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Enhancement of ethanol production from spruce wood chips by ionic liquid pretreatment

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HIGHLIGHTS

- ▸ Spruce chips and powder were pretreated by solvents: two ionic liquids and NMMO.
- ▸ The efficiency of pretreatment was examined by enzymatic hydrolysis and fermentation.
- ▸ 1-Ethyl-3-methylimidazolium acetate was the most efficient solvent.
- ▸ Ethanol yield of wood powder was improved from 9.7% to 81.5% of theoretical yield.
- ▸ Ethanol yield of wood chips was improved from 2.7% to 66.8% of theoretical yield.

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ABSTRACT

Pretreatment with three green cellulose solvents, N-methylmorpholine-N-oxide (NMMO), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]) were used for improvement of ethanol production. Spruce chips and powder were pretreated at 120 °C for 1, 3, and 15 h. In order to investigate the efficiency of the pretreatments, the pretreated materials were subjected to enzymatic hydrolysis at 45 °C for 72 h, followed by fermentation with *Saccharomyces cerevisiae* at 32 °C for 24 h. The best results were obtained from 15 h pretreatment with [EMIM][OAc]. The ethanol yield from the untreated spruce chips and powder were 2.7% and 9.7% of the maximum theoretical yield, respectively, whereas pretreatment of these materials with [EMIM][OAc] improved the ethanol yield to 66.8% and 81.5%, respectively.

1. Introduction

For bioethanol production from lignocellulosic materials, the cellulose and hemicellulose polymers should be hydrolyzed prior to fermentation. However, a pretreatment step is essential in order to reduce the high crystallinity and resistance of cellulose to enzymatic hydrolysis. Pretreatment is known as a key process for reduction of the bioethanol manufacturing costs from lignocelluloses by increasing the efficiency of the enzymatic hydrolysis [1–4]. Among the different pretreatment methods, treatment with cellulose solvents such as phosphoric acid [5–7], NaOH/urea [5,8], N-methylmorpholine-N-oxide (NMMO) [5,9], and ionic liquids (ILs) [5,10,11] have been suggested as the most efficient methods for enhancement of the saccharification yield. Pretreatments with these solvents are generally performed at milder conditions compared to other methods such as dilute acid, steam explosion, and alkali pretreatments; therefore, they result in less cellulose and hemicellulose degradation [5,8,12,13].

Ionic liquids (ILs) are composed of a cation and an anion, and the properties of IL can be adjusted by proper selection of the ions. ILs have received growing attention for pretreatment of lignocellulosic materials because of their unique properties in cellulose dissolution. ILs are considered as green solvents due to their low vapor pressure and high stability, which result in less impact on the environment. Furthermore, ILs are non-derivatizing solvents for cellulose and it is possible to recycle and reuse them [13–15]. Among the most efficient ILs, 1-butyl- and 1-allyl-3-methylimidazolium chlorides ([BMIM][Cl] and [AMIM][Cl]), and 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) are frequently used for dissolution of cellulose, among which the two latter ones show the best potential for lignocelluloses dissolution [13].

One of the characteristics of a suitable pretreatment process is the ability to modify the structure of wood chips as well as wood powder because of the significant energy costs of the milling process [1]. Pretreatment of triticale straw [16], switchgrass [17], rice

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straw [18], pure cellulose [19], and maple wood [20] by [EMIM][OAc] or [BMIM][OAc] have been resulted in significant improvement in the enzymatic hydrolysis of these materials. However, to our knowledge, there is no previous report on pretreatment of spruce wood chips with these ILs.

Size reduction of wood is a challenge in large scale ethanol production from lignocelluloses. An optimization is required to minimize the energy/cost of wood powder production and to increase the efficiency of the subsequent pretreatment process. Some pretreatment methods such as steam explosion, with or without addition of acid or SO_2, can be enough efficient on wood chips, while some other methods such as pretreatment with sodium hydroxide or hydrogen peroxide are not efficient on wood chips and they are effective only on the powder [21,22]. The main objective of this work was to investigate the efficiency of pretreatment processes with two new ILs, [EMIM][OAc] and [BMIM][OAc], and a cellulose solvent, NMMO, in enhancement of enzymatic hydrolysis and ethanol production from softwood (spruce) chips.

2. Materials and methods

2.1. Raw materials

Native spruce wood (*Picea abies*) was debarked, cut into smaller pieces, and milled with a garden waste mill to obtain chips with size of less than 2 cm. Then, the chips were partly ball-milled and screened to achieve wood powder with a size between 295 and 833 μm (20–48 mesh). Ionic liquids 1-ethyl-3-methylimidazolium acetate [EMIM][OAc] and 1-butyl-3-methylimidazolium acetate [BMIM][OAc] were obtained from Sigma–Aldrich. The ILs which are commercially available from BASF (Ludwigshafen Germany) were used for the pretreatment without further modifications.

Previous studies have shown that NMMO with 85% concentration, which is able to dissolve cellulose, is the best form of NMMO for pretreatment [23]. Thus, the commercial grade NMMO solution (BASF, Ludwigshafen, Germany), containing 50% NMMO, was concentrated by vacuum evaporation to 85% solution before pretreatment. All of these solvents exhibit very low vapor pressure in the applied concentrations. Physical properties of the solvents used for pretreatment as well as their molecular structure are summarized in Table 1. Two commercial enzymes, cellulase (Celluclast 1.5 L, Novozymes, Denmark) and β-glucosidase (Novozym 188, Novozymes, Denmark), were used for enzymatic hydrolysis. Cellulase activity was determined as 80 FPU/ml [24], while the activity of β-glucosidase was measured as 240 IU/ml using p-nitrophenyl-β-D-glucopyranoside as a substrate. In the mentioned assay, the amount of β-glucosidase enzyme that liberates one micromole p-nitrophenol per minute is defined as one IU [25].

2.2. Pretreatment

Cellulose solvents, [BMIM][OAc], [EMIM][OAc], and NMMO were used for the pretreatment. Mixtures containing 5% (w/w) of the softwood were prepared by addition of 1 g wood powder or chips to 19 g of the solvents in 100 ml laboratory glass bottles. The pretreatments were performed at 120 °C in an oil bath for 1, 3, and 15 h and manually mixed with a glass rod. Then, 20 ml boiling water was added in order to regenerate the treated wood. The temperature (120 °C) was selected in previous studies for the pretreatment by NMMO to provide the best dissolving conditions without the risk of solvent degradation [9,23]. The solvent was finally removed from the treated material by subsequent washing with water and vacuum filtration using a filter paper (Whatman No. 1) until clear filtrate appeared. This process eliminated the risk of inhibition of yeast growth by the solvents during the subsequent fermentation step. At the end, the collected regenerated wood was freeze-dried at −48 °C for 48 h and kept at 4 °C until use. All experiments were conducted in duplicates.

2.3. Separate enzymatic hydrolysis and fermentation (SHF)

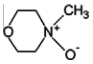
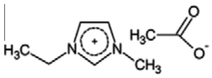
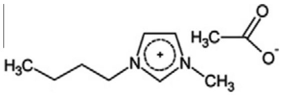
Enzymatic hydrolysis of the pretreated and untreated wood was performed at 45 °C for 72 h, and 5% (w/w) of the wood (based on the dry weight) in a shaker bath mixed at 120 rpm. An amount of 20 ml solution containing 50 mM sodium citrate buffer (pH 4.8) and 0.4 g/l sodium azide was autoclaved prior to the enzyme loading of 15 FPU cellulase and 30 IU β-glucosidase per g substrate [9]. Sodium azide was added to prevent the risk of microbial contamination during the sampling from the hydrolysis mixture. However, since the sodium azide is a metabolite inhibitor, a separate set of enzymatic hydrolysis was also performed without addition of sodium azide and without sampling and used for fermentation. After hydrolysis, the hydrolysates were separated by centrifugation from the solid fraction, and supplemented with all required nutrients for cell growth [26], autoclaved for 20 min at 121 °C, and subjected to fermentation [9]. The fermentations were conducted by 10 g/l flocculating strain of *Saccharomyces cerevisiae* (CCUG 53310 obtained from Culture Collection of University of Gothenburg, Sweden) at 32 °C for 24 h. The high cell density (10 g/l) was used to ensure complete consumption of all sugars. The strain maintenance and inoculum preparation were performed as previously reported by Shafiei et al. [9]. All experiments were conducted in duplicates.

2.4. Chemical characterization of spruce wood

The carbohydrate and lignin fractions of the native as well as the pretreated wood were determined using the two-steps acid hydrolysis procedure documented by National Renewable Energy Laboratory (NREL) [27]. Accordingly, cellulose and hemicellulose were hydrolyzed to their corresponding sugars and measured by HPLC. UV–Vis spectroscopy at 320 nm was used for determination of acid soluble lignin.

In order to investigate the effects of pretreatment on the wood structure, Fourier transform infrared (FTIR) spectra of the pretreated as well as untreated wood powder were used. FTIR

Table 1
The molecular structure of cellulose solvents, [BMIM][OAc], NMMO, and [EMIM][OAc], which were used for pretreatment.

Cellulose Solvent			
Physical property	NMMO	[EMIM][OAc]	[BMIM][OAc]
Purity	85%	⩾90%	⩾95%
Density (g/cm^3 at 25 °C)	~1	1.027	1.055
Flash point (°C)	Not available	164	153
Melting point (°C)	~78	<−20	<−20