Effective Biomass Delignification with Deep Eutectic Solvents

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Abstract. Efficient valorization of biomass is one of the most important aspects of circular economy. Obtaining valuable products from renewable materials, using ecological methods, is a key factor in natural environment protection. In many cases the first step of biomass valorization is removal of lignin from plant material. This process can significantly improve further transformations to final products, such as organic acids or monosaccharides. Deep eutectic solvents (DESs) are green chemicals, which have the ability to extract lignin from lignocellulosic material with high efficiency. In this paper we present an efficient method for lignin removal from plant material, using ecological deep eutectic solvents, based on choline chloride. We used waste biomass such as grass, rye straw and walnut shells. The best results were achieved in processes with DESs containing organic acids, such as lactic acid and malonic acid. Also, the impact of temperature of a process was examined—the largest amounts of extracted lignin were gained by delignification at 100 °C. The main goal of lignin removal from plant material is enhancing further transformation of biomass, e.g. enzymatic hydrolysis, in order to increase the yield of valuable products. We carried out the enzymatic hydrolysis of pretreated plant material with cellulase from *Aspergillus niger*. The effectiveness of biological transformations was improved, if delignification of biomass was previously performed. This effect was especially visible in case of soft material processing.

Keywords: Plant biomass · Biomass valorization · Deep eutectic solvents · Delignification · Enzymatic hydrolysis

1 Introduction

Plant biomass consists of three main components, which is cellulose, hemicelluloses and lignin. Cellulose is the most valuable raw material for further transformations to glucose and many other valuable compounds belonging to *Fine Chemicals*. The main problem in efficient biomass treatment is high lignin content in most plants. Lignin not

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only limits the access of enzyme to cellulose, but also can interact with the enzyme and inactivate it (deactivating effect of phenolic compounds), which decreases the efficiency of biomass transformations [1–3]. Because of that, lignin removal is necessary to achieve better results. Deep eutectic solvents (DESs) are a new type of ionic liquids, which have the ability to dissolve lignin, without any side effects on other parts of lignocellulose. Additionally, DESs are claimed to be much more ecological, than traditional solvents. They are obtained from natural components (such as choline chloride, betaine, lactic acid, citric acid), have lower toxicity and vapor pressure, and are biodegradable [4–6]. The next step of biomass valorization is its transformation to valuable products. It can be done with various methods, such as application of ionic liquids, acid and alkaline solutions or with biological agents. The last alternative is also compatible with green chemistry rules. By combining DESs pretreatment with enzymatic processing of plant waste, it is possible to develop ecological, environment-neutral system for obtaining chemical components, such as glucose, which has wide range of applications in many fields of industry.

The objective of this work was to evaluate the effectivity of different DESs, based on choline chloride, as lignin extraction media from plant biomass. Also, the role of delignification in further enzymatic treatment with cellulase was presented.

2 Materials and Methods

2.1 Materials

Grass (GR), rye straw (RS) and walnut shells (WS) were collected in Poland, air-dried and milled to obtain particles with size range 0.6–0.2 mm. Choline chloride (99%) was obtained from Acros Organics, lactic acid (85%) was from SigmaAldrich, malonic acid (99%) was form Apollo Scientific and citric acid (99%), ethylene glycol (pure), glycerin (pure), ethanol (99%) and acetone (pure) were purchased from Avantor Performance Materials Poland.

2.2 DESs Synthesis

DESs were prepared according to method described by Kandanelli et al. [7]. The abbreviations of each solvent with molar ratio of components are presented in Table 1. Choline chloride and hydrogen bond donor (lactic acid, citric acid, malonic acid, glycerin or ethylene glycol) were mixed in 80 °C with vigorous stirring, until transparent, stable at room temperature liquid was obtained. The water present in solvent was evaporated by rotary evaporator. DESs were stored at room temperature.

2.3 Biomass Delignification

2.50 g of biomass (grass GR, rye straw RS and walnut shells WS) was mixed with 50 ml of DES in a solid–liquid ratio 1:20 (w/v) in round bottom flask. Reactions were performed at 60, 80 or 100 °C in oil bath, with constant stirring (500 rpm), for 3 h. Each experiment was performed in triplicate. After delignification, samples were taken for

Table 1 DESs applied in this work

Hydrogen bond acceptor	Hydrogen bond donor	Molar ratio	Abbreviation
Choline chloride	Lactic acid	1:2	ChCl:Lac
	Citric acid	1:1	ChCl:Cit
	Malonic acid	1:2	ChCl:Mal
	Ethylene glycol	1:2	ChCl:Et
	Glycerol	1:2	ChCl:Gly

spectrophotometric analysis, at 420 nm, with DES as a standard solution. Concentration of lignin was calculated based on calibration curves, prepared separately for each type of biomass. Yield of extracted lignin was calculated as follows:

$$Lignin\ extraction\ yield\ (\%) = \frac{Mass\ of\ extracted\ lignin}{Mass\ of\ lignin\ in\ raw\ biomass} * 100 \quad (1)$$

Preparing the material for enzymatic hydrolysis was prepared according to Chen et al. [8]. After delignification, 50 ml of mixture acetone:water (1:1 v/v) was added to reaction vessel, to lower the viscosity of solution and facilitate filtration. Undissolved biomass was separated from solution by filtration. Acetone and water were evaporated and DES with dissolved lignin was obtained. 100 ml of mixture ethanol:water (1:9 v/v) was added to DES solution and left overnight in order to precipitate extracted lignin. Then, lignin was separated from solution by filtration. Ethanol and water were evaporated from DES, which allowed to recycle it to another delignification process.

2.4 Enzymatic Hydrolysis of Pretreated Biomass

Pretreated biomass was subjected to hydrolysis with cellulase from *Aspergillus niger* in citrate buffer (50 mM, pH = 5.0), solid to liquid ratio was 1:40 (w/v). 0.8 U/mg of cellulase was added to the reaction mixture. Reactions were performed at 50 °C, in incubator (Benchtop Shaking Incubator 222DS) with stirring (150 rpm), for 24 h. After hydrolysis, samples were taken for HPLC analysis. Glucose concentration was determined from the calibration curve, and the yield was calculated from the equation:

$$Glucose\ yield\ (\mathrm{mg/g}) = \frac{Mass\ of\ glucose\ in\ sample * Volume\ of\ a\ sample}{Mass\ of\ biomass}$$

2.5 Analytical Methods

The yield of extracted lignin was measured by spectrophotometric method, according to Skulcova et al. [9], with slight modification. Measurements were carried out on UV–VIS spectrophotometer (Jasco V-650) at 420 nm.

Glucose concentration was measured by HPLC method. The apparatus (Agilent 1200 HPLC system) was equipped with Phenomenex ROA-Organic acid H+ column and refractive index detector. 5 mM sulfuric acid was used as an eluent. Analysis was carried out at 60 °C, with flow rate of 0.5 ml/min.

3 Results and Discussion

3.1 The Effect of DESs Type and Temperature of a Process on Delignification Efficiency

In this study, different DESs were investigated to choose the best one for biomass delignification, which would ensure the highest yields of extracted lignin. The temperature of a process also had major impact on the results. Figures 1, 2, 3 present the comparison of results, shown as delignification degree of three types of biomass (grass GR, rye straw RS and walnut shells WS) with different DESs at 60, 80 and 100 °C.

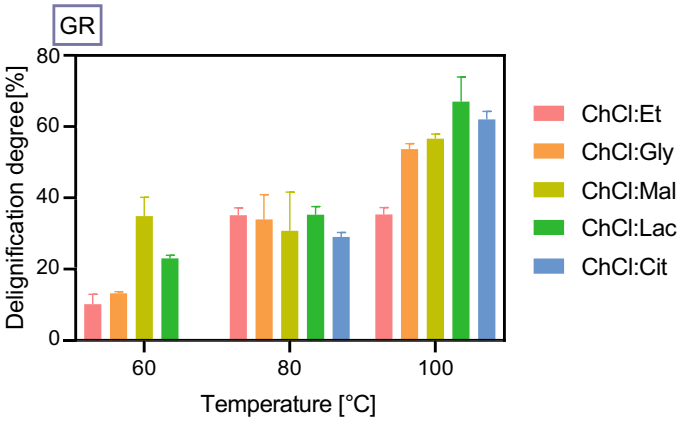

Fig. 1 Delignification degree of grass (GR) with DESs, at different temperatures

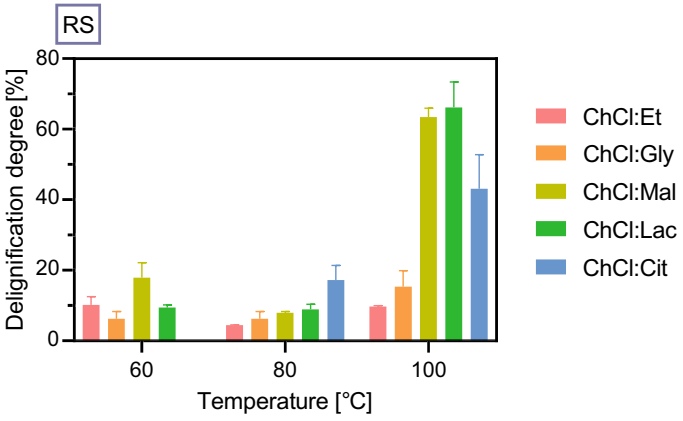

Fig. 2 Delignification degree of rye straw (RS) with DESs, at different temperatures

As can be seen for all types of plant material, it was possible to obtain higher lignin yields, if DES was composed of organic acids: lactic acid or malonic acid, compared to

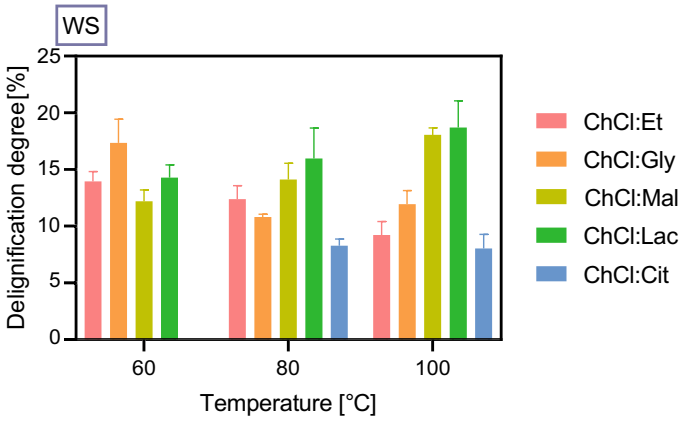

Fig. 3 Delignification degree of walnut shells (WS) with DESs, at different temperatures

DES with polyalcohols. It might be connected with the possible mechanism of delignification—in acidic conditions, ether bonds, present in lignin structure, tend to break more easily [10]. The maximum GR delignification degree was 67.02 ± 6.96%, for RS 66.19 ± 7.20% and for WS 18.70 ± 2.35%. These numbers could be achieved in process at 100 °C, with DES ChCl:Lac. In the case of solvent with citric acid, which also provides acidic environment, the delignification degree was lower. It can result from the high viscosity of this DES. Because of that it was impossible to carry out the experiments at temperatures below 80 °C for ChCl:Cit.

High viscosity is the main drawback of DESs. Because of that, biomass delignification should be performed at higher temperatures, which enhances mass transport and increases the yield of extracted lignin [11]. Additionally, at higher temperatures, lignin can be degraded to phenolic monomers, which results in faster polymer removal [12]. As it was mentioned before, for GR and RS, the highest delignification degrees could be achieved at 100 °C. This trend is not so obvious in the case of WS pretreatment—the results are quite random and not directly connected to the temperature of a process. The possible reason for that is the type of material—walnut shells are hard type of biomass, which is the most difficult to process. It can be seen that the maximum delignification degree is much lower, than for other types of biomass.

We have chosen DES composed of choline chloride and lactic acid for further experiments.

3.2 Enzymatic Hydrolysis of Pretreated Biomass

In order to examine the effect of biomass delignification on further transformations, we carried out the enzymatic hydrolysis of plant material with cellulase from *A. niger*. Since the best delignification was obtained in the process with DES ChCl:Lac, at 100 °C, we chose these conditions to pretreat the biomass before enzymatic transformation. Figure 4 presents the comparison of the amount of glucose obtained in enzymatic transformation of biomass without pretreatment and after pretreatment with ChCl:Lac.

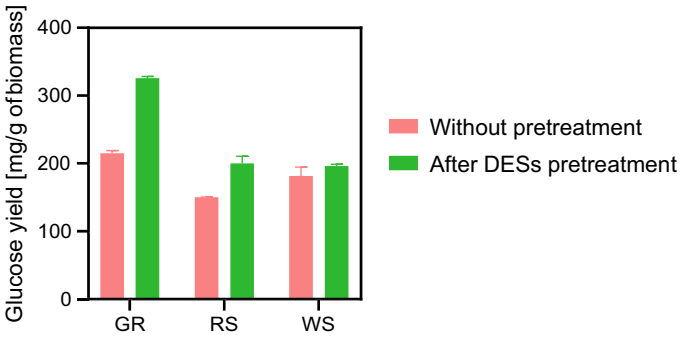

Fig. 4 The comparison between glucose yields, obtained by enzymatic hydrolysis of biomass (grass GR, rye straw RS and walnut shells WS) without pretreatment and after delignification with DES ChCl:Lac

Results presented in Fig. 4 show, that for soft types of plant material, delignification had positive effect on enzymatic hydrolysis efficiency. It was possible to increase the amount of glucose, especially in case of GR processing. The yield of a product increased from 215.10 ± 3.61 mg/g to 325.89 ± 2.35 mg/g. In enzymatic transformation of RS, it was possible to get higher glucose amounts after biomass pretreatment, but the rise was not so significant, as in GR processing. In the case of WS, delignification with DES did not significantly improve the further efficiency of transformation to glucose. It is connected with the hardness of raw material [13–15]. It is worth mentioning, that during delignification of WS, the amount of removed lignin was not so high, compared to the other types of biomass.

4 Conclusion

Application of deep eutectic solvents in biomass delignification is an ecological and efficient method for plant material pretreatment. It was possible to achieve high extracted lignin yields, especially for softer biomass processing. The best results were obtained in the presence of DESs consisted of choline chloride and carboxylic acids The hardness of material is still a major obstacle to overcome during plant waste treatment. The content of lignin in plant material has crucial effect on the outcome of further biomass processing. Delignification is an effective method for biomass valorization, to produce higher amounts of valuable chemicals.

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