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Biorefining brewery spent grain polysaccharides through biotuning of ionic liquids

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ABSTRACT

Brewery spent grain (BSG), a relevant waste from beer industry mainly composed of polysaccharides and lignin, is experiencing a surge in the production with its associated environmental impact. Thus, this manuscript bets in the application of aqueous solutions of a cholinium-based ionic liquid (IL) containing glycinate as anion ($[N_{1112OH}][Gly]$) for an efficient delignification pretreatment. The operation at 90 °C yielded drastic lignin reduction (75.89%), greater than the levels attained when a traditional imidazolium-based IL (1-ethyl-3-methylimidazolium acetate, $[C_2C_1im][C_1COO]$), was used (40.18%). The advantages of this pretreatment positively impacted the subsequent saccharification reaction, as the levels were increased up to about 1.5 times regarding the control (no IL) or the imidazolium-based pretreatment. ATR-FTIR spectrometry and scanning electron microscopy turned out to be useful tools to monitor the structural changes exerted. The results presented in this work make up the basis for a rational design of bio-ILs for delignification of lignocellulosic materials.

1. Introduction

During the elaboration of beer, highly useful derivatives are also obtained, including brewery spent grain (BSG), the lignocellulosic solid matter resulting from the filtering of wort obtained after the saccharification of the malted cereal grains (generally barley). Spain, with 36.469,219 hectoliters in 2016, is the fourth EU beer producer and the eleventh worldwide, which involves 600,000 tons of BSG/year (Ministerio de Agricultura y Pesca Alimentación y Medio Ambiente, 2016). The cell walls of BSG are opened to be hydrolyzed into sugars that could be employed as precursor of other added-value compounds or enzymes by microbial transformation (Mussatto, 2014). These facts underscore the potential of this byproduct, usually considered as a waste, to be used as a raw material in biorefinery processes.

Although thermal or chemical hydrolytic processes have been conventionally proposed to obtain sugars (pentoses and hexoses) from lignocellulosic biomass, Green Chemistry principles have urged the scientific community to invest more research efforts in the design of more sustainable strategies. In this scene, an ionic liquid (IL) pretreatment followed by an enzyme-catalyzed hydrolysis could be an appealing option. 1-ethyl-3-methylimidazolium acetate $[C_2C_1im][C_1COO]$ has already been considered the most effective IL for biomass pretreatment as it efficiently solubilize and alters the crystalline structure of cellulose and/or removes lignin, therefore increasing significantly the polysaccharides accessibility to enzymes and consequently improving the enzymatic hydrolysis (Chatel & Rogers, 2014; De Andrade Neto, De Souza Cabral, De Oliveira, Torres, & Morandim-Giannetti, 2016; Parveen, Patra, & Upadhyayula, 2016). Currently, this imidazolium family is the most important one in terms of sales, as the annual production of some of them exceeds the ton magnitude, and they were selected for improving existing industrial processes (e.g. BASIL, aluminum plating; Degussa, paint additives, Pionics, batteries) (Plechkova & Seddon, 2008). Nonetheless, although the negligible volatility of ILs is an asset to compete with conventional volatile solvents, their high stability and solubility in water could turn them into persistent pollutants if they are discharged/spilled on soils or aquatic environment (Deive et al., 2011). In fact, ILs consisting of imidazolium or pyridinium cations and halide-containing anions have already been demonstrated to be the families bearing more toxicity and environmental persistence (Petkovic, Seddon, Rebelo, & Silva Pereira, 2011), which together with their cost is still limiting their extensive application in different fields.

Accordingly, the production of non-toxic and environmentally friendly ILs from renewable materials is currently in the limelight (Liu,

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Hou, Li, & Zong, 2012), and their use for separation and environmental processes and biomass biorefining has been proposed (Álvarez et al., 2016; Dutta et al., 2017, 2018; Papa et al., 2017; Xavier et al., 2017; Xu, Sun, Xu, & Sun, 2013). In this context, our group has addressed the design of environmentally friendly cholinium-based ILs (N,N,N-trimethylhydroxyethyl ammonium, $[N_{1112OH}]^+$) (Deive et al., 2015), as the biodegradability, reasonable cost and chemical stability of this cation has already been ensured (Liu et al., 2012; Morandeira et al., 2017). Analogously, Ren, Zong, Wu, & Li (2016) pointed out the suitable role of these bio-ILs for pretreatment and fractionation of lignocellulosic biomass as they could efficiently dissolve lignin, while being poor solvents for microcrystalline cellulose and xylan.

The starting point of this work was the synthesis of a bio-IL derived from renewable and non-toxic natural material (choline hydroxide as the source for the cation; and one amino acid for the anion) through an economical and green route with water as the unique by-product. The data provided by Dutta et al. (2018) for other lignocellulosic materials (grass, hardwood and softwood) reveal the suitability of a polar aminoacid like lysine as anion, but the hypothesis that an apolar and cheaper aminoacid like glycine could be more suitable for delignification pretreatment is planned due to the apolar character of lignin. This IL was applied for the delignification of BSG at different temperatures and its efficiency as a hydrolytic promoter was compared with that achieved after a conventional imidazolium acetate-based pretreatment. The structural modifications imposed by the IL were analyzed in the light of ATR-FTIR spectra.

2. Materials and methods

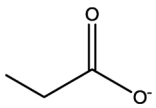
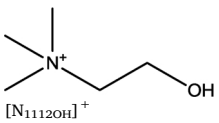
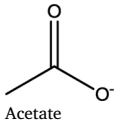
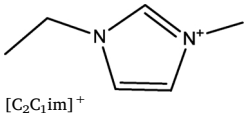
2.1. Materials

Brewery spent grain (BSG), with approximately 80% water content, was kindly provided by Letra (Vila Verde, Braga, Portugal). BSG humidity content was reduced in the laboratory in an oven (Celsius 2007, Memmert, Schwabach, Germany) at 50 °C for approximately 48 h to prevent microbial contamination during storage.

BSG was pretreated with cholinium glycinate $[N_{1112OH}][Gly]$. This IL was synthesized following the procedure reported by Deive et al. (2015) and its purity was checked by NMR data (> 95%) and its molecular structure is shown in Table 1a. 1-ethyl-3-methylimidazolium acetate $[C_2C_1im][C_1COO]$, which molecular structure is shown in Table 1b was purchased from Sigma-Aldrich (Steinheim, Germany) (> 95% purity). The selected ILs were vacuum-dried at reduced pressure and 50 °C for 3 days, and they were stored in amber glass vials with screw caps.

Commercial enzyme concentrates *Celluclast 1.5 L* and *Novozym 188*, with cellulase and *β*-glucosidase activities, respectively, were kindly provided by Novozymes, Denmark.

Table 1
Structure of the selected ILs.

	Anion	Cation
1a	 Glycinate	 $[N_{1112OH}]^+$
1b	 Acetate	 $[C_2C_1im]^+$

2.2. IL-assisted BSG fractionation

Ground samples of material (0.5 g) were treated in a 100 m L-glass bottle with 10 g IL (5% w/w) following the methodology reported by Ninomiya et al. (2015) with minor modifications. The mixture was placed into a sand bath, and heated on a hot plate VELP SCIENTIFICA (Usmate Velate, MB, Italy) with vigorous magnetic stirring, in the open atmosphere at 60, 90, 120 or 150 °C for 16 h. Afterwards, the mixture was diluted with 50 mL of acetone/water (1:1 v/v) and stirred for 30 min at room temperature, which resulted in the precipitation of carbohydrate-rich material (CRM), remaining in the liquid phase the lignin-rich material (LRM).

The suspension was centrifuged (Ortoalresa, Consul 21, EBA 20, Hettich Zentrifugen, Germany) at 2755 × g for 30 min, and residual CRM was separated from the supernatant by filtration using a nylon filter. The CRM was washed 4 times with 40 mL water to remove the IL and acetone; and centrifuged under the previously described conditions. The recovered CRM was dried in an oven (Oven Celsius 2007, Memmert, Schwabach, Germany) at 30 °C for 24 h, and gravimetrically measured (Denver Instruments, Bohemia, NY).

Acetone present in liquid phase was evaporated at room temperature causing the precipitation of LRM. After that, it was centrifuged, washed, dried and measured as previously described for CRM.

The streams containing the IL were mixed and vacuum-evaporated in a Büchi rotavapor R-215 (Frankfurt, Germany) at 50 °C, with variable pressure (from 100 to 40 mbar) to ease IL recovery and recycling.

2.3. Characterization of BSG and CRM

Previous to the characterization, BSG was washed with distilled water to avoid the interferences of free sugars from the brewery processes in the analyses. BSG was oven-dried (Binder-Model 53 ED, Tuttlingen, Germany) to constant weight at 105 °C in order to quantify the moisture percentage. Ash content was measured using a muffle furnace (Carbolite ELF 11/6B with controller 301, Derbyshire, United Kingdom) for 6 h at 575 °C. The composition of BSG was determined by quantitative acid hydrolysis (QAH) in two-stages (Pérez-Bibbins, Salgado, Torrado, Aguilar-Uscanga, & Domínguez, 2013). All parameters were performed in triplicate and standard deviations reported in the text.

The CRM was analyzed by QAH following the procedure described by Ninomiya et al. (2015) with slight modifications: 0.1 g of CRM were treated in a glass test tube with 2 mL sulfuric acid 72% (w/w) for 2 h at room temperature with regular stirring. Then, samples were diluted with 75 mL of water and autoclaved for 15 min at 121 °C. Finally, the two resulting fractions were analyzed as described above.

Glucose, xylose and arabinose were measured by HPLC (Agilent model 1200, Palo Alto, CA) equipped with a refractive index detector and an Aminex HPX-87H ion exclusion column (Bio Rad 300 mm × 7.8 mm, 9 m particles). Elution program with 0.003 M sulfuric acid was at a flow rate of 0.6 mL min^{-1} at 50 °C for 23 min.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis of the raw BSG and the CRMs obtained after pretreatment with $[N_{1112OH}][Gly]$ or $[C_2C_1im][C_1COO]$ with *Celluclast 1.5 L* and *Novozym 188* was assayed in order to evaluate the efficiency of the delignification. The cellulase activity and the *β*-glucosidase activity were ascertained by the filter paper activity test and by spectrophotometric measurements, respectively, following the methodology of Ghose (1987). The activity was expressed as Filter Paper Units per milliliter (FPU mL^{-1}) and International Units per milliliter (IU mL^{-1}), respectively.

Enzymatic hydrolysis was performed in 250 mL Erlenmeyer flasks using 0.1 M sodium citrate buffer (pH 4.85) with liquid-solid ratio 30 *g* g^{-1} at 48.5 °C and 150 rpm. The enzymatic cocktail tested was