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Characterization of agave bagasse as a function of ionic liquid pretreatment

Jose A. Perez-Pimienta [a,*], Monica G. Lopez-Ortega [a], Jose A. Chavez-Carvayar [b], Patanjali Varanasi [c,d], Vitalie Stavila [e], Gang Cheng [f], Seema Singh [c,d], Blake A. Simmons [c,d]

[a] Department of Chemical Engineering, Universidad Autónoma de Nayarit, Tepic, Mexico
[b] Instituto de Investigaciones en Materiales, UNAM, México D.F., Mexico
[c] Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Emeryville, CA, United States
[d] Sandia National Laboratories, Biological and Materials Science Center, Livermore, CA, United States
[e] Sandia National Laboratories, Energy Nanomaterials Department, Livermore, CA, United States
[f] College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, China

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ABSTRACT

Previous studies of agave bagasse (AGB-byproduct of tequila industry) presented unidentified crystalline peaks that are not typical from common biofuel feedstocks (e.g. sugarcane bagasse, switchgrass or corn stover) making it an important issue to be addressed for future biorefinery applications. Ionic liquid (IL) pretreatment of AGB was performed using 1-ethyl-3-methylimidazolium acetate ($[C_2mim][OAc]$) at 120, 140 and 160 °C for 3 h and a mass fraction of 3% in order to identify these peaks. Pretreated samples were analyzed by powder X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, field emission scanning electronic microscopy (FE-SEM), thermal analysis (TGA-DSC) and wet chemistry methods. Previous unidentified XRD peaks on AGB at $2\theta = 15°$, 24.5° and 30.5°, were found to correspond to calcium oxalate (CaC_2O_4) in a monohydrated form. IL pretreatment with $[C_2mim][OAc]$ was observed to remove CaC_2O_4 and decrease cellulose crystallinity. At 140 °C, IL pretreatment significantly enhances enzymatic kinetics and leads to ~8 times increase in sugar yield (6.66 kg m^{-3}) when compared to the untreated samples (960 g m^{-3}). These results indicate that IL pretreatment can effectively process lignocellulosic biomass with high levels of CaC_2O_4.

1. Introduction

Various physical and chemical pretreatment methods, including steam explosion, ammonium fiber expansion, dilute acid, lime, and organic solvent pretreatments, have been demonstrated to be capable of mitigating the biomass recalcitrance for subsequent enzymatic saccharification in order to obtain biofuels or value added products [1–3]. Most of these pretreatment methods are not selective and often produce

* *Corresponding author*. Tel.: +52 311 211 8821.
E-mail address: japerez@uan.edu.mx (J.A. Perez-Pimienta).
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undesirable by products (e.g. acetic acid or furfural) that can inhibit the downstream fermentative conversion of the monosaccharides into ethanol and other products [4]. In the last decade, numerous studies have focused on the dissolution of lignocellulose in a variety of certain ionic liquids (ILs) exhibiting a high solubility which have developed into a novel pretreatment process [5]. Currently, the costs of ILs production is high because they are produced on a relatively small scale; with vigorous research in the search of cheap and efficient ILs and increasing demand for ILs, the cost will drop in the near future. Most of the ILs used for biomass pretreatment are stable up to 300 °C with processing conditions within 80–180 °C and are relatively benign with minimal environmental impact in water and air pollution [6]. Microcrystalline cellulose can be readily solubilized in some ILs and recovered by the addition of an anti-solvent, such as water or ethanol. Crystallinity of the regenerated cellulose is lower than cellulose in the untreated biomass and is more susceptible to enzymatic hydrolysis [7].

Ionic liquids based on imidazolium cations (e.g., 1-butyl-3-methyl imidazolium chloride ([C_4mim]Cl) and 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) have been reported to solubilize a significant portion of lignocellulose at concentrations ranging from 5 to more than 15% (depending on temperature, nature of the IL, particle size and time) and enhance the saccharification kinetics of the recovered product [8]. Several studies have demonstrated that certain ILs can effectively solubilize lignocellulosic biomass such as switchgrass, poplar, pine, and corn stover [2], suggesting their potential for use in pretreating lignocellulosic feedstocks.

It was recently shown by Perez-Pimienta et al. [9] that [C_2mim][OAc] can effectively pretreat agave bagasse (AGB) by removing a significant amount of lignin (~45%) and generates a product that is highly amorphous. In the same report it was observed unidentified crystalline peaks of high intensity of x-ray diffraction in untreated AGB at $2\theta = 15°$, 24.5° and 30.5°, that are not reported in diffraction patterns of other biofuel feedstocks such as switchgrass or corn stover. After IL pretreatment with [C_2mim][OAc] the intensity of these unidentified peaks decreased. Since AGB is considered as a potential biofuel feedstock, it is necessary to characterize and analyze all its components, which may cause interference during pretreatment and subsequent downstream processes (enzymatic saccharification and fermentation).

In the present study a comprehensive characterization of AGB was conducted in order to determine the origin and role of the previously observed and unidentified crystalline components. *Agave tequilana Weber* is a plant that is cultivated in Mexico and used as a raw material in the production of the alcoholic beverage tequila. The AGB is a residual fiber obtained from the process and represents 40% of the harvested plant, with an annual generation in Mexico of about 112 kt in a wet basis, and at the moment just a small fraction is being used for soil composting or as plywood, while the rest is accumulated in landfills [10,11]. In this work, we sought to understand the physicochemical changes of AGB as a function of IL pretreatment with [C_2mim][OAc], with an emphasis on the elucidation of the unidentified crystalline peaks present in the biomass which has been reported by a number of research papers [9,12–15].

The experiments were designed to use a similar approach as in our previous report [9] thereby replicate the biomass behavior and obtain similar unidentified peaks using powder X-ray diffraction. Characterization of untreated and IL pretreated AGB was performed to determine glucan, xylan and lignin contents. Delignification and other chemical changes were tracked by Fourier Transform Infrared (FTIR) spectroscopy. X-ray diffraction (XRD) was used to compare the crystallinity of AGB before and after [C_2mim][OAc] pretreatment. Thermal properties was evaluated using thermogravimetric analysis and differential scanning calorimetry (TGA-DSC). Supramolecular structures of AGB were examined by field emission scanning electron microscopy (FE-SEM). Finally, the saccharification kinetics and overall sugar yields were determined.

2. Experimental section

2.1. Materials and sample preparation

Agave bagasse (AGB) was donated by Destilería Rubio, a Tequila facility from Jalisco, Mexico. This facility only used plants aged 7–8 years and the plant without the leaves is cooked about 18 h in an autoclave. After the cooking, the plant is milled and compressed to separate the syrup from wet bagasse. AGB samples were collected, washed thoroughly with distilled water and dried in a convection oven. The biomass was milled with a Thomas-Wiley Mini Mill fitted with a 400 μm screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA) and stored at 4 °C in a sealed plastic bag. Cellic® CTec2 (Cellulase complex for degradation of cellulose) and HTec2 (Endoxylanase with high specificity toward soluble hemicellulose) were a gift from Novozymes (Davis, CA). Ionic liquid, 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]), acetic acid, sodium acetate, sulfuric acid, 3,5-dinitrosalicylic acid (DNS), and sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO). Acetyl bromide and hydroxylamine hydrochloride were purchased from Alfa Aesar (Ward Hill, MA).

2.2. Ionic liquid pretreatment

A mass fraction of 3% of an AGB/IL mixture was prepared by combining 300 mg of milled AGB with 9.7 g [C_2mim][OAc] (used as received) in a 50 cm^3 autoclave vial. The vials and the contents were heated in an oven (Thelco Laboratory oven, Precision Instruments, VA) at 120, 140 and 160 °C for 3 h [2]. All experiments were conducted in triplicates. After 3 h of incubation, 30 cm^3 of deionized water was slowly added into the biomass[C_2mim][OAc]$^{-1}$ slurry to recover the pretreated AGB. A precipitate formed immediately, and the samples were centrifuged at 10,000 *g* for 10 min. The supernatant containing IL was removed, and the precipitate was washed five times with additions of water in order to ensure that excess IL had been removed. The washing process was continued until the concentration of IL in the supernatant, as measured by Fourier transform infrared (FTIR) spectroscopy using a previously established technique [9], was less than 0.2%.