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Short Communication

Influence of ozonolysis time during sugarcane pretreatment: Effects on the fiber and enzymatic saccharification

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HIGHLIGHTS

- Secondary cell wall is the structure most affected by ozonolysis treatment.
- High yield conversion of cellulose using OBU treatment.
- Fiber changes during increase in ozonolysis treatment time were observed.
- SEM and TEM techniques demonstrated changes in fiber cells after ozonolysis treatment.

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ABSTRACT

Modifications in sugarcane bagasse (SCB) from ozonolysis (O) NaOH (B) and ultrasound (U) (OBU) treatment for cellulosic ethanol production by enzymatic hydrolysis, were evaluated when increasing the exposure time of SCB to ozone. The lignin, cellulose, and hemicellulose after treatment were quantified: lignin removal and a consequent increase in cellulose content were shown using an infrared spectroscopic technique (ATR-FTIR) and chemical characterization. X-ray diffraction analysis (XRD) proved that OBU treatment does not affect the crystalline cellulose portion and electron microscopy techniques established that the fiber region most affected by the OBU treatment was the secondary cell wall, where the greatest lignin content is located. For OBU-60 treatment the lignin content was reduced and consequently there was a significant increase in cellulose content. After enzymatic hydrolysis, this pretreated SCB released 418 mg glucose/g, corresponding to six times more than untreated SCB and a yield of 93% of the cellulose available.

1. Introduction

The search for renewable energy has grown in recent decades, as a strategy to replace fossil fuels and for green energy policies. The lignocellulosic biomass has great potential for power production because it has low cost, is renewable, it is readily available and has high fermentable sugars content for ethanol production and is the main material used in a biorefinery (Travaini et al., 2016a). In the 2015/16 season, for example, Brazilian ethanol production reached 30.2 billion liters (UNICA, 2016) and, in 2015, the sugar plants injected, into the interconnected system, 5% more electrical energy than in 2014 (EPE, 2016). Biomass from sugarcane has an advantage over other lignocellulosic materials because it does not compete directly with food crops or require more field space. This is because, even after the use of sugarcane bagasse as an energy source in the co-generation of electricity, there is a considerable excess of residual biomass in the mills, as well as an abundance of straw deposited in the fields. This can be converted into ethanol by enzymatic hydrolysis and the subsequent fermentation of its polymerized sugars.

The fiber of sugarcane bagasse structure contains approximately 50% cellulose, 25% hemicellulose and 25% lignin (Travaini et al., 2014). However, the lignocellulosic material is highly recalcitrant, thus hindering the access of hydrolytic enzymes (Pandey et al., 2000). The cellulose present in vegetable fiber is divided into: (i) a crystalline portion formed from cellulose chains connected by a hydrogen bond in an organized way, and (ii) an amorphous

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portion of cellulose, less organized, more soluble and more easily degradable. To overcome lignocellulosic recalcitrance, different pretreatments of biomass are being investigated, such as steam explosion (STEX) (Scholl et al., 2015), liquid hot water (LHW) (Zhuang et al., 2016) and microwaves (Binod et al., 2012). This work has a particular focus on unpressurized processes, and among the possibilities in this area, ozonolysis is a promising alternative.

The ozone is a powerful electrophile due to an electron deficiency in one of the terminal oxygens during resonance. Thus, an ozone reaction with the lignocellulosic substrate mainly affects compounds with high electron density, such as double C—C bonds and aromatic rings, making the ozone attack the lignin structure in preference to the carbohydrates (Travaini et al., 2016a). The degradation of lignin and the consequent breakdown of the lignocellulosic structure increases the efficiency of the enzymatic hydrolysis procedure, making cellulosic ethanol production possible.

The alkali treatment was reported to be effective in the removal of hemicellulose and lignin fragments (Chaudhary et al., 2012) while treatment with ultrasound in an aqueous medium produces a phenomenon known as acoustic cavitation that can disrupt cell walls and facilitate the penetration of solvents in the cellulosic materials improving the alkaline effect in the treatment (Velmurugan and Muthukumar, 2012). The breakdown of lignin caused by ozonolysis, added to the effects of washing by alkaline assisted by ultrasound results in an efficient treatment.

Some studies using ozone as a biomass pretreatment obtained 80% (Li et al., 2015) and 84% (Travaini et al., 2016b), cellulose conversion to glucose after the enzymatic hydrolysis of the pretreated material. The combination of ozonolysis (O) and subsequent washing with NaOH (B) in ultrasound (U), produced excellent results with glucose conversion yields of 94% of the cellulose available, in previous work (Perrone et al., 2016). Ozonolysis in biomass treatment has been widely reported and the use of this oxidant has produced good results due to low formation of inhibitor compounds and also high glucose yields obtained during the enzymatic hydrolysis. However, there are no studies that demonstrate the effect of increased content of ozone during the treatment on the fiber. In this study, the modifications caused in the lignocellulosic material fiber due to an increasing ozonolysis time during sugarcane pretreatment were evaluated. The impact of different treatments on the physical structure of the sugarcane bagasse was evaluated by chemical compositional analysis. The X-ray diffraction technique (XRD) was used to verify changes in crystallinity. Infrared spectroscopy (ATR-FTIR) was used for the analysis of chemical changes in the functional groups from lignin, cellulose and hemicellulose, and microstructural analysis to understand the physical variations in the material morphology after treatments was done using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The yield of the enzymatic hydrolysis was also evaluated using high-performance anion-exchange chromatography (HPAEC-PAD).

2. Material and methods

2.1. Sugarcane bagasse and pretreatment

Sugarcane bagasse (SCB) was collected from Usina Vale, in Onda Verde, São Paulo State, Brazil (2013/2014 harvest). The SCB was washed three times with hot distilled water at 40 °C to remove any soluble sugar. The SCB was ground and sieved to obtain fibers less than 3 mm long. These fibers were dried in a fan oven at 40 °C for 24 h. Ozone gas was obtained using the Radast 10C generator, (Ozoxi-ozone, São Paulo State, Brazil) and the ozone flow was 32 mg O_3 min^{-1}, the same way as in the previous study (Perrone et al., 2016). In each test, 20 g of bagasse was pretreated in ozonolysis with exposure times of 5, 10, 20, 40, 60, 90, 120 and 180 min. After the process of ozonolysis, 2 g of this material were transferred to flasks (250 mL) containing 40 mL of NaOH 0.1 mol L^{-1} (B) and left to stand for 2 h. After that, the material was treated with ultrasound irradiation (U) for 5 min. using an ultrasonic probe (Fisher Scientific, Model 50 Sonic Dismembrator), operating at a frequency of 22 kWh and power of 50 W.

2.2. Physical analysis

ATR-FTIR spectra were collected using a Perkin-Elmer FTIR Spectrum Two. For ATR-FTIR analysis, approximately 0.1 g of the dried SCB samples were compressed to form discs (13 mm diameter and 1 mm thickness). The disc samples were pressed against the ATR interface with the same pressure for each sample. The air was used as background and all spectra were recorded in an average of 4 scans at a resolution of 4.0 cm^{-1}. Due to the heterogeneity of the SCB, the disc position was changed so that the infrared light could be pointed at four different places. XRD patterns of SCB were obtained on a Rigaku® diffractometer model MiniFlex 300 using CuKα radiation (λ = 1.54 Å) in an angular range of 5–40° at 2θ min^{-1} at 25 °C. The crystallinity index (Icr) was calculated following the method proposed by Seagal (Terinte et al., 2011). Structural carbohydrates, cellulose, hemicellulose and lignin fractions and ash of the bagasse were determined using the National Renewable Energy Laboratory–USA (NREL) analytical procedures NREL/TP 510-42618 (Sluiter et al., 2008).

2.3. Scanning electron microscopy (SEM)

The bagasse was fixed in 2.5% glutaraldehyde in a 0.1 mol L^{-1} phosphate buffer (pH 7.3) for 48 h at room temperature. Next, the material was washed with distilled water and post-fixed in 1% osmium tetroxide diluted in distilled water for 30 min at room temperature. Following fixation, the bagasse was dehydrated in a series of ethanol washes, critical point-dried with CO_2, and sputter coated with gold (Bal-Tec SCD 050). The samples were examined using an FEI Quanta 200 scanning electron microscope (FEI Company, Eindhoven, Netherlands) with an accelerating voltage of 12.5 kV.

2.4. Transmission electron microscopy (TEM)

The bagasse was fixed in a 2.5% glutaraldehyde and 4% paraformaldehyde solution in a 0.1 mol L^{-1} phosphate buffer (pH 7.3) for 24 h at room temperature and post-fixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature. After washing with distilled water, the material was block stained in 0.5% uranyl acetate for 2 h at room temperature. Next, the samples were dehydrated in a graded acetone series and embedded in Araldite® resin. Ultrathin sections were cut with a Leica ultramicrotome (EM UC7; Leica Microsystems, Wetzlar, Germany) and stained with uranyl acetate and lead citrate. The analyses were performed using a Tecnai Spirit transmission electron microscope (FEI Company, Eindhoven, The Netherlands). SEM and TEM analysis were performed at the Electron Microscopy Center of the Biosciences Institute (UNESP, Botucatu-SP, Brazil).

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis of 0.25 g of both dried untreated and pretreated bagasse was carried out in glass flasks with rubber stoppers with 10 mL of acetate buffer 0.1 mol L^{-1}, pH 5.0, containing cellulase (Prozyn®, São Paulo, Brazil) at a dosage of 32 FPU per gram of bagasse (dry basis). An enzyme reaction was performed at 60 °C,

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