Deep Eutectic Solvents: Fractionation of Wheat Straw

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Deep eutectic solvents (DESs) are a relatively new topic in science. Their usage is not yet clearly defined, and the areas in which DESs may be applied are constantly growing. A simple and clean fractionation of the main components of biomass represents a very important step in creating a clean, renewable carbon economy. A major challenge is the use of DESs for fractionation of biomass components at lower temperatures, without the use of expensive raw materials. In this work, wheat straw was pretreated with six different DES systems composed of choline chloride with urea (1:2), malonic acid (1:1), lactic (1:9; 1:10), malic (1:1), and oxalic acid (1:1). The pretreated biomass was characterized in terms of lignin content, ash, and holocellulose. A deep eutectic solvent, composed of choline chloride and oxalic acid, was found to produce the best delignification results. The solvents are not selective in the process of delignification.

Keywords: Deep eutectic solvents; Fractionation; Lignin; Biomass pre-treatment; Delignification

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INTRODUCTION

Biomass can be converted into diversified materials, biofuels, and biochemicals, especially through the modernization of existing biorefinery technologies, accompanied by the development of new procedures. Several methods have been developed for the pretreatment of lignocellulosic biomass (Zavrel *et al.* 2009; Surina *et al.* 2015) in order to obtain a high yield of the desired product. The methods must be adapted to the specific source of lignocellulosic biomass (Mäki-Arvela *et al.* 2010). The unit operations may cause significant degradation of various lignocellulosic components. In the course of degradation, the original material undergoes modification, the proportions of its individual components are changed, and it becomes thus more suitable for subsequent processing. The pretreated material may be utilized mainly in hydrolytic or fermentation processes. Moreover, the process of delignification and the substrate ability to interact with other chemicals used in these processes may be improved. Some pretreatment methods require extreme conditions such as high temperatures and pressures, or strong acids and bases, and special facilities are frequently required.

One of the most promising technologies for fractionation of components is the use of deep eutectic solvents. A deep eutectic solvent (DES) is a type of ionic solvent with special properties. It is composed of a mixture which forms a eutectic with a melting point much lower than either of its individual components (Abbott *et al.* 2004). Frequently, DESs are formed by mixing choline chloride with hydrogen bond donors,

such as amines, acids, and alcohols. Recently, various applications of DES have been reported (van Spronsen *et al.* 2011; Kroon *et al.* 2014; Kumar *et al.* 2015; Vigier *et al.* 2015). DESs were mentioned for the first time in the work of Abbott *et al.* (2003), which highlighted the good dissolution properties of these mixtures. DESs are highly sensitive (Kroon *et al.* 2014) and effective for extracting compounds from biological materials in high yields (van Spronsen *et al.* 2011).

Several papers describe the delignification of different biomass using DESs. Largo Garcia de Dios (2013) tested the delignification of pine wood and wheat straw. The authors used five different DESs, such as choline chloride and lactic acid; malic acid; oxalic acid; and lactic acid and tetramethylammonium chloride and 2-chloroethyltrimethylammonium chloride in varying proportions (eight experiments). The percentage of lignin obtained after delignification ranged from 0.6% to 7.8% for pine wood, and from 0.6% to 9.5% for wheat straw. The researchers found that, depending on the biomass, different types of lignin and lignin structures are extracted at different yields.

Kumar *et al.* (2015) found that the delignification of rice straw by choline chloride and lactic acid, after applying a pretreatment at the molar ratio of 1:5, resulted in a decrease in lignin content from 9.1 to 3.8%.

In the aforementioned papers, selective dissolution of the pure fractions (lignin, cellulose, and xylan) for lactic acid or betaine, and choline chloride was compared. The conclusion was that only lignin undergoes dissolution. Although evidence and published or patented applications make use of DES to dissolve the individual components of biomass, which have been known for decades, the development of this area is expanding significantly.

Similar to other investigations of actual or potential practical significance in the field of natural material valorization, three principal issues should be taken into account. One important issue is the environmental friendliness of the technology. Another is the yield of the desired products, and the last one concerns the selectivity of the process. Assessing the current published literature data, it is clear that in regards to yield and selectivity of extraction of individual components from biomass using DESs, the issues are open and detailed experiments must be performed and evaluated in order to reach a definite answer.

This work tries to contribute to the yield and selectivity investigation regarding the delignification of wheat straw using different types of deep eutectic solvents. Our aim is to estimate the effectiveness of delignification in removing lignin, as well as the selectivity of the process.

EXPERIMENTAL

Materials

Wheat straw was harvested from local agricultural fields in the Bratislava district of Slovakia. After harvesting, the straw was cleaned, grinded (600 mesh), and dried.

Before delignification, the wheat straw was extracted using the accelerated solvent extraction [according to NREL/TP-510-42619 (Sluiter *et al.* 2008)] and weighed, dried, and analyzed to determine the content of lignin, ash, and holocellulose (Table 1). The residual lignin content was determined as Klason lignin (TAPPI Method T222, 1998), and the extractive content was determined according to Sluiter *et al.* (2008).

Ash was determined using the TAPPI Method T211 (1998), and holocellulose was quantified with sodium chlorite treatment according to the procedure of Wise *et al.* (1946). Extracted, dried wheat straw was used as the lignocellulosic biomass and was treated with DES reagent.

Table 1. Composition of Wheat Straw

Wheat straw	Composition (%)
Extractives	7.3 ± 2.0
Ash	4.2 ± 0.4
Lignin	17.3 ± 1.0
Holocellulose	74.7 ± 0.2

Four replicates were measured, averaged, and evaluated.

DES Pretreatment

All chemicals were purchased from VWR® (www.vwr.com). Six different DESs were tested: choline chloride with urea, malonic, lactic, malic, lactic, and oxalic acid. The solutions were stirred in an oil bath to form a homogeneous liquid. Extracted straw (2.5 g absolute dry weight, STN EN ISO 638 (2009)) was added into individual DES at a ratio of 1:20 (wt/wt). DESs, choline chloride and urea (1:2), and malonic acid (1:1), and lactic (1:9; 1:10), malic (1:1) and oxalic acid (1:1) were used, and delignification was carried out for 24 h in a drying oven with a preset temperature of 60 °C. For DESs (choline chloride and urea; and choline chloride and malic acid) the temperature was 80 °C. The slurry was washed with an anti-solvent (water, ethanol – for malic acid), and after washing, the samples were dried at 105 °C. Properties of the prepared DESs are listed in Table 2.

Table 2. Properties of Prepared DESs

Reagent	ChCl/ Reag	Preparation time of DES (min)	Temperature of preparation (°C)	Refractive index at 25 °C	Density (kg/m^3) at 60 °C	Density (kg/m^3) at 45 °C
Urea	1:2	60	80	1.5117	1205	1213
Malonic acid	1:1	20	60	1.4861	-	-
Lactic acid	1:9	20	60	1.4432	1191	1202
Malic acid	1:1	40	80	1.4813	1292	1291
Lactic acid	1:10	20	60	1.4426	1210	1223
Oxalic acid x2.H2O	1:1	45	60	1.4662	1255	1267

Four replicates were measured, for refractive index at 25 °C standard deviation is less than 0.0005 units, for density less than 3 units, and for viscosity less than 0.35 units.

Refractive Index

The refractive index was measured using an Abbe refractometer at a temperature of 25 °C. Densities were determined with a pycnometer at 60 °C and 45 °C.

RESULTS AND DISCUSSION

The present research was focused on the application of deep eutectic solvent for the processing of wheat straw. The goal was to find an efficient solvent for lignin isolation. Table 3 shows the wheat straw residue yields after delignification by individual DES. The yield ranged from 59.1% to 94.9%. It is obvious from the results that each of the DESs was able to dissolve lignin from the used material. However the amount of dissolved lignin was different depending on the components of individual DES. The highest amount of the material was extracted by the DES composed of choline chloride and oxalic acid dihydrate. However, along with lignin, other biomass components were passed along to the DES.

Theoretically, the yield should reach 82.7% if all lignin present in wheat straw were dissolved. However, in this study, the determined yields of wheat straw residue after delignification were lower. These results clearly indicate that other wheat straw components (*e.g.*, holocellulose) became dissolved as well. Based on these results, it may be concluded that the used DESs did not act selectively. Rather, along with lignin, other biomass components became dissolved as well. Therefore, the present results contradict those reported by other authors (Kumar *et al.* 2015). Based on the yield, the cited article implies a high selectivity of the DES based on choline chloride with lactic acid and lactic acid with betaine. However, the authors have found that the process has not been well proven, and therefore the mechanism remains ambiguous.

In the published works on solubility of pure cellulose, xylan, hemicellulose, and lignin it was stated that cellulose, hemicellulose, and xylan are indissoluble in DESs (Kumar *et al.* 2015). Other works (Francisco *et al.* 2012) tested the solubility of lignin, cellulose, and starch in DESs. They found high solubility of lignin, but also negligible cellulose solubility.

Non-covalent interactions play an important role in the cellulosic microfibrils and between individual components. It is known that the cellulosic microfibril surfaces are coated with noncellulosic polysaccharides (Iiyama *et al.* 1994). These polysaccharides are hydrogen bonded to the surfaces of cellulose microfibrils. There are also possibilities for ionic and salt interactions between polysaccharides, proteins, and one another (Iiyama *et al.* 1994). Furthermore certain covalent interactions take place between different kinds of polysaccharides-polysaccharides, polysaccharides-lignin, polysaccharides-proteins, and lignin-proteins (Taherzadeh and Jeihanipour 2012). Also it is known that polysaccharides are covalently linked with lignin by covalently-linked bridging molecules. However, the lignin present in lignocellulosic biomass is concealed within the hemicellulose and cellulosic microfibrils, necessitating extraction for efficient processing (Cooper *et al.* 1984; Iiyama *et al.* 1994). Given the conditions, it seems likely that lignin and hemicellulose and cellulose are depolymerized and extracted by the DES-antisolvent mixture. It can be supposed that holocellulose will be degraded as well. These results were confirmed by the present work.

Table 3. Yield after Delignification by DESs and Content of Lignin in Initial Wheat Straw and Delignified Straw

Reagent	ChCl/Reag	Temperature of delignification (°C)	Lignin in wheat straw (g)	Yield after delignification (%)	Lignin in delignified wheat straw	Decrease of lignin content (%)
Urea	1:2	80	0.437	94.93	0.431	1.3
Malonic acid	1:1	60	0.433	90.57	0.417	3.8
Lactic acid	1:9	60	0.433	85.28	0.369	14.6
Malic acid*a	1:1	80	0.436	80.74	0.341	21.6
Lactic acid	1:10	60	0.433	83.49	0.307	29.1
Oxalic acid x 2 H_2O	1:1	60	0.435	59.07	0.183	57.9

*a - the slurry was washed with ethanol

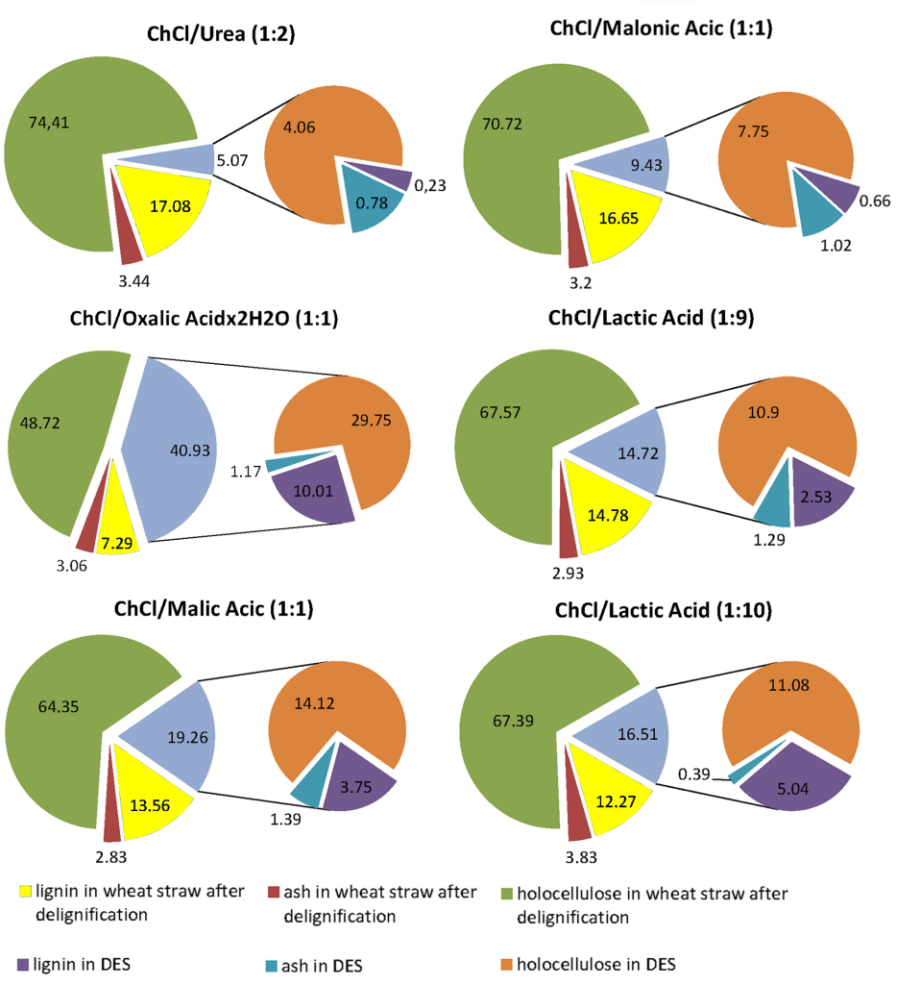

Fig. 1. Composition of delignified wheat straw and DES after delignification by choline chloride and urea (1:1), choline chloride and malonic acid (1:1), choline chloride and oxalic acid dihydrate (1:1), choline chloride and lactic acid (1:9), choline chloride and malic acid (1:1) and choline chloride and lactic acid (1:10) in %

The measured data documented that the highest portion of lignin was withdrawn using choline chloride and oxalic acid dihydrate. The applicability of oxalic acid is, however, restricted due to its toxic and caustic properties. Accordingly, DESs composed of ChCl/lactic acid (1:10) or ChCl/malic acid (1:1) are more suitable alternatives. It may be hypothesized that the reduction in delignification efficiency is a result of the presence of CH_2 groups in a long chain and NH_2. On the contrary, a higher content of the acid and a higher temperature can increase DES efficiency at delignification. The acid strength is an important factor, and it is a function of the solvent. Due to the acid environment the hydrolysis occurs and it leads to depolymerization of individual components. This contributes to the depolymerization and thus affects efficiency of DES.

Largo Garcia de Dios (2013) found that by increasing the choline chloride to lactic acid ratio, the amount of withdrawn lignin is increased. This was confirmed for a sample of wheat straw and beech wood, and is consistent with the results of the present study.

In Fig. 1, the composition of wheat straw after delignification and that of the used DESs are shown. When comparing the selectivity of lignin and holocellulose removal, it is obvious that the selectivity of DESs differed. When using ChCl/urea, the ratio of lignin removed to holocellulose (the ratio of the individual components after delignification by DESs) was 1:18, whereas it was 1:12 for ChCl/malonic acid, and 1:3 for ChCl/oxalic acid. In the case of ChCl/lactic acid (at molar ratio 1:9) the selectivity was 1:4.3, and for molar ratio 1:10 it was 1:2.2. When using ChCl/malic acid the ratio was 1:3.8.

From this viewpoint, the least suitable eutectic mixture was found to be ChCl/urea due to the lowest ratio of removed lignin. When comparing the ratio of removed lignin to holocellulose, and the portion of removed lignin, the best result was obtained by applying ChCl/lactic acid (molar ratio 1:10).

Singh *et al.* (2011) applied the soda pulping procedure to wheat straw. It was found that depending on conditions (130 to 160 °C, 0.25 to 2.5 h), the yield ranged from 35.70% to 56.25%, and the lignin content in pulp ranged from 2.62% to 12.85%. Within the present work, comparable results were reached using ChCl/oxalic acid dihydrate. In this case, 40.93% of wheat straw was dissolved in the eutectic mixture, *i.e.* the yield was 59.07%. However, Deniz *et al.* (2004) studied delignification of wheat straw using the kraft process with yield 42.6%. An interesting comparison has been reported in the paper of Rodriguez *et al.* (2008). Delignification of rice straw using the kraft process attained yields 32.9 to 42.1%, whereas when using a soda process the yields were 33.9 to 35.3%, and when using a soda-anthraquinone process the yield was 37.0%. Soda-anthraquinone cooking method of straw was used also in the paper of Ates *et al.* (2015) with the yield 37.9% (wheat straw) and 42.6% (rice straw).

These results suggest that eutectic mixtures behave similarly to those applied in classic delignification procedures. After delignification, the content of holocellulose and lignin in wheat straw was 48.7% and 7.3% in undissolved part and 29.8% and 10.1% in dissolved part (Fig. 1). Applying the other DESs, the content of holocellulose ranges from 64.35% to 74.71% that of lignin from 12.27% to 17.08% in undissolved parts, and the yield from 80.74% to 94.93%.

CONCLUSIONS

Deep eutectic solvents represent promising alternative solvent systems for the treatment of biomass. The distinct behaviors after the fractionation of wheat straw with various deep eutectic solvents, such as choline chloride and urea (1:2), and malonic (1:1), and lactic (1:9; 1:10), malic (1:1) and oxalic acid (1:1), were established in this study. Furthermore, the characterization of delignified samples was performed. There were clear differences between the samples obtained with differing DESs, especially when considering the content of removed of lignin and holocellulose.

1. The highest amount of lignin (57.9%) was removed using ChCl/oxalic acid dihydrate.
2. Based on the selectivity of lignin:holocellulose removal, (1:2.2) and the content of lignin (29.1%) removed from wheat straw, the best results was reached using ChCl/lactic acid at a molar ratio 1:10.
3. It was unveiled in this work that the investigated DESs do not act selectively during the delignification process.

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