



Enzymatic saccharification and structural properties of industrial wood sawdust: Recycled ionic liquids pretreatments



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ABSTRACT

Wood residues constitute a promising challenge for biochemical processing into bioethanol and chemicals with competitive costs. Here, we report the impacts of pretreatments in a hydrophilic ionic liquid ([C2mim][OAc]), onto the physicochemical properties and enzymatic saccharification of softwood (spruce) and hardwood (oak) sawdust. Enzymatic saccharification of IL- pretreated sawdust is significantly increased (up to 7 times) when compared to untreated ones. Methanol, ethanol or water can be used as polar anti-solvent for the recovery of a cellulose rich fraction after dissolution in IL (i.e regeneration step) without any effect on enzymatic saccharification. Chemical, textural and structural modifications possibly induced by the IL pretreatments have been investigated through various means (Infra-red spectroscopy, NMR, X-ray diffraction) in order to correlate the observed modifications in enzymatic saccharification. This mild pretreatment seemed to mainly act in a breakdown of lignocellulosic organization leading to better cellulase accessibility to cellulose thanks to the expansion of the powder and the creation of a large porous volume (5 times more apparent porous volume). Partial removal of lignin and extractives may also contribute to the best enzymatic performances. The recyclability and reuse up to 7 times of [C2mim][OAc] is shown without the need of strictly anhydrous conditions and any alteration of the pretreatment.

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1. Introduction

The biorefinery strategy is based on the use of carbon from renewable resource to produce platform molecules or biofuels [1,2]. Among all biomass, lignocellulose constitutes an abundant source of sugar and the biochemical process represents a promising way to produce bioethanol at competitive costs while respecting environmental requirements. Furthermore, agricultural and forest residues constitute an attractive lignocellulosic biomass feedstock as they are not used as foodstuffs and cheap. Among them, sawdust represents the major low-value by-product available in large amounts issued from wood transformation processes. In addition, it could be considered as pre-collected renewable feedstocks which may be converted on site into liquid biofuels [3].

Softwoods and hardwoods are highly recalcitrant and typically require a pretreatment step in order to enhance the enzymatic

digestibility of their constituting polysaccharides into fermentable sugars [4]. Different strategies have been proposed to reduce this recalcitrance enlisting diluted acid, organosolv, extrusion or steam explosion pretreatments [5–7]. The resulting enhancement in reactivity could originate from single or combined effects: (i) structural disorganization of lignocellulose leading to a better accessibility of constitutive cellulose, e.g. extrusion pretreatment of pine wood chips [7]; (ii) fractionation due to selective extraction of lignin and/or hemicelluloses, e.g. organosolv pretreatments of pine wood [8]; (iii) structural changes of cellulose by disruption of inter- and intra-chain H bond network [9,10]. However, several drawbacks must be overcome such as: (1) lower the amount of degraded by-products and/or (2) optimize processes for the recovery and reuse of solvents and/or (3) optimize the process conditions. Recently, an emergent pretreatment applied to various wood species (eucalyptus, pine, poplar, spruce etc.) is the dissolution in hydrophilic ionic liquids (ILs) [11]. According to the current environmental requirements, ILs constitute, at first glance, promising non-conventional solvents to perform eco-friendly pretreatments

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of lignocellulosic biomass thanks to their low-volatility, their ability to be recycled and their low toxicity for some of them [12–14]. The ability of an IL to dissolve lignocellulosic biomass essentially depends on the type of anion and cation, the type of biomass, the temperature/duration of the pretreatment and the biomass/IL ratio.

In this context, 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) was reported to be one of the best solvent for the dissolution of wood [14]. The efficiency of IL-pretreatments is often explained by a disruption of the three dimensional structure of lignocellulosic biomass leading to a selective removal of lignin [15,16]. At another level, interactions between IL and cellulose can lead to the disruption of its intra/inter-chain H bond network and thus, improving its enzymatic digestibility [10,17,18].

However, the validation of IL-based pretreatments in biorefineries pre-requests many technical and economic challenges are efficiently addressed. This enlists to (1) setup cost-effective technologies for the recovery and reuse of ILs, (2) reduce the related energy consumption, (3) optimize the lignocellulosic biomass to IL ratio, and (4) have a deeper understanding of their impact on industrial feedstock prior to enzymatic saccharification.

Previous studies demonstrate the feasibility to recycle imidazolium-based ILs by evaporation of anti-solvent previously introduced in the regeneration step of IL-pretreated samples [10,12,19]. However, these recycled ILs showed weakened efficiency for the pretreatment of lignocellulosic biomass [12].

In this study we investigated the impact of short duration pretreatments in pristine/recycled [C2mim][OAc] onto the chemical properties and enzymatic digestibility of industrial sawdust. [C2mim][OAc] was selected due to its low viscosity, low toxicity, its capability to dissolve wood and its ability to be reused [10,14,20]. Two distinct sawdust from hardwood (oak) and softwood (spruce) have been selected from a forest industry in Lorraine (France). The influence of the anti-solvent (i.e. water, ethanol, methanol) used for the regeneration of the IL and the recovery of the cellulosic fraction was studied. Chemical, textural and structural modifications possibly induced by the IL pretreatments have been checked through various means (infrared spectroscopy, solid state NMR, X-ray diffraction) in order to correlate the observed enzymatic saccharification performances.

2. Materials and methods

2.1. Raw materials

Industrial sawdust of oak (*Quercus petra*) and spruce (*Picea abies*) were provided by the forest industry SARL Husson Paul (Bathelémont, Lorraine, France). Native spruce and oak wood, respectively 80 and 180 years old, were obtained from the Château-Salins forest in Lorraine (France). Sawdust was produced during the sawing of wood logs dedicated to wood frame construction. On the industrial site, sawdust was stored in a warehouse at ambient temperature. For this present work, the collected sawdust was milled with a planetary ball miller (Retsch PM400) for 20 min at 300 rpm to achieve a size of less than 0.8 mm.

Cotton cellulose exhibiting a high purity degree was provided by Sigma–Aldrich (Steinheim, Germany) and was characterized by fibers size ranging from 125 to 400 μm (i.e. long cellulose fibers).

2.2. Reagents

1-Ethyl-3-methylimidazolium acetate ([C2mim][OAc], >98%) was purchased from Solvionic SA (Verniole, France). Cellulase from *T. reesei* (EC 3.2.1.4) was supplied by Sigma–Aldrich (Steinheim,

Germany) and presents a specific activity of 5 U/mg; one unit liberates 1.0 μmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 h of incubation). Anhydrous sodium acetate (99%) came from Fluka Sigma–Aldrich (Steinheim, Germany), sodium hydroxide aqueous solution (46/48%) from Fisher Scientific (Illkirch, France) and acetic acid (99%) from Carl Roth (Lauterbourg, France). Absolute ethanol (>99.8%) and methanol (>99.9%); standard arabinose, xylose, glucose and cellobiose were purchased from Sigma–Aldrich (Steinheim, Germany).

2.3. Pretreatments of sawdust and model cellulose samples with ILs – water mixtures

Freeze-dried sawdust or model cellulose (2% w/v) was suspended in various ILs/water ratios, the whole incubated at 110 °C (thermostated oil bath) under vigorous stirring for 40 min, and then cooled down in an ice bath. To each of these suspensions of pretreated sample was then added one polar solvent (absolute ethanol, absolute methanol or ultra-pure water) in 2:1 v/v solvent/IL ratio, under vigorous stirring for 30 min in ice bath, and the resulting suspension was then centrifuged (10,733g, 20 min, 4 °C with an Allegra 64R Beckman Coulter rotor: F0850). The solid recovered fractions were collected by vacuum filtration, thoroughly washed with the corresponding polar solvent and then freeze-dried.

Recovered permeate solutions (solvent-IL mixture) were recovered for subsequent IL-recycling.

2.4. Recovery and recycling of ILs

Each permeate solution of solvent-IL mixture was distilled by rotary evaporator (Büchi Rotavapor R-200) under vacuum at 80 °C for 16 h. Recovered IL was then stored at ambient temperature under dry atmosphere. The recovering yield was evaluated by volume measurement to be at least 90% per cycle.

The water content in IL was measured by Karl Fischer coulometry method (831 KF Coulometer, Metrohm) equipped with an oven at 140 °C (774 Oven Sample Processor, Metrohm). IL samples were weighed out into glass vessels and sealed. Accuracy of the measurement was verified with lactose standards. All values were corrected by subtraction of atmospheric water content. Each measure was three times repeated and results are expressed as mean values with standard deviations.

To determine the lignin content in each recycling IL solution, Klason lignin from industrial oak and spruce samples was used as standard as followed. Stock solution of Klason lignin was obtained by dissolution in a 50 mM NaOH aqueous solution at 70 °C for 12 h, then cooled down. Dilutions of this stock solution by 50 mM NaOH aqueous solution provide standard solutions. The lignin-free 50 mM NaOH aqueous solution is used as blank sample, and UV absorbance was recorded at 280 nm (Varian Cary 50 Bio UV/Visible spectrophotometer).

2.5. Enzymatic saccharification

The cellulase-catalyzed hydrolysis of the different (ligno)-cellulosic substrates (untreated or IL-pretreated) was carried out in stirred flasks (40 °C, 175 rpm) as described previously [10,21]. Typically, the final concentrations of lignocellulosic substrate and enzyme in 10 mL acetate buffer (50 mM, pH 4.8) were 2% (w/v) and 1 mg/mL, respectively. Concerning studies on IL-recycling and water content effects, it should be noted that enzymatic saccharification were performed in 1 mL Eppendorf tubes in the same other conditions. Each pretreatment and reaction was repeated 3 times. The yields of the substrate conversion into glucose were expressed as mean values with standard deviations (\pm).

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