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Structural and compositional changes in sugarcane bagasse subjected to hydrothermal and organosolv pretreatments and their impacts on enzymatic hydrolysis

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ABSTRACT

Economical sustainability of cellulosic ethanol technology still requires considerable improvements in efficacies of both pretreatment and enzymatic hydrolysis steps. In this work a number of physical techniques were applied to characterize sugarcane bagasse samples that underwent hydrothermal and/or organosolv pretreatments under variable conditions and to correlated the observed changes with the efficiency of enzymatic hydrolysis. Confocal and field emission scanning electron microscopy studies revealed morphological changes in lignin distribution in the plant cell wall. The hydrothermal pretreatment caused a disorder in the arrangement of the lignin, whereas organosolv pretreatment partially removed lignin from bagasse and fraction of it redeposited at the surfaces of cellulose fibers. The delignification process was also analyzed by both chemical composition analysis and nuclear magnetic resonance. Pretreatment conditions leading to a significant increase of the efficiency of enzymatic hydrolysis were identified. Our studies open avenues for further biophysical investigations of pretreated lignocellulosic biomass, which could lead to its improved enzymatic hydrolysis.

1. Introduction

Dwindling reserves of fossil fuels and growing concerns about greenhouse gas emissions and environmental impacts are increasingly encouraging the use of renewable resources and the development of alternative feedstocks for biofuel production worldwide. In Brazil, bioethanol production is still largely based on sugarcane juice fermentation. However, sugarcane bagasse, which is the residue from the milling process, is an energy-rich structure, containing cellulose, hemicelluloses and lignin (Soccol et al., 2010). The use of agricultural residues from the first-generation ethanol production, such as sugarcane bagasse and leaves, can contribute to the complete utilization of the raw material in the integrated biorefineries (Dias et al., 2012). Processing of lignocellulosic feedstock to ethanol usually involves four major unit operations: pretreatment, hydrolysis, fermentation, and distillation (Taherzadeh and Karimi, 2007), but these processes are still not fully efficient and should be optimized.

The sugarcane bagasse is mainly composed of tridimensional structural networks of cellulose intertwined by hemicelluloses and lignin (Rezende et al., 2011). Lignin is a phenolic, branched, and hydrophobic structure, highly resistant to degradation, that unproductively adsorbs enzymes and hinders cellulose accessibility during enzymatic hydrolysis of biomass (Achyuthan et al., 2010; Zhang and Lynd, 2004). Therefore, lignin content and distribution are recognized as important factors determining cell wall recalcitrance to enzymatic depolymerization (Paul, 2014). Because of this, different pretreatments are normally applied prior to enzymatic hydrolysis step in order to unstructure the cell walls and to partially remove hemicellulose and lignin. Pretreatments provide fractionation of lignocellulosic biomass, thus decreasing its recalcitrance and resulting in better yields of monomeric fermentable sugars released by the enzymatic hydrolysis step (Himmel et al., 2007; Kumar et al., 2009; Taherzadeh and Karimi, 2007). Currently, enzymatic hydrolysis is the most widely used method for bioethanol production, since it is a specific and environmentally

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friendly process, that can be run at low temperatures and does not produce by-products that may inhibit the subsequent fermentation step (Himmel et al., 2007; Sheehan and Himmel, 1999; Wingren et al., 2005). On the other hand, enzymes are expensive and contribute to the relatively high costs of the second-generation ethanol.

A choice of a particular pretreatment is important, because different pretreatments have different mechanisms of action that will affect the cell wall structure, the chemical species to be released and the inhibitory co-products to be generated (Himmel et al., 2007; Kumar et al., 2009; Soccol et al., 2010). Organosolv pretreatment efficiently removes lignin from the lignocellulosic materials through the partial hydrolysis of lignin bonds. It decreases the lignin content in the cell wall by breaking α-aryl ether and arylglycerol-β-aryl ether (β−O−4) bonds of lignin macromolecule (Nakagame et al., 2011; Sarkanen et al., 1981), imparting significant changes in the lignin structure, including increases in phenolic groups, and decreases the average molecular weight of the lignin (Gilarranz et al., 2000). This pretreatment could be more expensive than some others pretreatment processes, but can provide lignin-derived value-added products that might contribute to the economical viability of organosolv pretreatment in the context of the integrated biorefineries. Hydrothermal treatment has lower costs and reduced environmental impacts (Brodeur et al., 2011; Ma et al., 2014). This pretreatment only utilizes water at high temperatures, which mainly results in hemicelluloses solubilization and structural modifications of the biomass that lead to enhanced enzymatic hydrolysis. Hydrothermal pretreatment temperatures (typically ranging from 160 °C to 240 °C) and the biomass residence time will determine the types and the amount of sugars being released from the biomass (Yu et al., 2010). Hydrothermal pretreatment relies on autohydrolysis, which makes use of acetic acid liberated from hemicellulose's acetyl groups to catalyze the breakdown of polysaccharides into shorter chain oligosaccharides and simple sugars (Roos et al., 2009; Tunc and Van Heiningen, 2011). The hydrothermal pretreatment of lignocellulosic materials involves mostly solubilization of hemicelluloses, and also extractives, sugars and small fragments of lignin (Vázquez et al., 2005; Xing et al., 2011).

In this study the effects of two different pretreatments (organosolv and hydrothermal), applied independently and/or sequentially, to the sugarcane bagasse samples were analysed, aiming to find conditions and pretreatment combinations that lead to more efficient enzymatic hydrolysis. A comprehensive set of physical techniques combined with chemical composition analyses was applied to reveal the chemical and structural changes induced by the pretreatments in the sugarcane bagasse and their impacts on the efficiency of enzymatic hydrolysis. Insights into untreated and preteated biomass structure and composition, combined with the experimentally measured enzymatic hydrolysis yields, provide a unique opportunity for better comprehension of the impact of pretreatment-induced changes taking place in sugarcane bagasse samples on efficiencies of their enzymatic hydrolysis.

2. Material and methods

2.1. Material

Sugarcane bagasse from the last milling step for juice extraction was provided by the Cosan Group (Usina da Serra/Ibaté, São Paulo, Brazil). This material was milled using knife mill and rinsed with hot water (50 °C). Next, bagasse was dried in the oven at 60 °C for 24 h. Prior to each experiment, the moisture content was measured using an analytical balance (Shimadzu; Kyoto, Japan). All the analyses and measurements described in this work were performed with the same batch of samples.

2.2. Bagasse pretreatments

Sugarcane bagasse was pretreated using both hydrothermal and organosolv pretreatments. These pretreatments were applied separately and/or combined under different conditions. Hydrothermal pretreatment was applied using hot water as a reaction media in four distinct reaction times: 1 min (Hyd_1′), 30 min (Hyd_30′), 45 min (Hyd_45′) and 60 min (Hyd_60′) at 160 °C in a reactor (AU/E-20 model, Regmed). The pressure was kept at 7 bar and a 1:10 solid to liquid ratio (grams of bagasse/mL of water) was used. According to previous works, the temperature of 160 °C is close to the optimal for the sugarcane bagasse pretreatment (Yu et al., 2013), whereas at higher temperatures and more severe conditions, xylose and glucose degradation products were produced at significantly highier levels. Here, four distinct reaction times were applied to explore this particular temperature condition of the hydrothermal methodology.

In the case of organosolv pretreatments, previous studies also showed that they represent a potential method for delignification of plant biomass that simultaneously preserves the cellulose fraction (Novo et al., 2011). Novo and collaborators showed that one of the best pretreatment conditions for lignin removal by the organosolv method could be achieved at 190 °C. Furthermore, different reaction times have been applied and 150 min was found to be an efficient condition (Novo et al., 2011). In the present work, the study of shorter times were included to verify if they could be enough to introduce significant changes in the sugarcane bagasse structure. Thus the organosolv treatement was carried out in ethanol/water solutions (50% v/v) heated at 190 °C in a glycerin bath for three different reaction times: 50 min (Org_50′); 100 min (Org_100′) and 150 min (Org_150′).

Finally, the combined action of both hydrothermal and organolsolv pretreatments applied in sequence to increase the efficiency of enzymatic hydrolysis was also evaluated. Combined treatments consisted of a first hydrothermal step for 30 min or 60 min at 160 °C, followed by an organosolv step at 190 °C (Hyd_30′ + Org_50′; Hyd_30′ + Org_100′; Hyd_30′ + Org_150′; Hyd_60′ + Org_50′; Hyd_60′ + Org_100′; Hyd_60′ + Org_150′). After each pretreatment step the liquors were separated from the solid fractions by filtration, these were firstly rinsed with ethanol and then with tap water until reaching a neutral pH. The solids were dried in oven for 24 h at 60 °C and stored for characterization of its chemical composition and physical structure.

2.3. Chemical composition determination

The chemical composition for untreated and pretreated sugarcane bagasse solids was obtained following the protocol established by Rocha and coworkers (2011), with some modifications (Rezende et al., 2011). Initially, the solid samples were grounded using a knife mill, prior to passing through a 20-mesh sieve and had their dry matter weight determined. Extractives were removed from the untreated bagasse, using a 1:1 cyclohexane/ethanol solvent mixture under reflux in a Soxhlet apparatus for *ca.* 8 h, followed by water extraction for 5 cycles with 8 h each. Fiber extraction with cyclohexane-ethanol allows removal of waxes, lipids and tannins from the fiber surface and therefore has been applied (Park et al., 2008; Vallejos et al., 2012). After extraction, samples were dried and the content of extractives was determined.

Solid biomass samples (2 g) were hydrolyzed using 15 mL of a 72% H2SO4 solution at 45 °C, under constant stirring for 7 min. Then, 275 mL of distilled water were added to this mixture and the material was kept at 120 °C for 30 min. The remaining solids were separated from the reaction liquors by filtration in filter paper. The solid fraction was rinsed until a neutral pH was reached and then oven dried at 105 °C to a constant weight. This fraction corresponded to the biomass insoluble lignin and ashes. By calcination of this solid in a muffle furnace at 800 °C for 2 h, the ash (remaining inorganic fraction) and the lignin (calcinated fraction) amounts were determined. The acid soluble lignin was determined in the reaction liquor by UV-VIS, as previously described (Rocha et al., 2011). The composition of hydrolysate liquors including soluble sugars, organic acids and hydroxymethylfurfural was