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Short Communication

Effect of pretreatment and enzymatic hydrolysis on the physical-chemical composition and morphologic structure of sugarcane bagasse and sugarcane straw

Marcia Maria de Souza Moretti *, Olavo Micali Perrone, Christiane da Costa Carreira Nunes, Sebastião Taboga, Maurício Boscolo, Roberto da Silva, Eleni Gomes

Univ. Estadual Paulista – IBILCE/UNESP, São José do Rio Preto, São Paulo, Brazil

HIGHLIGHTS

- Physical and chemical changes were detected in the pretreated biomass.
- Microwave/acid glycerol solution pretreatment led to biomass morphologic change.
- Treated lignocellulosic material was more easily hydrolyzed by the enzymes.

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ABSTRACT

The present work aimed to study the effect of the pretreatment of sugarcane bagasse and straw with microwave irradiation in aqueous and acid glycerol solutions on their chemical composition, fiber structure and the efficiency of subsequent enzymatic hydrolysis. Thermogravimetric analysis showed that the pretreatment acted mainly on the lignin and hemicellulose fractions of the bagasse, whereas, in the straw, lesser structural and chemical changes were observed. The images from transmission electron microscopy (TEM) revealed that treating bagasse and straw with acid glycerol solution loosened the cell walls and there was a breakdown in the pit membrane. The treated material was submitted to hydrolysis for 72 h and higher yields of reducing sugars were observed compared to the untreated material (250.9 mg/g from straw and 197.4 mg/g from bagasse). TEM images after hydrolysis confirmed the possible points of access of the enzymes to the secondary cell wall region of the pretreated biomass.

1. Introduction

Brazilian production of sugarcane during the last harvest (2014/2015) was around 632 million tons, producing 28 billion liters of ethanol (anhydrous + hydrated). Around 30% of the sugarcane produced was stored as dry biomass in plants and was burned for energy co-generation (Perrone et al., 2016). The sugarcane bagasse and straw are the main by-products of the sugar and ethanol industry and can be an important source of sugar for use in biotechnological processes for obtaining high added value products (Wanderley et al., 2013).

The conversion of lignocellulosic residues to cellulosic ethanol is currently a topic of great interest around the world. This process consists of three steps: (***i***) pretreatment of the raw material to reduce the lignin content and to increase the polysaccharide exposure; (***ii***) enzyme hydrolysis to convert the polysaccharides into the glucose and xylose monomers; and (***iii***) fermentation of the sugars to ethanol. The main technical and economic challenges in this process are the development of inexpensive pretreatments that improves the accessibility of the enzymes to cellulose without the formation of compounds toxic to the fermentation processes, especially phenolics, and without loss of reducing sugar in the pretreatment step (Mesa et al., 2011).

Organosolv treatment is an effective technique in which lignin is extracted from lignocellulosic biomass through the use of an organic solvent such as ethanol, ethylene glycol or glycerol. The polar structure of glycerol can easily penetrate the lignocellulosic material providing an effective reaction medium for the delignification. As it can reach high temperatures at atmospheric pressure, this reduces the energy consumption (Novo et al., 2011). Micro-

* Corresponding author at: Department of Biology, Univ. Estadual Paulista – IBILCE/UNESP, Cristóvão Colombo, 2265, Jd Nazareth, CEP 15054-000 São José do Rio Preto, São Paulo, Brazil.

E-mail address: marciamoretty@yahoo.com.br (M.M.de S. Moretti).

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wave radiation is uniformly absorbed by the solution and provides an intense rotational movement of the water molecules generating heat, useful in promoting the disintegration of lignocellulosic complex (Chen et al., 2011). The pretreatment of rice straw with microwaves in a basic medium reduced the hydrolysis time by 50% to produce the same amount of reducing sugar compared to the conventional process (Zhu et al., 2006).

In a previous paper, Moretti et al. (2014) observed that, for sugarcane bagasse immersed in concentrated glycerol and pretreated with microwave irradiation, the chemical composition was altered (the lignin was reduced and the cellulose increased), when compared to samples immersed in distilled water or diluted phosphoric acid (pH 3.0). Structural modifications and improved hydrolysis were also seen. However, some issues remained like the possible use of aqueous glycerol solution instead of concentrated glycerol and the association of glycerol with sulfuric acid and also the effect of such treatment on sugar cane straw.

Microwave irradiation of sugar cane bagasse and straw in neutral and acid aqueous glycerol media at atmospheric pressure is a safe and economically feasible pretreatment and, in fact, this is the first study performed to evaluate the effects of an extract of *Myceliophthora thermophila* M.7.7 on the morphological changes in the fibers in this pretreatment and on the subsequent hydrolysis.

2. Material and methods

2.1. Materials

Sugarcane bagasse and straw were kindly provided by Guarani from their mill in Olimpia/SP-Brazil. The biomass was washed with distilled water until sugar-free, dried at 60 °C, ground to 1–3 mm sieve size and kept at room temperature protected from the light. The sugarcane bagasse and straw contain 47% and 43% de cellulose, 16% and 15% hemicellulose and 27% and 23% de lignin, respectively.

2.2. Microwave treatment of sugar cane bagasse

Five g of dry sugarcane bagasse or straw with a 1–3 mm sieve size were added to a 250 ml round-bottom flask and impregnated for 20 h with 30 ml of 70% v/v solution of glycerol in water or sulfuric acid (0.02 M). Then it was submitted to microwave irradiation (1300 W – 2450 MHz) for 2 min at 130 °C measured using an infrared thermometer. After microwave irradiation, 30 ml of distilled water was added to the flask, shaken and filtered. The liquid fraction was used for the quantification of reducing sugars (RS) and total phenolic compounds (TPC) released. The solid fraction was dried at 60 °C and used in the fiber analysis and enzymatic hydrolysis.

2.3. Chemical analysis

Total reducing sugars and total phenolic compounds released after pretreatment were quantified according to the methods described by Somogyi-Nelson (Somogyi, 1952) and Folin-Ciocalteu (Singleton et al., 1999), respectively. Cellulose, hemicellulose and lignin content were measured according to the laboratory analytical procedures of the National Renewable Energy Laboratory (NREL) for standard biomass analysis (Sluiter et al., 2011).

2.4. Morphological and physical analysis

The morphological analyses of the biomass were performed in a Philips transmittance electron microscope CM-100, strictly as reported by Moretti et al. (2014).

Thermal degradation was performed in a PerkinElmer TGA-4000 thermogravimetric balance, and the differential scanning calorimetry (DSC) was performed in a PerkinElmer DSC-8000 calorimeter. X-ray diffraction patterns were recorded in a Rigaku Miniflex 300 diffractometer, operating at 30 kV in an angular range of 3–70° at 2θ min^{-1}. The degree of crystallinity (CI) was calculated according to Eq. (1):

$$\mathrm{CI} = \frac{H_c}{H_a + H_c} \times 100 \quad (1)$$

where H_a corresponds to the height referring to the amorphous phase (2θ∼18°) and H_c corresponds to the height related to the crystalline phase (2θ∼22°) (Browning, 1967).

2.5. Enzyme hydrolysis

Enzyme hydrolyses of untreated and treated samples were carried out in 50 ml flasks with rubber stoppers containing 2.5% of dry substrates in a final reaction volume of 20 ml of enzyme solution. The samples were incubated for 72 h at 55 °C using a reaction mixture containing an acetate buffer (pH 5.0, 0.1 M) and enzyme solutions obtained from the cultivation of *M. thermophila* M.7.7. Tests were performed by taking, as a reference, the total protein of the enzyme solutions (5 mg/g of substrate). The activities of the enzyme solutions in units per gram of dry substrate were: endoglucanase 825 U/g; xylanase 6050 U/g and β-glucosidase 5 U/g.

3. Results and discussion

The pretreatment under microwave irradiation with acid glycerol solution (MW/H_2SO_4G) provided the highest release of RS (10.4 mg/g from bagasse and 4.3 mg/g from straw) and the highest quantity of TPC (19.0 mg/g from bagasse and 8.0 mg/g from straw). Similar results for sugar released (10.9 mg/g of dry biomass) were obtained by Linde et al. (2008), with wheat straw treated with steam explosion and sulfuric acid as solvent.

When the ratio between the amounts of sugar and phenols released is considered, the data show that the treatments with microwave/aqueous glycerol solution (MW/H_2OG) give an average proportion of 1.1 in contrast to an average of 0.5 from those treated with MW/H_2SO_4G. On the other hand, the solid fraction of all the treated samples showed slight changes in the biomass composition (the maximum observed was a 4% decrease in lignin in the pretreated bagasse sample with MW/H_2SO_4G). These results are significant because they indicate the selective action of the treatment, decreasing the toxicity of the fermentation broth by phenolic compounds.

TGA curves showed that the weight loss onset temperature $T_{(onset)}$ occurs at approximately 320 °C except for bagasse pretreated with MW/H_2SO_4G which started around 350 °C, indicating higher thermal stability for this material (Fig. 1A, B). DTG curves of all bagasse and straw samples showed two defined peaks at around 360 and 405 °C except for bagasse pretreated with MW/H_2OG (Insert Fig. 1A, B). The first peak can be attributed to the decomposition of hemicellulose and lignin whilst the second one corresponds to the cellulose decomposition (Perrone et al., 2016). Bagasse pretreated with MW/H_2SO_4G showed only one peak, which reached its maximum at 405 °C. The absence of the other peak (360 °C) is due to the degradation of lignin and hemicellulose

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