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Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation

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HIGHLIGHTS

- Ozonolysis in fixed bed is an efficient sugarcane bagasse pretreatment.
- Sample moisture and ozone concentration have a major impact on ozonolysis.
- Ozonolysis causes low carbohydrate degradation and few inhibitory compounds.
- Water washing is an effective detoxification alternative for bagasse ozonolysis.
- Electronic microscopy cleared morphological changes in bagasse.

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ABSTRACT

Sugarcane bagasse was pretreated with ozone to increase lignocellulosic material digestibility. Bagasse was ozonated in a fixed bed reactor at room temperature, and the effect of the two major parameters, ozone concentration and sample moisture, was studied. Acid insoluble and total lignin decreased whereas acid soluble lignin increased in all experiments. Pretreatment barely attacked carbohydrates, with cellulose and xylan recovery rates being >92%. Ozonolysis increased fermentable carbohydrate release considerably during enzymatic hydrolysis. Glucose and xylose yields increased from 6.64% and 2.05%, for raw bagasse, to 41.79% and 52.44% under the best experimental conditions. Only xylitol, lactic, formic and acetic acid degradation compounds were found, with neither furfural nor HMF (5-hydroxymethylfurfural) being detected. Washing detoxification provided inhibitor removal percentages above 85%, increasing glucose hydrolysis, but decreasing xylose yield by xylan solubilization. SEM analysis showed structural changes after ozonization and washing.

1. Introduction

Due to the economic, political, and environmental problems related to the use of fossil fuels, in recent years the search for and use of new fuel sources has been a recurrent theme at an international scale. According to *International Energy Agency* data (OECD, 2011), total final energy consumption increased from 4676 Mtoe in 1973 to 8353 Mtoe in 2009, a rise of more than 78% in a little over three decades. Moreover, in 2009, 41.3%, 15.2%, and 10.0% of this amount came directly from oil, gas and coal/peat respectively. Because of the problems related to the use of fossil fuels, action has been taken worldwide at an international, national and local scale to replace fossil fuels in an effort to cut these figures.

Among the many new energy production alternatives, the use of renewable sources proves the most promising. Due to their cost, abundance and environmental friendly nature, the production of second generation biofuels from lignocellulosic materials, such as agricultural and forest waste, provides an excellent substitute. In Brazil, which has the world's largest commercial biomass exploration program, *PROÁLCOOL* (*National Alcohol Program* – for sugarcane ethanol production), waste from the sugarcane industry is abundant. In the 2010 agricultural year, Brazil processed 719.1 million tons of sugarcane, 625 million tons for sugar and first generation ethanol, and the remainder for *cachaça* (white rum) and *rapadura* (a traditional Brazilian sweet), producing some 201

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million tons of sugarcane bagasse (Hofsetz and Silva, 2012). This bagasse is sometimes used for generating electricity by combustion or as animal feedstock. Yet, in certain cases it remains stockpiled in the field or in factories, and its degradation could pose an environmental problem (Cardona et al., 2010).

The use of lignocellulosic biomass to produce second generation biofuels requires at least three main steps: (1) pretreatment, to open the biomass lignocellulosic structure, releasing sugar polymers from their ligations to lignin, (2) enzymatic hydrolysis, to break sugar polymers down into their monomeric fermentable units, and (3) fermentation of hydrolysates to ethanol or other biorefinery products (FitzPatrick et al., 2010; Hendriks and Zeeman, 2009; Mosier et al., 2005).

The key factor in pretreatment is chemical and sub-microscopic transformation of bagasse, releasing cellulose and hemicelullose from lignin, opening pores, and thereby improving subsequent hydrolysis, whilst generating the smallest possible amount of anti-fermentative compounds (Martín et al., 2007b). Many types of pretreatment have been proposed, including physical, chemical, physical–chemical, biological, and sometimes a combination thereof (Carrasco et al., 2010; Gámez et al., 2006; Kaar et al., 1998; Martín et al., 2007a; Monte et al., 2011; Mosier et al., 2005).

In this work, chemical pretreatment of sugarcane bagasse, ozonization, was proposed for increasing enzymatic hydrolysis yields. Differences in the electronic characteristics between lignin and carbohydrates, mainly the double bonds and electric donor centers present in lignin, make ozonization a selective process, since ozone reacts 10^6 times faster with lignin than with carbohydrates (Maia and Colodette, 2003). Ozone attacks the double bonds, releasing soluble compounds of lower molecular weight, such as formic and acetic organic acids, changing the material pH from 6.5 to 2. Although organic acids can be formed, the scant generation of the inhibitors furfural and HMF is the most appealing feature of this pretreatment (Contreras Iglesias, 2003). Most references to ozone pretreatment are related to pulp paper industry bleaching experiments. However, the use of ozone as a lignocellulosic pretreatment to release fermentable sugars remains scarce.

Vidal and Molinier (1988) reported an increase from 0% to 57% in enzymatic hydrolysis yield of poplar sawdust after ozonolysis. Kojima and Yoon (2008) studied ozonolysis of newsprint and magazine pulps, and reported a significant decrease in lignin as well as improved enzymatic hydrolysis from 37% to 58%. Garcia-Cubero et al. (2009) increased enzymatic hydrolysis after ozonolysis of wheat and rye straw, with no furfural and HMF being detected. Working with wood pulp ozonolysis, Yu et al. (2011) reduced the total lignin content with loblolly pine and mixed southern hardwood pulps, with a maximum carbohydrate conversion in the latter of around 80%. Kaur et al. (2012) achieved a reduction of over 42% in cotton stalk lignin content using ozone pretreatment.

Hydrolysis of cellulose by cellulases acts synergistically with xylan hydrolysis, the most abundant hemicellulose sugar polymer, through xylanase enzymes. The action of the two groups is important vis-à-vis the overall efficiency of the saccharification process, and also because xylose, a product of hydrolysis, is an important biorefinery sugar (Buaban et al., 2010; FitzPatrick et al., 2010).

The aim of the current work was to evaluate ozonolysis as a pretreatment to enhance enzymatic hydrolysis of sugarcane bagasse. Two different operational parameters, bagasse moisture and ozone concentration, were evaluated working in a biomass fixed bed reactor. The impact of these parameters on ozone consumption, lignin and carbohydrate composition of pretreated bagasse, and enzymatic hydrolysis yield, was studied. The formation of acids and inhibitors was also analyzed, and a simple washing detoxification process was proposed to gauge their impact on sugar release yields. Electronic microscopy was used for qualitative evaluation of ozone attack on bagasse.

2. Methods

2.1. Materials

Sugarcane bagasse (surplus after milling) was donated by Usina Vale, city of Onda Verde, São Paulo State, Brazil. It was washed for particulate material removal, dried in a ventilated oven at 42 °C and ground in an agricultural crusher to a size of between 3 and 5 mm. The chemical composition of the sugarcane bagasse used in this study, analyzed following NREL (National Renewable Energy Laboratory – USA) laboratory analytical procedures (Sluiter et al., 2008), was 2.15 ± 0.09 moisture; 46.21 ± 0.10 cellulose (as glucose); 20.86 ± 0.05 hemicellulose (as xylose); 19.54 ± 0.03 acid insoluble lignin, 3.13 ± 0.04 acid soluble lignin; 1.54 ± 0.08 extractives (waxes, fats, non-structural carbohydrates, resins, tannins and colored substances) and 1.19 ± 0.10 ash. All the results are expressed as mass percentage.

Enzymatic complexes NS50013 (cellulase, xylanase) and NS50010 (β-glucosidase) were kindly provided by Novozymes (Denmark).

All chromatograph standards were analytical grade, and MilliQ Ultrapure water was used.

2.2. Ozonolysis

The raw material was ozonized in a fixed bed reactor (glass column 50 cm in height and 2.7 cm in diameter) under room conditions. In each test, 35 g of bagasse was adjusted to the required moisture value, mixing weighted amounts of bagasse and distilled water. The column was then filled with the moisturized bagasse and exposed to the air/ozone gas stream. Ozone was produced by a corona effect ozone generator, Sander 301, fed by dry air. Ozone concentration in gas phase was regulated by the electrical supply, and measured before starting each test with iodometric titration applying the APHA 2350 E method (APHA-AWWA-WEF, 1995). Air-flow rate remained constant at 60 L/h in accordance with previous findings (Garcia-Cubero et al., 2009). Residual ozone concentration in the reactor outlet was also measured by iodometric titration during the reaction. To standardize the ozonolysis time, each test was stopped 15 min after ozone breakthrough, that is, 15 min after ozone began to emerge from the fixed bed ozonization column. Experimental conditions are summarized in Table 1.

In tests where a detoxification washing process was applied, ozonated bagasse was shaken with distilled water (6% w/w) for 1 h, at 25 °C and 300 rpm.

Ozone-treated bagasse, washed or unwashed, was dried in a ventilated oven at 37 °C, and stored in a freezer at −18 °C until enzymatic hydrolysis or composition analysis.

All experiments were carried out twice.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of dried bagasse was performed in Erlenmeyer flasks with 6% (w/w) raw or pretreated sugarcane bagasse, washed or unwashed, and sodium citrate buffer 0.1 M (pH 4.8), containing an enzyme dosage per gram of cellulose (dry basis) of 10 FPU g^{-1} (NS50013) and 10 CBU g^{-1} (NS50010). Hydrolysis was performed at 50 °C, 300 rpm for 24 h. After hydrolysis, samples were withdrawn and the supernatant was put through a 0.22 μm filter and stored for analysis of sugars and other compounds (e.g. inhibitors and acids).

Xylanase supplementation was not investigated, since the enzyme complex NS50013 is produced by *Trichoderma reesei*, known to be a xylanase producer. Some works have reported xylanase activity of this complex as 55 U/mL with beechwood xylan as