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Research paper

Novel pretreatment pathways for dissolution of lignocellulosic biomass based on ionic liquid and low temperature alkaline treatment

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ABSTRACT

Pretreatment, fractionation and hydrolysis remains costly and challenging process steps in biochemical conversion of softwoods. Here, ionic liquid pretreatment using 1-ethyl-3-methylimidazolium acetate (EMIM-OAc) at high temperature (100 °C, 6 h) and alkali based (NaOH/urea) pretreatment at sub-zero temperature (−18 °C, 24 h) were compared and combined in studies of Norway Spruce biomass deconstruction. Both treatments significantly improved the enzymatic digestibility of the biomass. EMIM-OAc gave higher glucan than mannan digestibility, indicating a more pronounced effect on the cellulose polymer than on the hemicellulose polymer. In contrast, low temperature alkali pretreatment using NaOH or NaOH + urea gave a more pronounced effect on mannan than on glucan digestibility. By combining the two methods the total monosugar yield after enzymatic hydrolysis was improved by 20−50% as compared to using ionic liquid or alkali based pretreatment alone. Lignin dissolution was low for both methods under the conditions studied.

1. Introduction

Lignocellulosic biomass represents the most abundant biomaterial found in nature, and is expected to be a central raw material in the industrial processes foreseen in the future bioeconomy. Lignocellulosic biomass is generally composed of the three main components cellulose, hemicelluloses and lignin. Following the biochemical conversion pathway of lignocellulose to biofuels and biochemicals, pretreatment and fractionation represent central starting points in biomass processing. Due to the recalcitrant nature of lignocellulose, these steps can be both technically and economically challenging. In particular, softwoods remain a challenging raw material for cost-effective conversion to biofuels and chemicals, following the biochemical conversion route. Thus, the aim of this work was to expand our understanding of these challenging process steps in our strive to develop cost-effective pretreatment schemes for conversion of recalcitrant Norway Spruce lignocellulosic biomass.

Several pretreatment methods are being studied in the ongoing development of the future biorefinery processes, and are previously well described in the literature. This includes either mechanical, thermal, chemical and biological methods or a combination of two or more of these [1–5]. Focus of much work has been on the conversion of annual plants (e.g. bagasse, straw) and numerous methods have been shown to give effective deconstruction of such lignocellulosic biomass, followed by effective saccharification of the biomass carbohydrates. However, wood biomass, in particular softwoods, still represent a challenging case when it comes to development of cost-effective pretreatments.

Ionic liquids have been reported by many researchers [6–10] to represent possible solvents in the pretreatment of recalcitrant lignocellulosic biomass. The pretreatment efficiency is dependent on the ionic liquid used, the lignocellulosic biomass (type, moisture, size and load), pretreatment temperature and time, and antisolvent used [10]. A detailed literature review on lignocellulosic biomass fractionation by ionic liquids is given by da Costa Lopes et al. 2013b, describing biomass dissolution using i.a. different ionic liquids, biomass regeneration methods, treatment temperature and time, biomass type, particle size and water content.

Ionic liquids such as 1-allyl-3-methylimidazolium chloride [Amim]Cl and 1-ethyl-3-methylimidazolium [C_2mim]OAc have proven highly effective in the dissolution of cellulose, lignin, and hemicellulose in different types of lignocellulosic biomass including corn stover, switch grass, rice straw, and various hard and softwoods [10–12]. Investigating pretreatment of Norway Spruce

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using four imidazolium-based ionic liquids, Gräsvik et al. (2014) showed that the best effect for Norway Spruce was obtained with acetate based ionic liquid [7]. Furthermore, results by Khalil et al. (2012) showed that ionic liquids were able to selectively extract the rigid lignin from the lignocellulose while yielding a highly amorphous cellulose fraction [13].

Ionic liquid pretreatment is comparable to other mainstream pretreatment methods in sugar yields, but requires no harsh operating conditions such as high temperature or high pressure. Ccurrently, however, the high price and uncertainty regarding the toxicity of ionic liquids represent serious drawbacks in the application of these solvents. Thus alternatives for biomass pretreatments are sought, including switchable ionic liquids, distillable ionic liquids and deep eutectic solvents (DES). Recently, Morais and co-workers demonstrated that the use of imidazole, a commonly used precursor of imidazolium based ILs, gave selective separation of wheat straw cellulose, hemicelluloses and lignin, thus enhancing the enzymatic digestibility of the cellulose rich fraction [14].

Alkaline pretreatment processes are also applied to lignocellulosic biomass (e.g. Ref. [4]). Sodium hydroxide (NaOH) alone or in combination with urea, have been used for low temperature (<0 °C) dissolution of cellulose [15–18]. These solvent systems represent a relatively cheap, easy to handle treatment, commonly used in a variety of industrial processes. Although dissolution of **cellulose** using alkaline or alkali/urea based solvent systems is rather well reviewed, dissolution of **lignocellulosic** materials (i.e. lignin containing biomass) is studied only to a limited extent. A previous study by Zhao and colleagues in 2008 describes use of this pretreatment to improve the enzymatic hydrolysis of wood fibres from spruce [19]. In 2014, Shi and colleagues described the effect of varying both lignin and hemicelluloses contents on the dissolution of wood pulp using such solvent systems [18]. Both studies indicate that the use of alkali/urea solvent systems may represent a feasible pretreatment and fractionation route for lignocellulose, not only pure cellulose. Also, the content of lignin in the wood chips or pulps did not prevent the dissolution of the wood cellulose in the alkali/urea solvent [18].

The focus of the present work has been to study pretreatment and fractionation of highly recalcitrant softwood biomass from Norway Spruce by use of ionic liquid and/or alkali/urea solvent systems. Initial trials were performed using the ionic liquid 1-ethyl-3-methylimidazolium acetate (EMIM-OAc). Further trials were focused on the use of sub-zero temperature NaOH based solvent systems in the presence or absence of urea. Additionally a sequential combination of ionic liquid and the alkali based pretreatment was also elucidated. The saccharification potential of the pretreated materials was used as the main evaluation parameter. Lignin dissolution following the various pretreatment schemes was also studied.

2. Materials and methods

2.1. Materials

ATMP fiberized Norway Spruce chips prepared as described by Johansson and colleagues were hammer milled to particle sizes between 0.7 and 1.7 mm [20]. Extractives were removed using Soxhlet extraction (6 h, 9:1 cyclohexane:acetone mixture, both from Romil Ltd.). Ionic liquid pretreatment was performed using EMIM-OAc (Sigma-Aldrich, purity >90%, <0.5% water). Alkali based pretreatments were performed using sodium hydroxide (J.T. Baker) and/or urea (ProLabo). Enzymatic hydrolysis trials were performed using a mixture of Celluclast and Novozyme 188 (both from Novozymes). Bovine serum albumin (BSA, New England BioLabs Inc) was added to the hydrolysis reaction.

2.2. Characterization of biomass

The raw material and the precipitate after pretreatment were analysed with regard to raw material composition according to NREL/TP-510-42618, dry matter content according to ISO 638 and saccharification potential according to NREL/TP-510-42629 with a few modifications as described below. The filtrate obtained after the pretreatment experiments was analysed with regard to lignin content according to NREL/TP-510-42618.

For compositional analyses the cellulose and hemicellulose contents were calculated from the monosugar contents according to the following:

Cellulose = Glucose-1/3 Mannose
Hemicellulose (Galactoglucomannan) = 4/3 Mannose + Galactose

2.3. Ionic liquid pretreatment

Prior to use, both EMIM-OAc and the lignocellulosic material were dried under vacuum at 40 °C for 24 h ensuring water contents <0.5% for the ionic liquid and <5% for the biomass. Subsequently, the samples were mixed under inert nitrogen atmosphere, at a wood:ionic liquid weight ratio of 5 wt%. The sample was stirred at 500 rpm using a Radleys Tech (05–1170) electric heater at 100 °C for 6 h, as found to be optimal in initial optimization trials, performed at incubation times of 3, 6, 12 and 24 h and temperatures 80, 100 and 120 °C. Optimal digestibility both for glucan and mannan were obtained following treatment at 100 °C for 6 h.

After pretreatment the dissolved material was recovered by precipitation in deionised water (10× the volume of EMIM-OAc) under vigorous mixing, followed by filtration (GF/A). The precipitated material was washed (deionised water) and dried. Dry matter content was measured. A schematic illustration of the process layout is shown in Fig. 1, left hand side (route A). The ionic liquid pretreated samples were then either (i) subjected to enzymatic hydrolysis to study the enzymatic saccharification potential or (ii) further processed in a subsequent alkaline, urea or alkaline + urea treatment (route B, Fig. 1).

2.4. Low temperature alkaline, urea and alkaline + urea pretreatment

Alkaline pretreatment (7% NaOH), urea pretreatment (12% urea) or alkaline + urea pretreatment (7% NaOH+12% urea) was performed at −18 °C for 24 h as previously described by Zhao and colleagues in their optimization study on alkaline pretreatment on spruce [19]. The weight ratio of liquid:wood solids was 20. The pretreated material was washed with deionised water and neutralized to pH 7. The neutralized sample was dried and its dry matter content measured. The sample was then subjected to enzymatic hydrolysis (see Fig. 1, right hand side; Route B).

The pretreatments were performed either as solitary experiments on the hammer milled spruce chips, or following ionic liquid pretreatment of the spruce chips.

2.5. Enzymatic hydrolysis/saccharification potential

The enzymatic hydrolysis of the pretreated lignocellulosic materials was performed according to NREL/TP-510-42629, with a few modifications; A biomass sample equal to the equivalent of 0.1 g cellulose was dissolved in sodium citrate buffer (0.1 M, pH 4.8) to a concentration of 2% cellulose (w/v). Subsequently, 2 mg of a sodium azide solution (2%) was added to prevent growth of