



# Combined ethanol and methane production using steam pretreated sugarcane bagasse



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## ABSTRACT

Efficient energy production relies on complementary use of crop residues, to enhance the amount of energy obtained per unit biomass. In this frame, sugarcane bagasse (SB) was pretreated and the resulting slurry and liquid fraction served, respectively, for simultaneous saccharification and fermentation (SSF) at high solid concentration (15%), and anaerobic digestion (AD). More specifically, SB was subjected to twelve pretreatments to enhance fiber deconstruction and subsequent energy output: steam explosion alone (195 °C for 5, 10 and 15 min), after impregnation with 0.4% and 0.7% Ca(OH)<sub>2</sub>, and at 205 °C for the same three times after 0.7% Ca(OH)<sub>2</sub> addition. After pretreatment, enzymatic hydrolysis was carried out on washed solid fraction; glucose and xylose were determined on this fraction as well as residual liquid fraction. On this latter, inhibitors (acetic and formic acid, furfural and 5-hydroxymethylfurfural) were also determined. Based on high glucose yield in enzymatic hydrolysis, three pretreatments were selected for SSF of the slurry. The same pretreatments underwent AD of the liquid fraction. Inhibitors augmented at increasing time and temperature, although never achieved critical levels. Lignin removal (range, 17–38%) was enhanced by lime addition, whereas increasing temperature and time did not contribute to delignification. Glucose yield in washed solid fraction varied accordingly. SSF exhibited the highest ethanol yield with mild lime addition (60% of theoretical) vs. steam alone (53%). However, modest yields were generally evidenced (average, 55%) as a result of high viscosity, especially in the case of high lime dose in SSF at high solid concentration. Combined energy yield (ethanol, methane and solid residue) proved lime effectiveness as catalyst in steam explosion of SB, beside two intrinsic advantages: low water consumption in SSF at high solid concentration, and the possibility of lime removal from downstream effluents through carbonation.

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## 1. Introduction

Many studies have been conducted on the use of sugarcane bagasse (SB) as a source for second generation bioethanol (2G EtOH) (Sambusiti et al., 2015; Barakat et al., 2013; Da Silva et al., 2010). A wide consensus supports the use of SB for 2G EtOH production in terms of environmental benefits (Dias et al., 2011; Furlan et al., 2013), compared to combustion in order to power a first generation

ethanol plant. However, a few studies demonstrate the economic advantages of integrating first and second generation ethanol process (Dias et al., 2011; Furlan et al., 2013;). Owing to this, a large number of experiments have been carried out to investigate the best route to obtain 2G EtOH from SB, as reported by Macrelli et al. (2012).

In this frame, pretreating biomass is a prerequisite to maximize the enzymatic convertibility of SB cellulose and hemicellulose into fermentable sugars (Galbe and Zacchi, 2007). Steam pretreatment, also known as steam explosion, is one of the most studied and promising methods (Toor et al., 2013). It is often preceded by impregnation with a catalyst, generally consisting of acidic gases or liquids (e.g., sulfur dioxide, sulfuric acid) (Martin et al., 2002; Sassner et al., 2007; Carrasco et al., 2010). Catalyst addition determines a higher pretreatment efficiency than steam explosion alone; this latter is also called auto-hydrolysis process (Bondesson et al., 2013). However, using a catalyst impacts on the environment

**Abbreviations:** 2G EtOH, second generation bioethanol; AA, acetic acid; AD, anaerobic digestion; ANOVA, analysis of variance; FA, formic acid; HMF, 5-hydroxymethylfurfural; Log R<sub>0</sub>, severity factor; LSD, least significant difference; P, pretreatment; SB, sugarcane bagasse; SSF, simultaneous saccharification and fermentation; TOC, total organic carbon; TS, total solids; VS, volatile solids; WIS, water insoluble solids.

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because of water and chemical supply that generate large amounts of downstream waste to be disposed. Compared to this, it would be advisable to use a catalyst securing high yields of 2G EtOH, null impact on the environment, and whose by-products have a market value.

In general, chemical catalysts involve issues, such as equipment corrosion and the need of processing downstream effluents, resulting in high water consumption (Ramos, 2003). Among chemical catalysts, acids do not remove lignin, a fraction of biomass that is not converted into ethanol, hindering enzymatic hydrolysis (Jorgensen et al., 2007). Lignin may block enzymes activity by restricting access to cellulose and hemicellulose, resulting in rate limited enzymatic hydrolysis and subsequent curbing of potential ethanol yield (Chang and Holtzapfle, 2000; Rabelo et al., 2011). Compared to acids, alkaline pretreatments can effectively abate lignin in agricultural residues as SB (Fuentes et al., 2011), although they did not prove satisfactory in processing recalcitrant substrates as softwood (Chandra et al., 2007).

Lime, i.e., calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ), is an alkaline catalyst suited to enhance degradation of lignocellulosic biomass of agricultural origin. More to this, it can be removed by carbonating the waste water with  $\text{CO}_2$  (Chang et al., 2001). The resulting  $\text{CaCO}_3$  may be used for several applications as mitigating the drought stress in tomato (Patanè et al., 2012), thus constituting a valuable by-product instead of a downstream waste.

The yeast strain *Saccharomyces cerevisiae* is well suited for simultaneous saccharification and fermentation (SSF) of hydrolysed lignocellulosic material. However, *S. cerevisiae* ferments hexoses but not pentoses. Hence, adopting a pathway to convert pentose sugars into additional energy is crucial to improve the energy output of the whole process. SSF of both hexoses and pentoses with engineered yeast strains is seen a promising option in the near future (Olofsson et al., 2008). At present, methane production through anaerobic digestion (AD) of pretreated liquid appears the most reliable practice (Kaparaju et al., 2009; Dererie et al., 2011) to complement 2G EtOH production through SSF of the solid fraction. However, there is still a paucity of information concerning the effects of pretreatments aimed for high ethanol yield in SSF (slurry), on methane yield in AD (liquid fraction). It is feared, thereby, that pretreatments most beneficial for SSF might adversely reflect on AD. This could offset pretreatment advantages, in terms of combined energy output.

Given these premises, the aims of the present study were to compare the effects of lime used as catalyst in SB impregnation before steam explosion, on sugar yields after pretreatment and enzymatic hydrolysis. Time, temperature and catalyst concentration during pretreatment were varied. Pretreatments that in the enzymatic hydrolysis had showed top glucose yields, underwent separation of the slurry from the liquid fraction. The slurry was subjected to SSF at high solid concentration (15%); the liquid fraction to AD. Finally, combined energy yield was calculated as the sum of the energy contained in ethanol, methane and the solid residue after SSF.

## 2. Material and methods

### 2.1. Process configuration

SB was impregnated with/without lime prior to steam explosion. Steam explosion pretreatment was carried out at residence times of 5, 10 and 15 min and at temperatures of 195 and 205 °C. Pretreatment conditions are described in Section 2.3 and presented in Table 1. Upon completion, pretreated samples were separated into a condensed slurry and a liquid fraction, as described in Section 2.3. The slurry was washed repeatedly with distilled water

**Table 1**

Experimental conditions and respective severity factors ( $\text{Log } R_0$ ) in sugarcane bagasse pretreatment.

Pretreatment	$\text{Ca}(\text{OH})_2$ % (w/w)	Time (min)	Temperature (°C)	$\text{Log } R_0$
1	–	5	195	3.5
2	–	10	195	3.8
3	–	15	195	4.0
4	0.4	5	195	3.5
5	0.4	10	195	3.8
6	0.4	15	195	4.0
7	0.7	5	195	3.5
8	0.7	10	195	3.8
9	0.7	15	195	4.0
10	0.7	5	205	3.8
11	0.7	10	205	4.1
12	0.7	15	205	4.3

to remove potential inhibitors, resulting in a washed solid fraction that was subjected to enzymatic hydrolysis. Thereafter, the slurries whose solid fractions had showed highest glucose yield in enzymatic hydrolysis, were selected for SSF. The corresponding liquid fractions were subjected to AD. Fig. 1 describes the process configuration from raw biomass to final energy carriers, including enzymatic hydrolysis as side process to select slurries and liquid fractions for subsequent energy conversion.

### 2.2. Raw material

SB was air dried (total solids, 93%) and chopped into pieces of approximately 0.5 mm size for the analysis of structural carbohydrates, lignin (Klason lignin and acid soluble lignin), ash and extractives (Sluiter et al., 2005, 2008a).

### 2.3. Pretreatments

The 12 pretreatments investigated in the experiment (Table 1) can be divided into two main groups: autohydrolysis, consisting of steam alone at increasing time (P1–P3); alkaline pretreatment (P4–P12), consisting of nine combinations of  $\text{Ca}(\text{OH})_2$  concentration (0.4 and 0.7%), temperature (195 and 205 °C) and time (5, 10, 15 min).

The raw material (20–50 mm size) was immersed for one hour either in water (P1–P3) or in an aqueous solution (P4–P12) containing  $\text{Ca}(\text{OH})_2$  at 0.4% or 0.7% (w/w). In both cases, a liquid to solid ratio of 20:1 (w/w) was used. After 1 h impregnation, the wet SB was dewatered in a 3 L press (Tinkturenpressen HP5M, Fischer Maschinenfabrik GMBH, Germany), to reach a dry matter content of 45–50%.

After the dewatering process, the steam pretreatment was then performed in a 10 L reactor described by Palmqvist et al. (1996), loaded with an amount of wet SB corresponding to 400 g dry matter. More in detail, the steam unit was composed of a 10 L reactor connected to a controlling computer and the flash chamber.

Pretreated material was filtered through a 2.5  $\mu\text{m}$  sieve, resulting in a liquid fraction and a condensed slurry. The slurry was washed and then analyzed for structural carbohydrates and lignin, while the liquid fraction was analyzed for the content of total sugars, monomers (glucose and xylose) and inhibitors (acetic acid (AA), formic acid (FA), furfural and 5-hydroxymethylfurfural (HMF)), according to a method from the US National Renewable Energy Laboratory (Sluiter et al., 2008a,b). The content of water-insoluble solids (WIS) was determined using the method developed by Weiss et al. (2010). All the chemical and physical traits were analyzed in duplicates.

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