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Research paper

Fractional pretreatment of raw and calcium oxalate-extracted agave bagasse using ionic liquid and alkaline hydrogen peroxide

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ABSTRACT

Occurrence of calcium oxalate (CaC_2O_4 – CaOX) crystals has been observed in more than 215 plant families. However, very little is known about the effects of calcium oxalate on biomass pretreatment and saccharification. Agave bagasse (*AGB*) was used as a model material due to its natural high levels of CaOX. To understand the physicochemical changes in function of biomass pretreatment, both raw *AGB* and CaOX-extracted agave bagasse (*EAB*) were subjected to ionic liquid (*IL*) with 1-Butyl-3-methylimidazolium chloride [C_4C_1Im][Cl] and alkaline hydrogen peroxide (*AHP*) pretreatments. Physicochemical changes were monitored by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and wet chemistry methods. Results show that free CaOX crystals affected negatively (by ca 39%) the saccharification of *AHP*-pretreated *EAB* compared to *AGB*. On the other hand, *IL* pretreatment achieved higher sugar yield (7.8 g dm^{-3}) and lower crystallinity (14%) with *EAB* than for *AHP* (5.4 g dm^{-3} and 29%, respectively).

1. Introduction

Lignocellulosic biomass is currently considered the most promising long-term feedstock for biofuels production, however, it is highly recalcitrant to breakdown and offers limited accessibility to enzymatic degradation of cell wall sugars and subsequent fermentation [1]. Pretreatment by deconstructing the biomass is crucial, but is still a quite costly process that liberates fermentable sugars from biomass. A suitable pretreatment process involves: (1) disrupting hydrogen bonds in crystalline cellulose, (2) breaking down cross-linked matrix of hemicelluloses and lignin, (3) raising the porosity surface area of cellulose, and finally, (4) avoiding the formation of byproducts that are inhibitory to subsequent processes [2,3].

Several pretreatment technologies are employed to overcome lignocellulose recalcitrance and can offer high selectivity in deconstructing biomass to desired end products by partially breakdown the plant cell wall to improve enzymatic accessibility. A number of processes are currently available to pretreat lignocellulosic biomass; some key examples use liquid catalysts such as acids (H_2SO_4 or HCl), ammonia, bases (such as NaOH or H_2O_2), ionic liquids (such as 1-Butyl-3-methylimidazolium chloride [C_4C_1Im][Cl] or 1-ethyl-3-methylimidazolium acetate [C_2C_1Im][OAc]) or simply water. It has been a considerable challenge to clarify the physicochemical effects of the diverse types of pretreatments upon subsequent hydrolysis and fermentation [4].

Lignocellulosic feedstock such as Agave plants growing in arid and semi-arid lands could be a sustainable response to the growing demand for renewable fuels that do not conflict with food production. Such plants use Crassulacean Acid Metabolism (CAM) and therefore have low water requirements and are productive in semiarid regions. Therefore as carbon is assimilated overnight thereby decreasing the diffusive gradient of water out of the leaves and improve water use efficiency [5,6]. Due this efficient use of resources, CAM plants have recently been introduced as potential bioenergy crops [7]. The high soluble carbohydrate reserves that

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CAM plants contain, requires less energy for conversion to fuels hence resulting in a better quality material. With regard to future climate change, species of agave may have an advantage over other bioenergy crops (such as sugarcane or corn stover) because CAM physiology adapts to extreme temperatures and drought. Interestingly, the areas in the world that have been identified as most suitable for Agave plantations as feedstock (Mexico, Australia, and South Africa) are also areas where the variation in the temperature is relatively low [8]. Another important attribute is the high estimated average annual productivities for Agave species of 10–34 Mg ha^{-1} $year^{-1}$ in comparison to switchgrass (15 Mg ha^{-1} $year^{-1}$) and poplar wood (11 Mg ha^{-1} $year^{-1}$) [9].

In plants, calcium oxalate deposition is common. Members of more than 215 plant families accumulate crystals within their tissues [10]. Oxalate-producing plants, which include many crop plants, accumulate oxalate in a large mass fraction range (3–80% of their dry weight) [11–14], where as much as 90% of the total calcium of a plant can be found as an oxalate salt [10]. It has been reported that CAM species such as Agave bagasse have higher concentration of calcium oxalate (CaC_2O_4 – CaOX) than most of the current biofuel feedstocks [15] such as grasses (switchgrass), agricultural (sugarcane bagasse) or forestry residues (pine wood) [14,16,17]. Furthermore, these feedstocks does contain realty low to non-measurable elemental calcium content [18–21]. Meanwhile, it has been reported from different Agave species (*americana*, *atrovirens*, *deserti*, *fourcroydes*, *lechugilla*, *salmiana*, *tequilana*, and *utahensis*) ranging from 1.4 to 6.1% of calcium concentration [14,22,23].

Calcium oxalate functions in plants include calcium regulation, plant protection, detoxification (e.g. heavy metals or oxalic acid), ionic equilibrium and tissue support/plant stiffness, even light gathering and its reflection [24]. Another interesting property of calcium oxalate is its exothermic reaction or incompatibility with strong oxidizers such as hydrogen peroxide or ozone, both widely used as solvents or chemicals for biomass pretreatment [24–26]. The presence of high levels of calcium oxalate in agave bagasse could have an effect (positive or negative) on pretreatment performance; hence, this is clearly an important issue to be addressed for future biorefinery applications.

The main objective of this study is to apply raw agave bagasse (*AGB*) as a model (due to its natural high level of CaOX) and CaOX-extracted agave bagasse (*EAB*) to understand the physicochemical changes with both samples in function of biomass pretreatment. An oxidative process, alkaline hydrogen peroxide (*AHP*) and ionic liquid (IL) pretreatments were employed. Lignin removal, crystallinity index using X-ray diffraction (XRD) and chemical fingerprint tracked by Fourier transform infrared spectroscopy (FTIR) were used as response variables, besides, CaOX crystals distribution with scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) were carried out. Finally, we conclude with the comparison of sugar yield kinetics of untreated and pretreated biomass.

2. Experimental section

2.1. Experimental design

A 2^4 factorial design with 3 replicates plus controls was used to determine the effect of calcium oxalate on saccharification yields. Four different pretreatment conditions were used namely: *AHP* using two hydrogen peroxide concentrations (*AHP*-A = 125 g kg^{-1} of biomass and *AHP*-B = 500 g kg^{-1} of biomass) and *IL* using two temperatures (*IL*-120 °C and *IL*-160 °C). Two different biomass materials with different calcium oxalate content in agave bagasse samples were applied. On the one hand, named *AGB* (raw agave bagasse without any manipulation that have natural high calcium oxalate concentration) and *EAB* (agave bagasse that was subjected to an extraction process that removed the calcium oxalate). Untreated *AGB* and *EAB* were used as controls. The response variables tested were lignin removal, crystallinity and sugar production.

2.2. Materials and preparation

Agave bagasse was donated by Destilería Rubio, a Tequila plant from Western Mexico in the state of Jalisco. This facility has a year-round process with a real possibility for continues use (6–15 Mg day^{-1}). The central fruit (stem or "piña") was received from defoliated agave plants aged 7–8 years (20°52 46.374 N; 103°49 8.138 O, altitude: 1180 m above sea level, annual rainfall mean 1073 mm; semi-arid climate), located near Tequila, Jalisco. The stems were cooked for 18 h in an autoclave, then milled and compressed to separate the syrup from wet bagasse. Samples of the wet bagasse were collected, washed thoroughly with distilled water and dried in a convection oven at 40 °C. The biomass was milled in a Thomas-Wiley Mini Mill fitted with a 400 μm screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA). The ground biomass was stored at 4 °C in a sealed plastic bag prior to their use. Cellulases from *Trichoderma reesei* (Celluclast 1.5L with 97 FPU cm^{-3}), β-glucosidase from *Aspergillus niger* (Novozyme 188 with 320 CBU cm^{-3}), 1-Butyl-3-methylimidazolium chloride [C_4C_1Im][Cl], hydrogen peroxide, hydrochloric acid, sulfuric acid, 3,5-dinitrosalicylic acid (DNS), and sodium hydroxide were purchased from Sigma–Aldrich (Mexico). Catalase was purchased from Merck (Mexico).

2.3. Analytical methods and procedures

2.3.1. Calcium oxalate extraction

In order to obtain the extracted agave bagasse (*EAB*) a total calcium oxalate extraction was performed using 1 g of *AGB* into a 250 cm^3 Erlenmeyer flasks and 50 cm^3 of 2 mol dm^{-3} HCl. The flasks were placed in a shaking water bath at 80 °C for 30 min. The extracts where further diluted with 50 cm^3 of deionized water and then transferred into 15 cm^3 centrifuge tubes and centrifuged at 10600× *g* for 10 min [27]. The supernatants were filtered through Whatman #1 filter paper, washed with deionized water and the recovered biomass was vacuum oven dried at 45 °C for 48 h before compositional analysis.

2.3.2. Pretreatment processes

2.3.2.1. IL pretreatment. The ionic liquid [C_4C_1Im][Cl] was purchased from Sigma–Aldrich (≥95% pure), and used without further purification or drying. Pretreatment initiate by mixing 0.3 g of milled biomass with 9.7 g of [C_4C_1Im][Cl] in a 25 cm^3 autoclavable vial using *AGB* and *EAB*. The vials and their contents were heated in an oven (Binder KBF Laboratory oven) at 120 °C and 160 °C for 3 h [28]. All experiments were conducted in triplicates. After 3 h of incubation, 40 cm^3 of deionized water was slowly added into the biomass [C_4C_1Im][Cl] slurry to recover the pretreated biomass. A precipitate immediately formed, and the samples were centrifuged at 10600× *g* for 20–25 min. The supernatant containing *IL* was removed, and the precipitate was washed with water to remove any *IL* excess. The washing process was continued until the concentration of *IL* in the supernatant as measured by Fourier transform infrared (FTIR) spectroscopy was less than 0.2%. The recovered product was vacuum dried at 45 °C for 48 h before compositional analysis.

2.3.2.2. AHP pretreatment. A solution of hydrogen peroxide (H_2O_2) diluted from a commercial 30% stock (Sigma–Aldrich ACS Reagent Grade) was adjusted to pH 11.5 ± 0.2 with 5 mol dm^{-3} NaOH and