applying 2,5-dihydroxybenzoic

acid (DHB) matrix solution (10 mg mL^{-1} in 50% acetonitrile/0.1% trifluoroacetic acid, *v/v*, 1 μL) directly on a MTP AnchorChip 800/384 TF MALDI target (Bruker Daltonik, Bremen Germany). Then, before drying the matrix solution, the sample (10 mg mL^{-1} in 1 M NaOH, 1 μL) was added and allowed to dry at room temperature. External mass calibration was performed with a calibration standard (Bruker Daltonik, Bremen Germany) for the range *m/z* 700–4000 (9 mass calibrant points): 0.5 mL of calibrant solution and DHB matrix previously mixed in an Eppendorf tube (1:2, *v/v*) were applied directly on the target and allowed to dry at room temperature.

Both the pristine and recovered xylans were analyzed by a size-exclusion chromatography (SEC) system equipped with an online degasser, a refractive index (RI) detector, and a set of columns comprising a Shodex OHpak SB-G guard column, OHpak SB-802.5HQ and OHpak SB-804HQ columns. The xylans were eluted at a flow rate of 0.5 mL min^{-1} with 0.1 M sodium acetate (aq)/0.02% NaN_3). Before the injection (50 μL), the samples were filtered through a nylon membrane with 0.20 μm pores. The system was calibrated with narrow polyethylene glycol (PEG) standards and the polymer molecular weights (M_n^{SEC}) and Đ (M_w/M_n) were determined by conventional calibration using Clarity software version 2.8.2.648. The samples were injected four times.

Elemental analyses of both pristine and recovered xylans were performed on a Leco Truspec 630-200-200 equipment with a sample size of up to 10 mg, a combustion furnace temperature of 1075 °C, and an afterburner temperature of 850 °C.

Hemicellulose extraction from *E. globulus* wood biomass

Extraction assays from *E. globulus* pre-extracted biomass (with ethanol/toluene) were performed using the best conditions according to the solubility assays performed previously. Two solid/liquid ratios were used, 0.1 and 0.04. The assays were performed in a Radleys Tech carousel and the temperature was fixed at 90 °C and the agitation at 600 rpm to ensure the constant agitation of the biomass. After a 24 h extraction, the biomass was separated from the solvent through vacuum filtration with a cellulose filter and excess ethanol (twice the volume of the filtrate) was added to precipitate the hemicelluloses. After the precipitation occurred, the samples were filtered again using a nylon Whatman filter with a 0.45 μm pore and the precipitate was dried in a ventilated oven overnight. The samples were then weighted and taken to FTIR-ATR in to confirm the hemicelluloses structure. Triplicates were made for all extractions.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biorefinery · deep eutectic solvents · extraction · green solvents · xylans

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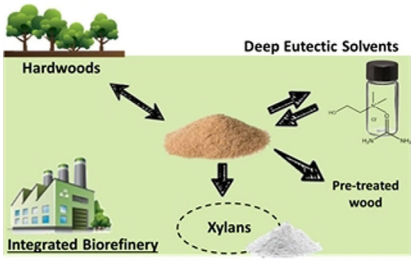
New media provides the solution: Deep eutectic solvents formed by choline chloride and urea in aqueous solutions display excellent ability to solubilize hardwood xylans and show great potential for the extraction of these polysaccharides from hardwoods in an integrated biorefinery context.



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Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans