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# Enzymatic hydrolysis of steam-exploded sugarcane bagasse using high total solids and low enzyme loadings

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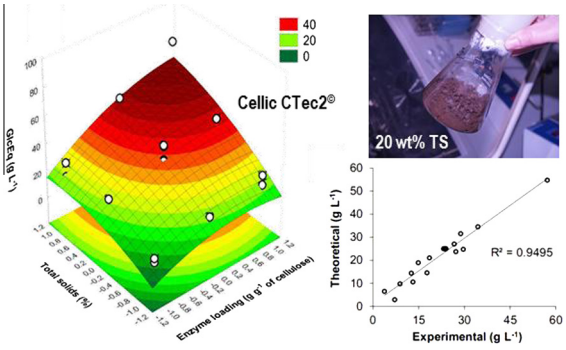
## HIGHLIGHTS

- Hydrolysis of pretreated cane bagasse was performed at high total solids.
- Cellic CTec2 was very efficient at high total solids and low enzyme loadings.
- The enzymatic hydrolysis results were nicely fit by a quadratic model.
- The best conditions resulted in the release of 76.8 g $L^{-1}$ of glucose equivalents.
- C6 fermentation of substrate hydrolysates could increase ethanol production by 39%.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Hydrolysis of phosphoric acid-impregnated steam-treated sugarcane bagasse was pre-optimized using a face-centered central composite design in which the process variables were the substrate total solids (TS, %), agitation intensity (AI, rpm) and enzyme loading (EL, g $g^{-1}$). Pretreatment was carried out at 180 °C for 10 min using cane bagasse with 50 wt% moisture content containing 9.5 mg of $H_3PO_4$ per gram of dry biomass. Hydrolyses were performed for 96 h at 50 °C using Cellic CTec2® and water-washed steam-treated substrates. The highest amount of fermentable sugars was obtained with 20 wt% TS, producing 76.8 g $L^{-1}$ of glucose equivalents, which corresponded to a total glucan conversion of 69.2 wt% and to a theoretical net increase of 39% in ethanol production from the same sugarcane tonnage without considering the use of leaves, tops and the additional yields from C5 sugars.



## 1. Introduction

Cellulosic ethanol can be obtained from different types of biomass such as sorghum (Shen et al., 2012), corn straw (Yang et al., 2011), rice straw (Ran et al., 2012) and sugarcane bagasse (Maeda et al., 2011), among others. This conversion process occurs in several steps including biomass preconditioning, pre-treatment, water-washing to remove inhibitors, enzymatic hydrolysis, fermentation of hexoses and pentoses, ethanol recovery, and effluent treatment. When hydrolysis and fermentation are performed separately (SHF, separated hydrolysis and fermentation), there is a possibility to optimize each step individually (Olofsson et al., 2008; Sánchez and Cardona, 2008). However, the integration of at least two of these steps is important to reduce the overall

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process cost, which still represents one of the major obstacles to overcome for its full commercialization (Chovau et al., 2013).

Four possible integration strategies have been investigated in recent decades: simultaneous saccharification and fermentation (SSF), co-fermentation of pentoses and hexoses (CF), simultaneous saccharification and co-fermentation (SSCF) and the consolidated bioprocess (CBP) (Taherzadeh and Karimi, 2007). However, efforts in process integration are still limited by obstacles such as the need for industrial microorganisms that are capable to ferment pentoses and hexoses efficiently, the built-up of inhibitory compounds in process streams, and differences in the optimal conditions for two or more processes that are to be carried out simultaneously in a single bioreactor. In this scenario, further developments in cellulosic ethanol must seek for improvements in the utilization of the C5 stream, reduction in the inhibitory impact of substrate hydrolysates on both enzymes and microorganisms, continuous recovery of ethanol from SSF reactors, and reduction in the energy requirements for process integration (Chandel et al., 2012). Recently, Ishola et al. (2013) developed a new proposal for process integration named simultaneous saccharification, filtration and fermentation (SSFF). They claimed that SSFF has advantages over SHF and SSF because enzyme inhibition can be avoided and both enzymes and microorganisms can be used at their optimal conditions. In addition, SSFF allowed the recovery and reuse of the fermenting microorganisms for at least five consecutive times.

The development of enzymatic hydrolysis at high total solids (TS) has been considered a fundamental step to reduce the process cost by decreasing the water consumption and increasing the concentration of sugars in the fermentation broth. Preliminary studies indicated that, by increasing the glucose concentration in substrate hydrolysates, the cost of distillation can be considerably reduced after fermentation (Lin and Tanaka, 2006). However, large amounts of substrate may cause a decrease in hydrolysis yields due to much lower agitation efficiencies, lower contact between substrate and enzymes, nonspecific adsorption on non-cellulosic components such as lignin, and loss of catalytic activity by shearing effects (Ramachandriya et al., 2013). For these reasons, hydrolysis at high TS has been normally carried out at high enzyme loadings, which also involves additional problems such as the increased competition for substrate sites available for hydrolysis and enzyme jamming on the substrate surface (Xiao et al., 2004; Várnai et al., 2013). According to Kim et al. (2008) and Hodge et al. (2008), the extent of enzymatic hydrolysis at high TS is strongly affected by the accumulation of inhibitory compounds such as sugars (mostly, glucose, cellobiose and xylooligomers) and organic acids (acetic and phenolic acids), as well as by serious mass transfer limitations. However, even when lower glucose yields are obtained, this strategy remains attractive because may lead to high concentrations of fermentable sugars (Zhang et al., 2009).

The substrate accessibility to enzymatic attack is not only related to substrate and enzyme related factors but also to the good mixing between the solid and liquid phases. Samaniuk et al. (2011) suggested that the mechanical action reduces the substrate particle size and increases substrate available surface area, while Lenting and Warmoeskerken (2001) attributed the higher conversion rates to an apparent increase in the availability of more accessible substrate sites (amorphous cellulose). Hence, the technology used for high TS must be capable of mixing the phases uniformly in order to achieve high conversion efficiencies and low energy consumption in relatively short reaction times without causing denaturation of the cellulolytic enzymes (Zhang et al., 2009).

The aim of this work was to develop a pre-optimization study for the enzymatic hydrolysis of lignocellulosic materials at high TS and low enzyme loading (EL). The enzyme used for this purpose was the Cellic CTec2 from Novozymes (Bagsværd, Denmark), which was developed specifically for the hydrolysis of lignocellulosic materials, thus presenting important advantages over other existing enzymes such as lower enzyme requirements for hydrolysis, lower cost and greater stability against inhibitors (Novozymes A/S, 2010). Furthermore, there is no need to enrich this enzyme preparation with exogenous β-glucosidases such in the case of Celluclast 1.5L FG, which was for many years considered a standard preparation for the enzymatic hydrolysis of cellulose when in combination with an external source of β-glucosidase activity (Novozym 188). The substrate used in this study was obtained from sugarcane bagasse by phosphoric acid-catalyzed steam explosion (Aguiar et al., 2013).

## 2. Methods

### 2.1. Material

The sugarcane bagasse was obtained from the São Martinho Group (Pradópolis, São Paulo – Brazil) with the logistical support from the Sugarcane Technology Center (CTC – Piracicaba, SP, Brazil) and Novozymes Latin America (Araucária, PR, Brazil). In total, 300 kg of bagasse (wet basis) were collected after crushing and washing to remove water soluble carbohydrates (mostly sucrose). This material, containing about 50 wt% of moisture in relation to its dry mass, was dried in an oven with air circulation at 50 °C, separated in smaller sample sizes and stored in a cold chamber in vacuum-sealed plastic bags. The moisture content of the stored material was in the range of 8 wt% (wet basis).

The cellulase complex Cellic CTec2 was donated by Novozymes Latin America (Araucária, Brazil). The enzyme loading (EL) in the hydrolysis experiments was expressed in relation to its wet mass, as recommended by the manufacturer.

### 2.2. Pretreatment of sugarcane bagasse

The pretreatment conditions used in this study were developed as part of CaneBioFuel project (http://cordis.europa.eu/result/rcn/53336_en.html) using diluted phosphoric acid to impregnate the bagasse prior to steam explosion. The acid impregnation consisted of spraying dilute phosphoric acid (13.4 g $L^{-1}$) on cane bagasse with 8 wt% moisture to achieve a final moisture content of 50 wt% (wet basis) and a concentration of 9.5 mg of $H_3PO_4$ per gram of dry matter. The impregnated material was stored at 4 °C overnight.

The steam explosion was performed in a 10-L stainless steel reactor immediately after the acid-impregnated material was brought up to room temperature. Nearly 1 kg of cane bagasse (dry basis) was charged into the preheated reaction chamber and subjected to high pressure steaming at 180 °C (13 atm) for 10 min. Then, the reactor content was decompressed abruptly into a cyclone for sample collection. The pretreated bagasse (TS in the range of 18–22 wt%) was recovered and drained in Büchner funnel to the lowest water retention level and the retained fibers were dispersed in water at a 5 wt% TS. The suspension remained under mechanical stirring for 1 h at room temperature, when it was filtered once again in Büchner funnel up to the lowest water retention level. The resulting material was designated as phosphoric-acid impregnated steam-exploded cane bagasse ($H_3PO_4$/SECB). This material was stored in vacuum-sealed plastic bags at 4 °C with a typical moisture content of 70 wt% until its use for chemical characterization and enzymatic hydrolysis.

Total moisture and ash contents were determined gravimetrically in both as-received and $H_3PO_4$/SECB samples using the NREL/TP-510-42621 (Sluiter et al., 2008a) and NREL/TP-510-42622 (Sluiter et al., 2008b) methods, respectively. Total extractives were also determined gravimetrically in the as-received cane bagasse