Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

# Sugarcane hybrids with original low lignin contents and high field productivity are useful to reach high glucose yields from bagasse

*D.F. Laurito-Friend, F.M. Mendes, F.M. Reinoso, A. Ferraz, A.M.F. Milagres* *

*Departamento de Biotecnologia, Escola de Engenharia de Lorena, Universidade de São Paulo, 12602-810 Lorena, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 5 January 2014
Received in revised form
17 November 2014
Accepted 10 February 2015
Available online 27 February 2015

*Keywords:*
Sugarcane
Lignin-depleted plants
Alkaline-sulfite pretreatment
Sulfonation
Cellulases
Glucose

ABSTRACT

Five sugarcane hybrids plus a reference material were evaluated according to the glucose yields obtained after alkaline-sulfite pretreatment and enzymatic hydrolysis. Sugarcane hybrids with varied original chemical compositions were used to assess how contrasting samples might influence the integrated pretreatment and hydrolysis process. The hydrolysis efficiency of six samples treated at three different chemical loads, suggested that lignin and hemicellulose removals during the pretreatment were not the single factor necessary to reach high cellulose conversion levels in the enzymatic hydrolysis step. Pretreated samples with the highest total acid contents (mainly sulfonic acids) were also the most digestible materials. The glucose yields were heavily dependent not only on the digestibility of the pretreated materials but also on the field productivity of the plants. One of the hybrids, presenting high glucan yields after pretreatment and high digestibility, produced low glucose yields because it presented very low biomass productivity. In contrast, one of the hybrids that presented low glucan yield after pretreatment, but was highly digestible and presented high biomass productivity, provided the highest glucose yields in the data set, producing 4192 and 5629 kg of glucose per hectare after enzymatic hydrolysis for 24 h and 72 h, respectively.



## 1. Introduction

Sugarcane is largely used for ethanol production because it yields up to 150 kg of sucrose per ton of wet plant [1–3]. The cell wall polysaccharides from sugarcane bagasse are also recognized to be a useful source of monomers for production of fuels and chemicals [4]. However, like in any lignocellulosic material, the complex cell wall ultra structure restricts the access of enzymes to the polysaccharides, which makes pretreatment necessary to reach high glucose yields from lignocellulosic biomass [5–9].

Chemithermomechanical (CTM) pretreatments employing sodium sulfite as delignification agent are useful to remove part of the original lignin and hemicellulose from the lignocellulosic biomass, resulting in a pretreated material that is more hydrophilic and contains partially sulfonated residual lignins [8,10,11]. The degree of dissolution of each individual lignocellulose components depends on the reaction severity, but optimized processes can provide glucose yields as high as

* *Corresponding author*. Tel.: +55 12 31595019; fax: +55 12 31533165.
E-mail address: adriane@debiq.eel.usp.br (A.M.F. Milagres).
http://dx.doi.org/10.1016/j.biombioe.2015.02.015
0961-9534/

90% after enzymatic digestion of the pretreated material [8,11,12]. The origin of the CTM pretreatments is the pulping processes used to produce high yield pulps. These processes are well established at industrial level with proved machinery, including on line chemicals impregnation and disk refining. Under alkaline medium, the sulfite CTM process is very selective for lignin removal up to 50% of delignification, preserving most of the cellulose from the original biomass [13]. Therefore, the use of alkaline sulfite in pretreatment processes for biomass saccharification seems logical because lignin removal of the order of 50% is enough to provide very digestible materials for the subsequent enzymatic hydrolysis step [14,15]. In comparison with other currently evaluated pretreatment processes employing only NaOH as delignification agent, alkaline-sulfite proved to be more efficient to decrease lignocellulose recalcitrance and to preserve more cellulose when a target delignification level is defined [8,13]. Comparing with traditional auto-hydrolysis and acid processes [6], alkaline-sulfite pretreatment of sugarcane bagasse provides a more swollen and partially delignified substrate which presents higher digestibility at low enzyme dosages [8,10].

The use of originally less recalcitrant lignocellulosic materials may be associated with less severe pretreatments to provide efficient conversion of the polysaccharides into useful monomers at high yields [3,16–19]. Several studies show that genetic modifications providing diminished lignin contents or altered lignin structure may result in benefits for the pulping or the biofuel industries. However, genetic modification of the plants or breeding for less recalcitrant lignocellulosic materials requires an integrate evaluation of the glucose yields with basis not only on the pretreatment and the enzymatic digestion steps. Plant productivity is also a key factor determining how much glucose can be obtained from planted biomass.

In the present work, five sugarcane hybrids plus a reference material, which presented varied chemical composition and plant productivity, were evaluated under an integrated process including alkaline-sulfite CTM pretreatment performed with different chemical loads followed by enzymatic digestion with commercial enzymes. The digestibility of the pretreated materials by commercial enzymes were correlated with the original characteristics of the samples as well as with the chemical changes occurred during the pretreatment. A final integrated process was analyzed in the light of total glucose yields computing plant productivity, potential available glucan and glucan conversion to glucose.

## 2. Materials and methods

### 2.1. Raw materials

The bagasse samples used in the experiments were obtained from sugarcane hybrids previously selected with basis on contrasting chemical compositions and biomass productivity [3]. The origin of the hybrids was the RIDESA consortium (Academic Network for the Development of Sugar-Alcohol Sector) associated with the Federal University of Viçosa, MG, Brazil. Sample preparation and storage were identical to that described in Masarin et al. [3]. In addition to the experimental hybrids, sugarcane bagasse samples collected in a sugar and ethanol mill that crushes a mix of commercial sugarcane cultivars was used as reference material. One set of industrially available sugarcane bagasse was used in the first experimental trial of enzyme loading affecting hydrolysis efficiency (data reported in Fig. 1). Another set was used for all the other reported results. Both sugarcane bagasse samples were collected at the mill and air-dried to a final humidity of 12% (referred to as “mill bagasse” in the present work).

### 2.2. Biomass pretreatment

The pretreatments were performed with 20 g of bagasse impregnated with the alkaline sulfite liquor at a bagasse/liquor ratio of 1:10 (w/v). Impregnation was performed by applying vacuum to the air-dried biomass contained in a Buchner flask for 30 min, then the liquor was displaced into the flask and an additional 15 min of vacuum was applied. The alkaline sulfite liquor corresponded to loads of 2.5, 3.75 and 5 g of NaOH (per 100 g of dry bagasse) combined with 5, 7.5 and 10 g of $Na_2SO_3$ (per 100 g of dry bagasse), respectively. Impregnated biomass was cooked at 120 °C for 120 min. The cooked material was filtered through filter paper and the liquor was discarded. Retained solids were washed with 1 L of distilled water and particles, smaller than 0.2 mm passing through the filter paper, were pumped back to the Buchner funnel. Recirculation of the filtrate permitted the formation of a fiber mat over filter paper that retained fines. Water recirculation was stopped when wash-water was free of turbidity. The retained solids were blended with additional 750 mL of water in a laboratory blender for 15 min. Yield of solids after pretreatment was calculated by measuring initial and final biomass dry weights. Pretreated material was stored at −18 °C up to use in subsequent experiments.

### 2.3. Chemical composition of the bagasse samples

Approximately 3 g of milled samples were extracted with 95% ethanol for 6 h in a Soxhlet apparatus. Extracted samples were hydrolyzed with 72% (w/w) sulfuric acid at 30 °C for 1 h (300 mg of sample and 3 mL of sulfuric acid) as described by Ferraz et al. [20]. The acid was diluted by addition of 79 mL of water and the mixture was heated at 121 °C at 1 atm for 1 h. The resulting material was cooled and filtered through a porous glass filter number 3. The solids were dried to a constant weight at 105 °C, which was determined as the insoluble lignin. The soluble lignin in the filtrate was determined by UV spectroscopy at 205 nm. An absorptivity value of 105 L/g.cm was used to calculate the amount of acid-soluble lignin present in the hydrolyzate. Concentrations of monomeric sugars in the soluble fraction were determined by HPLC using a BIO-RAD HPX87H column at 45 °C eluted at 0.6 mL/min with 5 mM sulfuric acid. Sugars were detected with a temperature-controlled RI detector.

### 2.4. Determination of acid groups

Approximately 1.5 g (dry basis) of each sample was stirred with 50 mL 0.1 M HCl for 45 min for complete protonation of