



## Continuous pretreatment of sugarcane biomass using a twin-screw extruder



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### ABSTRACT

Although the area of biomass pretreatment has been improved in the last decades, there are challenges regarding the process scale up, the development of continuous processes and the formation of biomass conversion inhibitors. An option to circumvent these shortcomings would be the use of extrusion for biomass pretreatment. This study evaluated the pretreatment of sugarcane biomass in a twin-screw extruder by taking into consideration the use of additives such as water, glycerol, ethylene glycol, and Tween<sup>®</sup> 80, the selected additive load, the process temperature, rotating speed, number of passes and screw design. Results showed that using a biomass:glycerol ratio of 1:0.75 for bagasse and 1:0.53 for straw the pretreatment efficiency, measured by the biomass hydrolysis yields, was increased after multiple extrusion passes and, more importantly, the insertion of a reverse element in the screw configuration, resulting in a straw hydrolysis yield of 68.2%. These results indicate clearly the potential of extrusion for sugarcane biomass pretreatment.

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### 1. Introduction

The biorefinery concept is based on the conversion of biomass into a wide range of products. It can integrate different processes to produce fuel, power, heat and chemicals using a diversified range of renewable feedstocks. The implementation of biorefineries would allow the fully exploitation and optimization of the biomass potential along with the minimization of wastes and emissions (Rabelo et al., 2011; Kokossis et al., 2015). The expected result is to provide higher amount of fuel produced by the biomass grown area, increasing the sustainability of the process (Duque et al., 2014a).

Lignocellulosic biomass processing via biochemical routes aims at converting, by means of enzymatic hydrolysis, the carbohydrate content of raw materials into biomass sugars syrups that can be further processed via microbial or chemical routes into a

collection of bioproducts. Enzymatic hydrolysis applies biomass-degrading enzymes that can convert cellulose and hemicellulose into monosaccharides (Kokossis et al., 2015). However, the biomass of plants consists predominately of cell walls that are highly recalcitrant towards enzymatic hydrolysis in its native state. Besides, the anatomical tissue complexity of lignified biomass makes it extremely resistant to microbial attack. That is why enzymes applied to untreated biomass usually produce very low hydrolysis yields. Therefore, before enzymatic hydrolysis can effectively take place, the biomass must undergo a pretreatment step to open up the cell wall structures, successfully addressing its recalcitrance character (Singh et al., 2015). Thus, the pretreatment step has the function of increasing the enzyme accessibility to cellulose and hemicellulose (Duque et al., 2014b).

Physical, chemical, physico-chemical, and biological pretreatments have been studied to improve the enzymatic saccharification of biomass (Alvira et al., 2010; Behera et al., 2014; Asakawa et al., 2016; Zhou et al., 2016). Recently, studies on lignocellulosic biomass extrusion have shown a significant improvement in biomass enzymatic saccharification. Although the extrusion of lignocellulosic biomass is mostly reported in combination with

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chemical pretreatments (Karunanithy and Muthukumarappan, 2009, 2011c; Chen et al., 2011; Silva et al., 2013a; Negro et al., 2015), extrusion alone has also been studied as a physical pretreatment (Karunanithy and Muthukumarappan, 2010, 2011a,b; Lamsal et al., 2010; Lee et al., 2010a).

Extrusion is a continuous pretreatment process presenting a higher processing capacity when compared to batch systems (Cha et al., 2015). Additionally, fermentation inhibitors are not produced during biomass extrusion (Karunanithy and Muthukumarappan, 2010, 2011a), except when the extrusion is carried out in combination with chemical pretreatments (Karunanithy and Muthukumarappan, 2009, 2011c). Other advantages that favor the implementation of extrusion as a pretreatment for biomass on large scale processes include short residence time, the use of moderate temperatures, and the fact that the pretreated material does not require washing and other conditioning steps, as no inhibitors of the enzymatic hydrolysis and fermentation are formed during the pretreatment (Mood et al., 2013). Furthermore, and most importantly, extrusion is easily adaptable to commercial-scale operation (Lee et al., 2010b; Cha et al., 2015).

Extrusion promotes structural changes in biomass due to shearing, mixing, and heating, which all cause a certain degree of biomass disruption that results in greater exposure of cellulose surface, decrease in crystallinity, and increased porosity and surface area (Karunanithy and Muthukumarappan, 2011b; Mood et al., 2013; Cha et al., 2015; Menardo et al., 2015). These structural changes are mainly promoted in the mixing zones of the screws that are formed by successive kneading elements connected with a slight deviation angle between them, which depends upon the extrusion purpose. However, the extrusion of lignocellulosic materials requires the use of an additive mixed with the biomass to improve the flow properties of the material, thereby making its transportation possible. Otherwise, clogging may occur, especially in the mixing zone, which can damage the equipment and cause biomass degradation (Silva et al., 2013a). Substances with cellulose affinity, such as glycerol, ethylene glycol, and dimethyl sulfoxide (DMSO), can be good options as extrusion additives because they effectively fibrillate wood cell walls, thereby opening its structure and lowering the equipment torque (Silva et al., 2013b).

The present study aims at evaluating the extrusion pretreatment of sugarcane bagasse and straw in a twin-screw extruder using water, glycerol, Tween<sup>®</sup> 80, and ethylene glycol as biomass additives, as well as examining the effect of different screw configurations and process operating conditions. Sugarcane bagasse and straw were chosen due to their high availability in Brazil as agricultural residues. Indeed, in 2015 the total production of sugarcane bagasse and straw was, approximately, 79 and 63 million tons (dry basis), respectively, from a total sugarcane production of 632.1 million tons (UNICA, 2016).

## 2. Materials and methods

### 2.1. Materials

Sugarcane bagasse and straw were kindly provided by Catanduva Mill (São Paulo, Brazil) and by Itarumã Mill (Goiás, Brazil), respectively. The sugarcane bagasse was open-air dried reaching a moisture content of  $10.40 \pm 0.37\%$  and  $8.9 \pm 0.30\%$  for lots 1 and 2, respectively, while straw was received with a moisture content of  $12.05 \pm 0.36\%$  and  $10.34\% \pm 0.26\%$  for lots 1 and 2, respectively. Air dried bagasse was used to avoid microorganism contamination during storage, to enable its grinding, to test in similar moisture conditions as sugarcane straw and mainly to test the selected additives without water interference. After that, the biomasses were milled in a cutter mill (Retsch SM 300, Germany) to reduce particle

sizes to less than 2.0 mm. The milled material was sieved through a 0.20 mm sieve and the fraction from 0.20 mm to 2.0 mm was selectively recovered diminishing the particle sizes heterogeneity and the contamination of the sieved material with soil wastes.

Glycerol, ethylene glycol, and Tween<sup>®</sup> 80 were purchased from commercial sources and used as extrusion additives. All other chemicals were also purchased from commercial sources and used without any further purification. The commercial enzyme Acremonium cellulase was provided by Meiji Seika Co. (Japan).

### 2.2. Chemical composition of sugarcane bagasse and straw

The determination of the carbohydrate, lignin, extractives and ashes content of raw sugarcane bagasse and straw was done as previously reported (Sluiter et al., 2011). Cellulose and hemicellulose derived monosaccharides were identified and quantified using a Thermo Scientific Dionex ICS-5000 system (Canada) using high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD). Data acquisition and treatment were controlled by Chromeleon Chromatography Data System software. The guard cartridge and analytical column used were the CarboPac PA1 (4 mm x 50 mm and 10  $\mu\text{m}$  particle sizes) and CarboPac PA1 (4 mm x 250 mm and 10  $\mu\text{m}$  particle sizes), respectively, obtained from Thermo Scientific Ltd. (USA). The column temperature was 30 °C, and the mobile phase was composed of phase A (type 1 reagent grade deionized water) and phase B (300 mM NaOH solution). The gradient programs used for the separation were as follows: 0.0–32.0 min, 0% B; 32.0–32.1 min, 0–85% B; 32.1–42.0 min, 85% B; 42.0–42.1 min, 85–0% B; and 42.1–52.0 min, 0% B. The mobile phase was filtered through a 0.2  $\mu\text{m}$  filter and degassed by purging with ultra-pure nitrogen gas for 5 min prior to use. The flow rate was 1.0 mLmin<sup>-1</sup> and the injection volume was 5  $\mu\text{L}$ . The system was also equipped with a post column addition of 400 mM NaOH solution with a flow rate of 0.3 mLmin<sup>-1</sup>.

### 2.3. Extrusion pretreatment

#### 2.3.1. Effect of additive type and load and number of extrusion passes

All extrusion pretreatments were conducted using a co-rotating twin-screw extruder (HAAKE PolyLab OS, Thermo Scientific, Germany). The barrel presented a diameter of 16 mm and the screws length/diameter ratio (L/D) was 25. The screw configuration initially used in this study is shown in Fig. 1. The screws contain three different elements: forward screw elements, which enable the continuous movement of the material from inlet to outlet; kneading screw elements, which exert an effective mixing and shearing effect on the biomass; and reverse screw elements, which move the material backwards and increase its residence time to improve the pulverization effect. All extrusion experiments were carried out without a matrix support.

The first set of experiments, carried out to choose the best additive, were conducted at 50 °C and 30 rpm using a mixture of milled sugarcane biomass with either water, glycerol, ethylene glycol, or Tween<sup>®</sup> 80 with a biomass:additive ratio of 1:0.5, previously kept for 24 h in a cold room. The pretreated materials were submitted to enzymatic hydrolysis, as described in Section 2.4, and the chosen additive was selected for the continuation of the work.

In the second set of experiments, the influence of the amount of the chosen additive was evaluated by testing biomass:additive ratios of 1:0, 1:0.25, 1:0.53, 1:0.75, and 1:1, in order to select the appropriate load of additive for each biomass. The bagasse:additive ratio of 1:0.75 and straw:additive of 1:0.53 was chosen for the continuation of the work.

A third set of experiments was carried out using the selected biomass:additive ratios for sugarcane bagasse and straw in order

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