

## COMPARISON BETWEEN WET OXIDATION AND STEAM EXPLOSION AS PRETREATMENT METHODS FOR ENZYMATIC HYDROLYSIS OF SUGARCANE BAGASSE

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Alkaline wet oxidation and steam explosion pretreatments of sugarcane bagasse were compared with regard to biomass fractionation, formation of by-products, and enzymatic convertibility of the pretreated material. Wet oxidation led to the solubilisation of 82% of xylan and 50% of lignin, and to a two-fold increase of cellulose content in the pretreated solids, while steam explosion solubilised only 60% of xylan and 35% of lignin and increased cellulose content in the solid material by one third. Wet oxidation formed more aliphatic acids and phenolics, and less furan aldehydes in the liquid fraction than steam explosion did. A better enzymatic convertibility of cellulose was achieved for the wet-oxidised material (57.4 %) than for the steam-exploded material (48.9 %). Cellulose convertibility was lower for the whole slurry than for the washed solids in both pretreatments, but more significantly in steam explosion. This investigation demonstrates the potential of wet oxidation as a promising pretreatment method for enzyme-based bagasse-to-ethanol processes.

*Keywords:* Sugarcane bagasse; Ethanol; Pretreatment; Wet oxidation; Steam explosion; Enzymatic hydrolysis.

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### INTRODUCTION

Sugarcane bagasse, the solid residue left after extraction of the juice from sugar cane (*Saccharum officinarum*), can be considered as a potential substrate for the production of bioethanol, a fuel that is increasingly interesting due to environmental concerns. As in the case of other lignocellulosic bioresources, bagasse contains cellulose and hemicelluloses, which ought to be hydrolysed, either by acids or by enzymes, for obtaining fermentable sugars. Acid hydrolysis is a technologically mature process, but it is hampered by problems associated with corrosion of equipment and relatively high cost for acid recovery when concentrated acid is used and by the decomposition of hemicellulose sugars when diluted acid is used (Galbe and Zacchi 2002; Taherzadeh and Karimi 2007a). Enzymatic hydrolysis of lignocellulosic materials is very promising, but a pretreatment must be implemented prior to hydrolysis in order to increase the reactivity of cellulose by disrupting its close association with hemicelluloses and lignin (Sun and Cheng 2002; Galbe and Zacchi 2002; Taherzadeh and Karimi 2007b).

A number of pretreatment methods of different nature have been investigated for different kinds of lignocellulosic materials (Sun and Cheng 2002; Mosier et al. 2005; Hu

et al. 2008). Steam explosion (STEX), a process based on heating up lignocellulose by high-pressure steam followed by an explosive decompression, is a pretreatment method that has been investigated for sugarcane bagasse (Kaar et al. 1998; Martín et al. 2002). Although STEX is very effective (Saddler et al. 1993), it has the drawback of formation of by-products that are inhibitory for the enzymatic hydrolysis of cellulose and the fermentation of hydrolysates (Palmqvist et al. 1996; Martín et al. 2002). Wet oxidation (WO) is a pretreatment method consisting in treating biomass with water and air or oxygen at temperatures above 120°C (McGinnis et al. 1984; Schmidt and Thomsen 1998). An advantage of WO, especially when combined with an alkali, is the very limited formation of fermentation inhibitors, such as furan aldehydes (Bjerre et al. 1996a) and phenol aldehydes (Klinke et al. 2002). WO pretreatment has been investigated for wood (McGinnis et al. 1984), wheat straw (Schmidt and Thomsen, 1998; Klinke et al. 2002), corn stover (Varga et al. 2003), and more recently for sugarcane bagasse (Martín et al. 2007a).

In this work, the effect of wet oxidation and steam explosion on the fractionation of sugarcane bagasse, the formation of by-products, and the enzymatic convertibility of cellulose and xylan were compared systematically.

## **EXPERIMENTAL**

### **Raw Material**

Sugarcane bagasse from the 2003 harvest was generously donated by “Juan Ávila” sugar mill (Matanzas, Cuba). The bagasse was air-dried, milled to pass a 2-mm screen (Bie & Berntsen AS, Jylland, Denmark), and stored in plastic bags in a dark chamber at room temperature. A portion of the raw material was milled a 1-mm particle size and used for analysis of the chemical content.

### **Pretreatment**

Wet oxidation was performed in a 2-L loop reactor with continuous circulation and stirring (Bjerre et al. 1996a). Sixty grams of bagasse (93.9 % dry matter (DM)) were mixed with 1 L of water and with 2 g of Na<sub>2</sub>CO<sub>3</sub> in the WO reactor. Previous experience indicates that higher biomass concentrations can render mechanical stirring and circulation more difficult. Oxygen was supplied at a pressure of 12 bar. The material was heated up to 195°C, and it was maintained at this temperature for 15 min. Upon completion of the pretreatment time and cooling to room temperature, the reactor was emptied by suction. For the steam explosion pretreatment, 100 g of bagasse was loaded into a 4.9-L pressurised vessel, which is connected to a boiler supplying steam saturated up to 40 bar, and the material was heated up to 205°C for 10 min. When the pretreatment time had elapsed, the pretreated material was recovered in a cyclone connected to the outlet of the reactor. Each experiment was performed in duplicates.

The pretreated slurries obtained after both WO and STEX were separated by vacuum filtration (SUE 300, Heto-Holten A/S, Denmark) giving a cellulose-enriched filtration cake and a hemicellulose-containing filtrate. The filtration cakes were washed

with deionised water. Both the filtration cakes and the filtrates were analysed and compared to untreated bagasse (Fig. 1).

### Analysis of the Solid Fraction

The filter cakes obtained after separation of the slurries were dried to 90-92% dry matter (DM) content in a climatisation chamber (Binder WGB 20, Tuttlingen, Germany) at 20°C and 65% relative humidity. The material was ground in a coffee grinder (Krupps 75, Mexico) and milled to pass a 1-mm screen. The DM content was analysed using a halogen moisture analyser (HR73, Mettler Toledo, Switzerland). The chemical composition of the pretreated material and of the raw bagasse was determined by a two-step analytical acid hydrolysis procedure (Kaar et al. 1991) followed by HPLC analysis (Shimadzu Corp., Kyoto, Japan). In the first step the materials were hydrolysed with 72% H<sub>2</sub>SO<sub>4</sub> at 30°C during 1 h. After that, water was added and a posthydrolysis was performed with diluted H<sub>2</sub>SO<sub>4</sub> at 121°C during 1 h. The hydrolysates were separated by vacuum filtration, and glucose, xylose and arabinose were determined by HPLC. Prior to HPLC analysis, the sulphate anions were precipitated by an equivalent amount of Ba(OH)<sub>2</sub> and removed by centrifugation. Glucose, xylose, and arabinose were separated on an Aminex HPX-87H column (Bio-Rad, Hercules, CA) using 4 mM H<sub>2</sub>SO<sub>4</sub> as mobile phase at a flow of 0.6 ml/min at 63°C and detected using an RI detector (Shimadzu Corp., Kyoto, Japan). The content of cellulose, xylan, and arabinan in the solid material was determined by multiplying the concentration of the sugars in the hydrolysate by a factor considering water addition during hydrolysis (Puls 1993). The filtration residues were dried at 105°C overnight, weighed for determining the content of Klason lignin, and incinerated at 550°C during 3 h for determining the ash content.

The content of non-cell wall material (NCWM) was analysed by cooking in a neutral detergent according to a modification of the sequential gravimetric method of Goering and van Soest (van Soest and Robertson 1980).

### Analysis of the Liquid Fraction

The filtrates obtained after pretreatment were stored frozen at -20°C until analyses. Free sugars, acids, and furan aldehydes were determined by HPLC. Before HPLC analysis, the filtrates were adjusted to pH 2 with dilute H<sub>2</sub>SO<sub>4</sub>, centrifuged at 10 000 rpm for 10 min (4-15 Sigma Laboratory Centrifuges GmbH, Germany), and filtered to crimped vials. For determination of the total content of sugars in the filtrates, acid posthydrolysis (4 % H<sub>2</sub>SO<sub>4</sub>, 121°C, 10 min) was applied (Bjerre et al. 1996b). Sulphate anions were removed as indicated above. Acetic, formic, malic, succinic, and glycolic acids were analysed using the above-mentioned HPLC conditions, except that the temperature was 40°C. Furfural and hydroxymethylfurfural (HMF) were quantified by HPLC using a Nucleosil 5C-18 25 mm column (Macherey-Nagel, GmbH, Germany) and a UV-detector at 280 nm. The total content of phenolic compounds was determined by the Prussian blue method (Graham 1992), using a Power Wave X spectrophotometer (Bio-tek Instruments, Inc., USA).

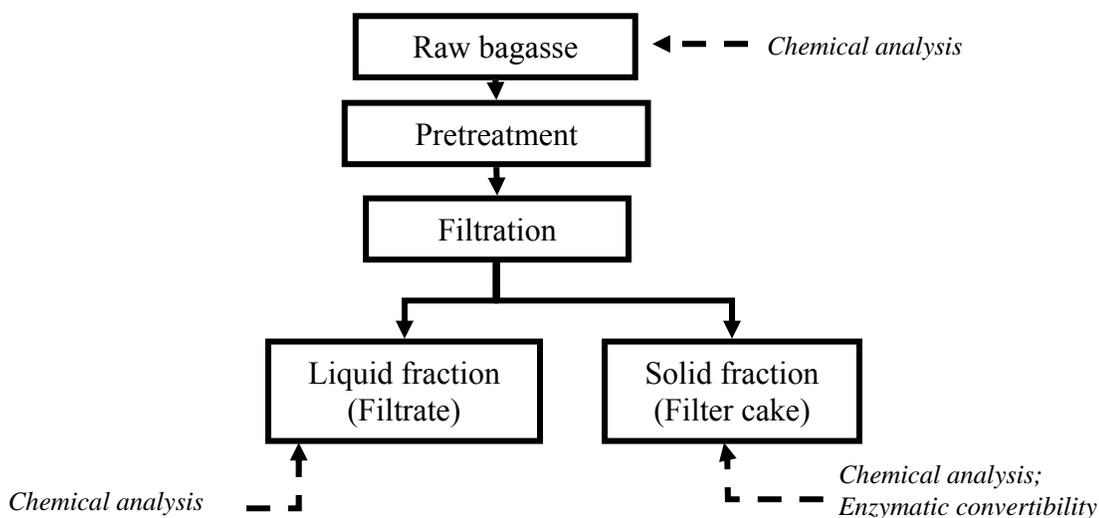
### Enzymatic Convertibility

For evaluating the enzymatic convertibility of cellulose, the method of Bjerre et al. (1996a) was used. Approximately 100 mg of the washed pretreated solid fraction was

suspended in 5 mL of 0.04 M acetate buffer (pH 4.8) corresponding to 2% DM content. A commercial preparation of *Trichoderma reesei* cellulases (Celluclast 1.5L) and a  $\beta$ -glucosidase preparation (Novozym 188), both kindly donated by Novozymes A/S (Bagsværd, Denmark), were added. The enzyme activity of Celluclast 1.5L was 82 filter paper units (FPU)/g, the  $\beta$ -glucosidase activity of Novozyme 188 was 450 IU/g, and the enzyme loading were 25 FPU/g DM and 0.46 CBU/ml, respectively. The reaction mixture was incubated in a shaking incubator (Lab-Therm, Adolf Küchner, AG, Switzerland) at 50°C and 150 RPM for 24 h. At the end of the hydrolysis the hydrolysates were separated by centrifugation and filtered into HPLC vials. Glucose and xylose were determined by HPLC, and the results were used for calculating the enzymatic convertibility of cellulose and xylan. In a parallel experiment, the convertibility of the whole slurry was also assayed. In that experiment, 100 mg of the washed filtration cake was re-suspended in the filtrate, which had previously been adjusted to pH 4.8, and the hydrolysis was performed under the same conditions. All of the experiments were performed in triplicates. The experimental results were processed with the software STATGRAPHICS Plus 2.1 for Windows for performing analysis of variance, which made it possible to compare the results and to determine if the measured differences were statistically significant.

## RESULTS AND DISCUSSION

Bagasse was pretreated either by wet oxidation or by steam explosion according to scheme shown below (Fig. 1). The pretreatment conditions were selected based on previous studies of WO (Martín et al. 2007a) and STEX (Martín et al. 2001) of bagasse. The composition of the solid and liquid streams obtained after each pretreatment was analysed, and the enzymatic convertibility of the pretreated material was assessed.



**Fig. 1.** Scheme for pretreatment and analysis of sugarcane bagasse

### Composition of the Solid Fraction

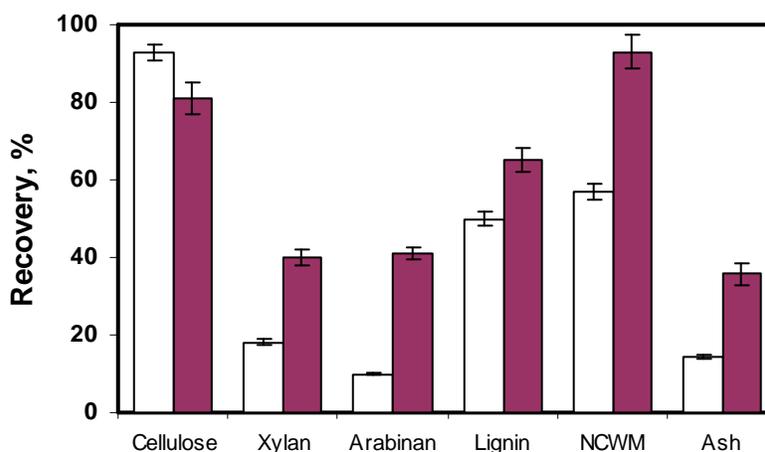
For both pretreatments, an important fraction of the material was not recovered in the solid material. The analysis of the composition of the filtration cakes revealed that this was due to the partial solubilisation of hemicelluloses and, to a lesser extent, lignin. The highest solubilisation occurred after wet oxidation, where only 52.9% of the initial dry matter was preserved in the filtration cake, whereas the yield of dry matter after the steam explosion was 61.5% (Table 1).

**Table 1.** Relative contents of Different Components of the Raw Bagasse and of the Solid Fractions Obtained after Pretreatment, %.

Component	Raw bagasse	Wet oxidation	Steam explosion
Pretreated solids	-	52.9 <sup>1</sup> (31.7) <sup>2</sup>	61.5 <sup>1</sup> (61.5) <sup>2</sup>
Cellulose <sup>3,5</sup>	34.3 (0.31)	60.5 (0.24)	45.0 (1.19)
Xylan <sup>3,5</sup>	19.9 (0.92)	6.9 (0.18)	12.9 (0.65)
Arabinan <sup>3,5</sup>	2.1 (0.01)	0.4 (0.00)	1.4 (0.01)
Klason Lignin <sup>3,4,5</sup>	21.8 (0.32)	20.4 (0.47)	23.1 (0.41)
NCWM <sup>3,5</sup>	8.6 (0.15)	9.2 (0.42)	13.0 (0.55)
Ash <sup>3,5</sup>	1.4 (0.01)	0.4 (0.00)	0.8 (0.00)

<sup>1</sup>Yield (%) of pretreated solids. <sup>2</sup>The total amounts of the solid fractions are shown in parentheses. Average of two (<sup>3</sup>) and four (<sup>4</sup>) replicates. <sup>5</sup>Standard deviation shown in parentheses.

The solubilisation of hemicelluloses was more remarkable after wet oxidation, where most of xylan and arabinan was solubilised, leaving only 18 and 10%, respectively, of those hemicellulose components in the solid fraction (Fig. 2). A lower solubilisation of hemicelluloses occurred during steam explosion, where the recovery of both xylan and arabinan in the fibres was around 40%.



**Fig. 2.** Recovery of the main components in the solid fraction after pre-treatment, % (w/w). Light bars, WO; dark bars, STEX. Error bars represent standard deviations.

Wet oxidation led to a solubilisation of approximately 50 % of the initial lignin (Fig. 2), while a lower delignification was induced by STEX, since 65 % of the initial lignin was recovered in the steam-exploded fibres. The higher delignification occurring during wet oxidation can be explained by the reactivity of phenol-like compounds under WO conditions (Bjerre et al. 1996a). The alkaline pH is an important factor favouring delignification during wet oxidation, as was revealed by a previous work by this group (Martín et al. 2007a).

As a result of hemicelluloses and lignin solubilisation, a cellulose enrichment of the solid material was achieved with both pretreatments. That enrichment was higher for wet oxidation, which led to an almost two-fold increase of cellulose content, while after steam explosion cellulose content increased only in 31% (Table 1). Furthermore, most of the cellulose (93%) contained in the raw bagasse remained in the fibres after WO, while the recovery of cellulose in the steam-exploded solid material was only 81%, indicating that around one fifth of cellulose was solubilised during steam explosion (Fig. 2). The increase of cellulose content in the solid material and the high preservation of cellulose are positive traits of wet oxidation pretreatment in view of economic considerations.

The solid materials obtained after both pretreatments contained a higher percentage of non-cell wall material (NCWM) than the initial bagasse (Table 1). The calculation of the recovery showed that most of the NCWM (93%) was recovered in the solid material resulting from the STEX pretreatment, whereas 43 % was solubilised during WO (Fig. 2). The elucidation of the factors behind the difference in removal of NCWM during wet oxidation and steam explosion deserves further attention.

A significant solubilisation of the mineral components happened in both pretreatments. The solubilisation was more substantial for wet oxidation, where only 14.3% of the initial ash was recovered in the filtration cake (Fig. 2). The high degree of ash removal is another positive feature of wet oxidation if the lignin-rich residue generated after enzymatic hydrolysis is intended to be used as a fuel.

No significant visual differences were detected in the structural character of wet-oxidised and steam-exploded bagasse. Both materials were dark brown, and their consistence was compact.

### **Yield of Sugars in the Liquid Fraction**

For both pretreatments, the concentrations of free monosaccharides in the liquid fraction were much lower than the expected concentrations according to the high hemicelluloses solubilisation detected in the analysis of the solid fraction (Table 2). This is an indication of a limited hydrolysis of hemicelluloses during pretreatment. The content of free sugars was lower in the filtrate obtained by wet oxidation (hereafter referred to as WO filtrate) than in the filtrate obtained by steam explosion (hereafter referred to as STEX filtrate). This result is in agreement with previous information about the tendency of wet oxidation to catalyse the transfer of hemicelluloses from the solid phase to the liquid phase without a major hydrolysis of the solubilised hemicellulose molecules. According to McGinnis et al. (1984), monosaccharides are oxidised to carboxylic acids by WO, while oligosaccharides are more resistant to oxidation due to the stability of the glycosidic linkages. Therefore, sugar oligomers instead of monosaccharides are present in wet oxidation filtrates.

The presence of oligosaccharides was confirmed by the increase of the content of monosaccharides observed when the filtrates were subjected to acid posthydrolysis (Table 2). Although the yields of total sugars were comparable in both filtrates, it was evident that more xylose was formed by wet oxidation and more glucose was formed by steam explosion. This is in conformity with the low recovery of hemicelluloses detected for wet oxidation and the low recovery of cellulose detected for steam explosion (Fig. 2).

**Table 2.** Yield of Sugars in the Liquid Fraction Obtained after Pretreatment.

		Wet oxidation	Steam explosion
Glucose, g/100 g	Free <sup>1</sup>	0.5 (0.02)	0.8 (0.03)
	Total <sup>2</sup>	1.3 (0.01)	2.0 (0.11)
Xylose, g/100 g	Free <sup>1</sup>	2.4 (0.13)	3.4 (0.16)
	Total <sup>2</sup>	9.7 (0.08)	8.9 (0.42)
Arabinose, g/100 g	Free <sup>1</sup>	0.9 (0.00)	1.0 (0.00)
	Total <sup>2</sup>	1.0 (0.01)	1.0 (0.01)

Free sugars, measured directly in the filtrates; Total sugars, measured after acid posthydrolysis of the filtrates. Average of two (<sup>1</sup>) and four (<sup>2</sup>) replicates. Standard deviation shown in parentheses.

The yields refer to raw bagasse.

### Overall Recovery of Carbohydrates

The quantitative analysis of recovered polysaccharides is a practical approach for estimating the losses of cellulose and hemicelluloses during pretreatment. The overall recovery included the carbohydrates recovered in both the filtrate and the filtration cake, and it was calculated based on mass balances as shown below:

$$\text{Cellulose (\% (w/w))} = \frac{\text{Cell}_{\text{SF}} (\text{g}) + \text{Glucose}_{\text{LF}} (\text{g})/1.11}{\text{Cell}_{\text{UB}} (\text{g})}$$

$$\text{HC (\% (w/w))} = \frac{\text{Xylan}_{\text{SF}} (\text{g}) + \text{Arabinan}_{\text{SF}} (\text{g}) + [\text{Xylose}_{\text{LF}} (\text{g}) + \text{Arabinose}_{\text{LF}} (\text{g})]/1.14}{\text{Xylan}_{\text{UB}} (\text{g}) + \text{Arabinan}_{\text{UB}} (\text{g})}$$

where: Cell<sub>SF</sub>: Cellulose in the solid fraction;

Glucose<sub>LF</sub>: Glucose in the liquid fraction;

Cell<sub>UB</sub>: Cellulose in the untreated bagasse;

HC: Hemicelluloses;

Xylan<sub>SF</sub> and Arabinan<sub>SF</sub>: Xylan and arabinan in the solid fraction;

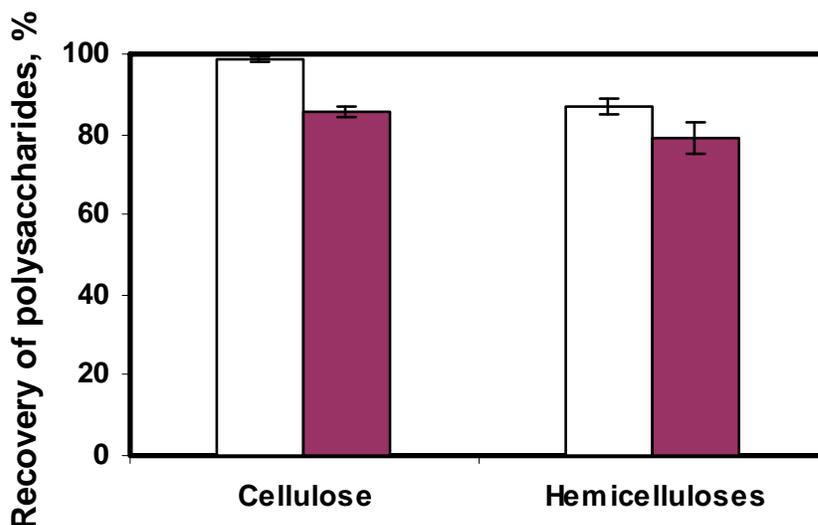
Xylose<sub>LF</sub> and Arabinose<sub>LF</sub>: Xylose and arabinose in the liquid fraction;

Xylan<sub>UB</sub> and Arabinan<sub>UB</sub>: Xylan and arabinan in the untreated bagasse;

1.11 and 1.14: Conversion factors considering water addition during hydrolysis of polysaccharides to monomeric sugars.

Wet oxidation was more favourable than steam explosion regarding preservation of the polysaccharides, since higher overall recoveries of both cellulose and hemicelluloses were achieved for the former pretreatment than for the latter (Fig. 3).

Cellulose was almost completely recovered by wet oxidation, while 13% of it was lost by steam explosion. The lower cellulose recovery for STEX might be linked to its higher solubilisation during pretreatment, which led to a longer exposure of the solubilised oligosaccharides to the degrading effects of elevated temperature and acidic pH (Ramos 2003).



**Fig. 3.** Overall recovery of cellulose and hemicelluloses in the whole pretreated material, % (w/w). Light bars, WO; dark bars, STEX. Error bars represent standard deviations.

For both pretreatments, hemicelluloses were more sensitive to destruction than cellulose, but they were better recovered for wet oxidation than for steam explosion (Fig. 3). That phenomenon might be related to the low hydrolysis degree of hemicelluloses during wet oxidation. Contrarily to the sensitivity to degradation monosaccharides, the hemicelluloses-derived oligosaccharides contained in the WO filtrate are rather stable under pretreatment conditions.

### Formation of By-Products in the Liquid Fraction

By-products, such as aliphatic acids, furan aldehydes, and phenolic compounds, were formed during pretreatment. The pattern of by-product formation was different for each pretreatment method. The main by-products formed during wet oxidation were aliphatic acids, accounting for 6.7 g/100 g, while the contribution of phenolic compounds (2.7 g/100 g) was relatively less significant, and the amounts of furan aldehydes were negligible (Table 3). On the other hand, steam explosion led to the formation of approximately similar amounts of carboxylic acids (2.2 g/100 g) and phenolic compounds (2.1 g/100 g) with an important contribution of furan aldehydes (0.8 g/100 g).

**Table 3.** Formation of By-products during Pretreatment, g/100 g Raw Bagasse

	Wet oxidation	Steam explosion
Succinic acid	0.2 (0.00)	0 (0.00)
Glycolic acid	1.2 (0.00)	0.1 (0.00)
Formic acid	2.1 (0.09)	0.4 (0.01)
Acetic acid	3.2 (0.14)	1.7 (0.10)
Phenolic compounds	2.7 (0.11)	2.1 (0.11)
Furfural	0.2 (0.01)	0.6 (0.02)
HMF	0 (0.00)	0.2 (0.00)

Average of two replicates. Standard deviation shown in parentheses.

The concentration of aliphatic acids in the WO filtrate was three times higher than in the STEX filtrate (Table 3). The high formation of acids was a consequence of the oxidation of sugars, phenols, and other compounds produced during the hydrolytic stage of WO process (McGinnis et al. 1984), and to the release of acetic acid during the initial hydrolysis of acetyl groups in hemicelluloses (Mishra et al. 1995). The acetic acid found in the STEX filtrate was derived from the hydrolysis of acetyl groups during steam pretreatment, while formic acid was generated by degradation of the sugars.

The concentration of the furan aldehydes in the STEX filtrate was four times higher than in the WO filtrate, where HMF was not detected. Furfural and HMF are formed by dehydration of pentoses and hexoses, respectively, under thermal and acidic conditions (Kholkin 1989). The low formation of furfural during alkaline wet oxidation is in agreement with previous reports (Bjerre et al. 1996a; Schmidt and Thomsen 1998; Klinke et al. 2002; Martín et al. 2007a).

The low concentration of furfural and HMF in WO filtrates makes wet oxidation a promising pretreatment method for ethanol production, since these compounds are well-known inhibitors of ethanolic fermentation (Palmqvist and Hahn-Hägerdal 2000). The good fermentability of WO-pretreated bagasse was demonstrated in a recent report by this group (Martín et al. 2006)

The formation of phenolic compounds was slightly higher for wet oxidation than for steam explosion (Table 3). Phenolic compounds are formed as a result of the partial degradation and solubilisation of lignin. They are undesirable in lignocellulosic hydrolysates because of their inhibitory effect on the ethanologenic microorganisms (Jönsson et al. 1998). The lower formation of phenols during steam explosion might be an outcome of the lesser extent of delignification occurred during that pretreatment (Fig. 2). Recently, we showed that the solubilised lignin fraction is further degraded by WO than by STEX, as high concentrations of oxidised phenols lacking side chains were found in WO filtrates, whereas the phenols detected in STEX filtrates maintain their side chains (Martín et al. 2007b).

### Enzymatic Convertibility

The effectiveness of the pretreatments was revealed by the enzymatic convertibility of cellulose, which was approximately five times higher in the washed solids obtained after either of the pretreatments than in untreated bagasse (Table 4). Evidently, the solubilisation of hemicelluloses and lignin and the destruction of their association with cellulose have increased the accessibility of cellulose and have made it more

reactive towards the enzymes. The improvement of the enzymatic convertibility might also be attributable to other factors, such as a partial destruction of the crystalline structure and a decrease of the degree of polymerisation of cellulose with the consequent increase of the available chain ends (Converse 1993).

Wet oxidation, with 57.4% of cellulose being converted to glucose, was more effective than steam explosion, where the convertibility was 48.9%. The differences between the enzymatic convertibilities of the materials obtained by both pretreatments were found to be statistically significant. The better cellulose convertibility of the wet-oxidised material might be due to its higher cellulose content and the lower contents of lignin and hemicelluloses (Table 1). Cellulose represented more than 60% of the dry matter in the solid material obtained by wet oxidation, whereas it represented only 45% in the STEX filter cake. On the other hand, the solid fraction obtained by steam explosion contained two times more hemicelluloses and approximately 15% more lignin than the WO filtration cake. It should be noted that the enzymatic convertibility was lower than what was obtained previously in separate experiments with bagasse pretreated by wet oxidation (79.2 %) (Martín et al. 2006) and steam explosion (75.5 %) (Martín et al. 2001). The differences might be attributed to particularities of the raw material, since in those studies other sorts of bagasse, from different places and harvest years, were used.

**Table 4.** Enzymatic Convertibility of Cellulose and Xylan in Raw Bagasse and in Bagasse Pretreated by Wet Oxidation and by Steam Explosion, %.

	Raw bagasse	WO-WSM	WO-Slu	STEX-WSM	STEX-Slu
Cellulose	11.1 (0.5)	57.4 (2.3)	54.3 (2.6)	48.9 (1.7)	39.0 (2.0)
Xylan	8.7 (0.4)	44.8 (1.9)	74.9 (3.4)	38.8 (1.9)	52.3 (2.5)

WSM, washed solid material; Slu, slurry. Average of three replicates. Standard deviation shown in parentheses.

The hydrolysis of the whole slurry instead of the washed solid material is important for techno-economical reasons, since it represents a lower consumption of fresh water, and it eliminates the need of using a separation device after pretreatment. Therefore, the enzymatic convertibility of the slurries was also assayed. The slurries were prepared by mixing the solid and the liquid fractions obtained after pretreatment. For both pretreatments the cellulose convertibility of the slurry was lower than that of the washed solid material, but for wet oxidation the difference was less significant than for steam explosion (Table 4).

The lower cellulose convertibility achieved in the slurries, compared with the washed solid material, might be explained by the negative effect of degradation products from hemicellulose and lignin contained in the filtrate. As was discussed before, the liquid fraction contains aliphatic acids, phenolic compounds, and furan aldehydes, which are recognised inhibitors of the microbial processes including enzymatic hydrolysis (Palmqvist et al. 1996). Furan aldehydes seemed to be more inhibitory than other compounds, since the lowest enzymatic convertibility was achieved in the STEX slurry, where the content of those compounds was four times higher than in the WO slurry. On the other hand, the better convertibility of the WO slurry might be an indication of a less

inhibitory effect of aliphatic acids, whose content in the WO filtrate was three times higher than in the STEX filtrate (Table 3). However, the elucidation of the actual inhibitory effect of furans and acids is beyond the scope of this work, and it would require a different experimental approach for avoiding the interference of the dissolved cellulose oligomers. Unpublished results by this group revealed that the gluco-oligosaccharides solubilised during pretreatment are readily hydrolysed with commercial cellulases, which somehow compensates for the inhibition by the degradation products.

Xylan was hydrolysed by the enzymes as a result of the xylanase activity of *T. reesei* cellulase preparation, which is in agreement with previous reports (Thygesen et al. 2003). In contrast to what was observed for cellulose, xylan convertibility of the slurries was better than that of the washed solid material (Table 4). Xylan convertibility in the WO slurry was 67% higher than in the WO washed solid material. For steam explosion xylan convertibility in the slurry was also higher than in the washed solid material, although the increase was more modest (35%). The higher xylan convertibility in the slurries was due to the contribution of the xylan contained in the filtrate. A correlation between xylan convertibility in the slurries and content of dissolved xylan in the filtrate was found in a previous study (Martín et al. 2007a).

## CONCLUSIONS

1. This study helped to elucidate the differences between wet oxidation and steam explosion regarding the fractionation of biomass, the formation of by-products, and the enzymatic convertibility of the pretreated material.
2. Major differences in biomass fractionation and by-product formation were found. Wet oxidation (WO) led to a major solubilisation of hemicelluloses and lignin and to an almost complete recovery of cellulose, whereas steam explosion led to a minor solubilisation of hemicelluloses and lignin and to an incomplete recovery of cellulose. During WO, aliphatic acids were produced in high amounts and the production of furan aldehydes was very low, whereas during STEX the production of aliphatic acids was low, but furan aldehydes were produced in considerable amounts.
3. The solid material obtained after wet oxidation displayed higher enzymatic convertibility than the solid material obtained by steam explosion. Although for both pretreatments the enzymatic hydrolysis of the slurry was inhibited compared to the hydrolysis of the washed solid material, the inhibition was more evident for STEX than for WO.
4. This study provides strong evidence of the potential of wet oxidation as a way of pretreating sugarcane bagasse prior to the enzymatic hydrolysis in a bagasse-to-ethanol process.

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