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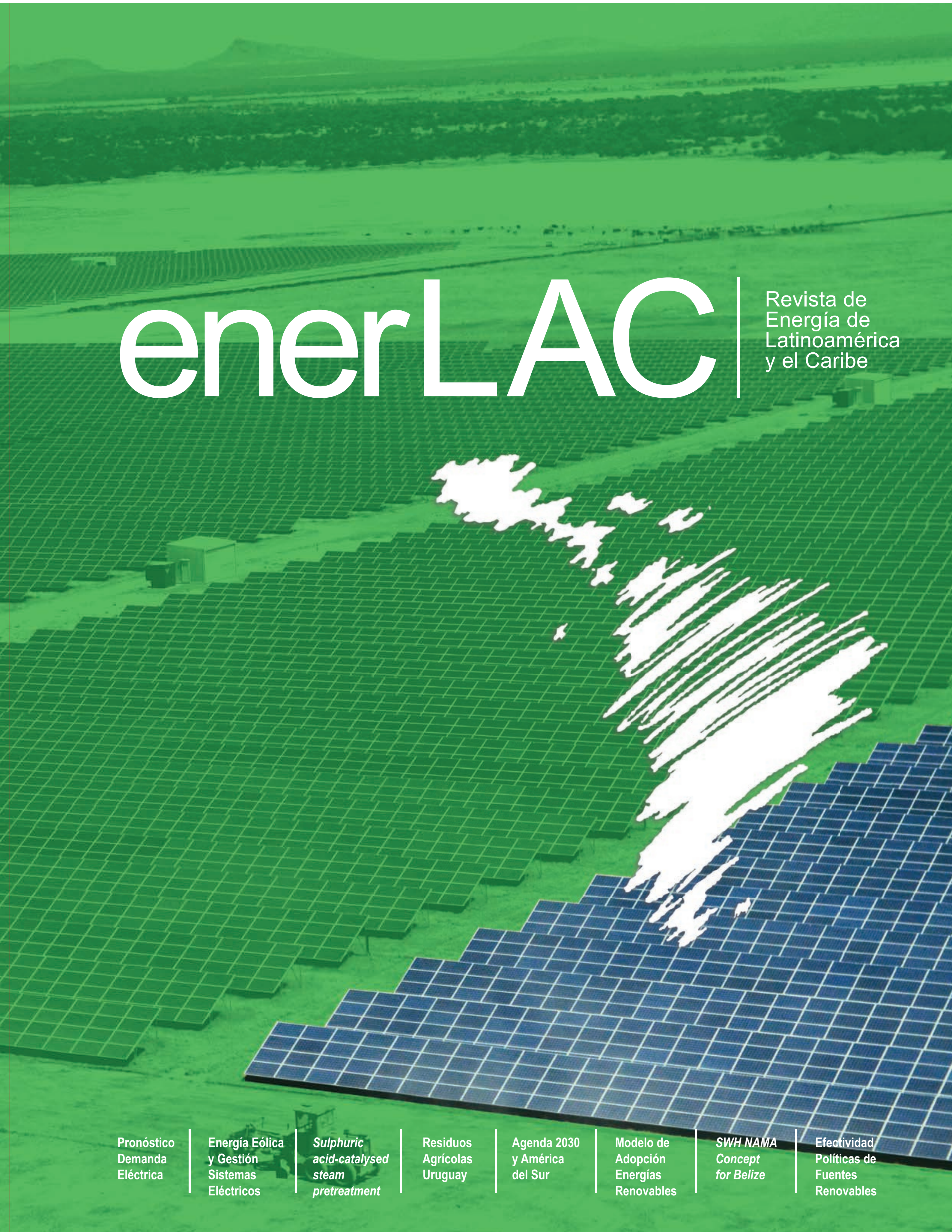

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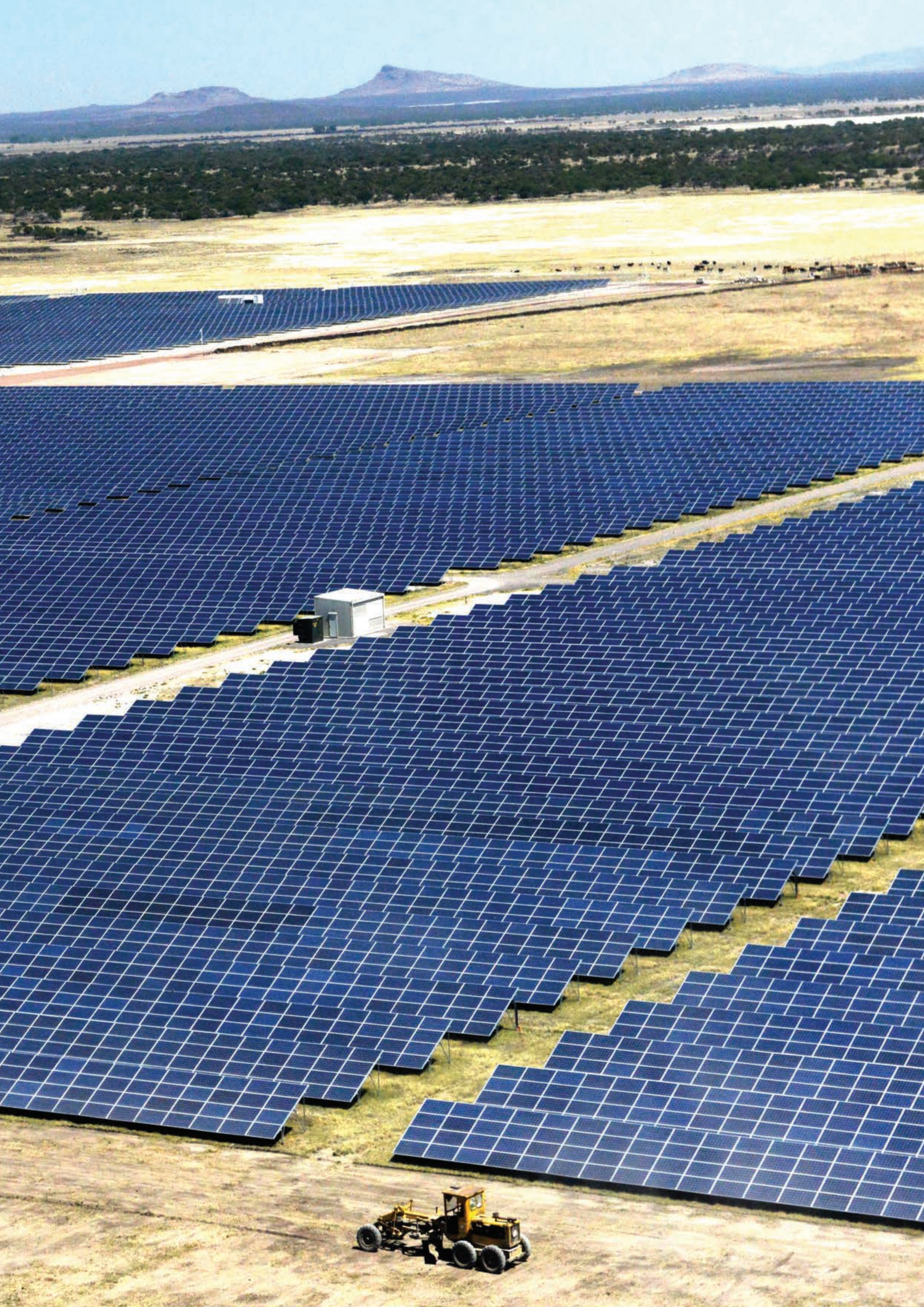

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# STUDY OF SULPHURIC ACID-CATALYSED STEAM PRETREATMENT OF THE HARDWOOD *ANADENANTHERA COLUBRINA*

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## ABSTRACT

Hemicellulose from lignocellulosic materials constitutes a large potential source of fermentable sugars to be used for fuel production. The hardwood Anadenanthera colubrina is used in the South American forest industry. The wood (and its residues) has a high carbohydrate content (cellulose 43% and hemicellulose 21%) and may be of interest as a lignocellulose feedstock for fuel production. The aim of the present study was to determine conditions for a good recovery of hemicellulose, primarily pentose sugars from that material. *A. colubrina* hardwood was subjected to steam pretreatment using dilute sulphuric acid ($H_2SO_4$ = 0.5 or 1.5 % (w/w)), at a temperature between 180 to 220 °C, a holding time of 5 or 10 min and different moisture contents (40 – 60%). Acid hydrolysis gave a good recovery of pentose sugars, with a xylose yield of 68%, and only minor amounts of degradation products in terms of furan compounds. Only minor proportion of the lignin was solubilised. Acid-catalysed steam pretreatment thus appears to be a suitable pretreatment process for recovery of hemicellulose sugars from this feedstock to be used in fermentation processes.

**Keywords**: *Anadenanthera Colubrina*, Ethanol, Steam Pretreatment

## *RESUMEN*

*La hemicelulosa proveniente de residuos lignocelulósicos constituye una fuente potencial de azucares fermentables para la producción de combustibles. La especie de madera dura Anadenanthera colubrina es utilizada en Suramérica para la industria forestal. Esta madera (sus residuos) tiene un alto contenido de carbohidratos (celulosa 43% y hemicelulosa 21%) siendo una posible materia prima lignocelulósica para la producción de combustibles. El objetivo del presente estudio fue determinar las condiciones para alcanzar una alta recuperación de hemicelulosa,* *principalmente pentosas provenientes de este material. La madera dura A. colubrina fue sujeta a pretratamiento de vapor utilizando ácido sulfúrico diluido ($H_2SO_4$ = 0.5 a 1.5 % (p/p)) entre temperaturas de 180 a 220 °C, un tiempo de residencia de 5 a 10 min y diferentes contenidos de humedad (40 – 60%). La hidrolisis ácida alcanzo una buena recuperación de pentosas, con un alto rendimiento de xilosa del 68%, y con pequeñas cantidades de productos de degradación en términos de furanos. Solo una pequeña proporción de lignina fue solubilizada. El pretratamiento de vapor catalizado con ácido sulfúrico aparece como un proceso de pretratamiento sostenible para la extracción de azucares de hemicelulosa a partir de materias primas lignocelulósicas que puede ser utilizado para procesos fermentativos.*

***Palabras Clave**:* Anadenanthera Colubrina*, Etanol, Pretratamiento de Vapor*

**Latin American and the Caribbean countries (LAC) are potential suppliers of raw materials such as lignocellulosics for bioethanol production, since these raw materials have well-established cultivation procedures, as well as technology for harvesting and their transportation.**

## INTRODUCTION

Over the last years there has been an increasing interest in using renewable energy as a substitute for fossil fuels. A major reason behind this interest is the concern about the effects of greenhouse gases and the associated risks for global warming (Cao, 2003; de Campos et al., 2005). In the context of renewable resources, lignocellulosic feedstocks constitute important sources of fermentable sugars to be used for fuel production. Xylan is the most abundant no cellulosic polysaccharide present in several biomasses (about 20-40%), including agricultural residues, herbaceous crops, and deciduous (hardwood) tress (Ebringerová et al., 2005). The lignocellulosic sector of Latin America and the Caribbean (LAC) is a potential supplier of feedstocks for bioethanol, since these feedstocks already have well-established cultivation procedures in place, as well as technology for harvesting and transportation (McMillan, 1994; Zhan et al., 2005; IICA, 2007). The costs of the feedstock normally depend on, for example, plant location, size and the method of procurement (Zhan et al., 2005).

In the forest sector, South America possesses large wood reserves (23% of global forests) predominantly dominated by hardwood trees. Numerous of these trees species are used to produce energy either by being burnt directly or in the form of charcoal or pellets (Juslin & Hansen, 2002; ECLAC et al., 2013). One of the species is *Anadenanthera colubrina* (Vell.) Brenan, which is widely distributed in Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay and Peru (Prado & Gibbs, 1993; Delgobo et al., 1998; Carrasco, 2013). In Bolivia, *A. colubrina* (also known as Curupaú) is an important commercial hardwood, and large quantities of forest and mill residues, such as sawdust and chips, are produced in the Bolivian forest industry. *A. colubrina* has a high carbohydrate content and together with the facts above, this makes it an interesting substrate for bioethanol production (Carrasco, 2013).

One of the most widely used pretreatment methods is the steam pretreatment, which hydrolyses most of the hemicellulose into monomeric sugars (D-xylose, L-arabinose, D-galactose, and D-mannose). The addition of catalyst as $H_2SO_4$ and $SO_2$ during steam pretreatment can significantly improve the hemicellulose hydrolysis in terms of pentoses removal in comparison to autohydrolysis pretreatment (i.e. treatment without catalyst) (Grohmann et al., 1986; Galbe & Zacchi, 2007; Carrasco et al., 2010). Catalysis by sulphuric acid has been most extensively studied, including feedstocks as aspen (Mackie et al., 1985; Grohmann et al., 1986; Josefsson et al., 2002; De Bari et al., 2007), eucalyptus (Carrasco et al., 1994; Emmel et al., 2003), poplar (Carrasco et al., 1994), oak (Carrasco et al.,1994), *Salix* (Sassner et al., 2008), and willow (Eklund et al., 1995). In general, steam pretreatment generates xylose-richliquors (hydrolysates) as effluent due to the hydrolysis of hardwood hemicellulose sugars. The presence of high amounts of *O-acetyl* groups facilitates the catalytic hydrolysis of the hemicellulose sugars by acetic acid formation (Dekker, 1987). The composition of hydrolysates furthermore depends on the pretreatment conditions such as catalyst concentration, reaction temperature, liquid-to-solid ratio (L/S) and residence time. Ideally, the cellulose polymer should be easily accessible to enzymatic hydrolysis after steam pretreatment. In addition to mixed sugars and oligosaccharides, inhibitory compounds suchas organic acids, furans and numerous phenolic compounds are also likely to be present in the pretreated feedstocks (McMillan, 1994; Galbe & Zacchi, 2007).

The objective of the present study was to investigate the potential of the hardwood *A. colubrina* as a feedstock for bioethanol production. The work focused on production of sugars from the hemicellulose during steam pretreatment, with the aim of reaching as high pentose sugars yield as possible, with low formation of by-products. Steam pretreatment experiments using $H_2SO_4$ as catalyst were made in the temperature range 180 to 220$^{o}$C, reaction times of 5 or 10 minutes, and moisture contents between 40 and 60%.

## MATERIALS AND METHODS

### Feedstock preparation

Fresh sawdust *A. colubrina* was supplied by a sawmill, MARSA SRL (La Paz, Bolivia). The collected material was stored at room temperature (15°C) awaiting milling. Before washing, the woody material was screened to remove the oversized material, which was sent to a re-milling. Here the lignocellulosic was hammer-milled through a sieve size of 1.2 mm, after which the feedstock was washed with water (to remove dirt, sand and other solid residues). Following this, different preparations of moisture content (MC) were made. The sawdust material was dewatered by pressing to reach approximately 60% of high moisture content (HMC), and was drying at room temperature, reaching 40% of low moisture content (LMC). The prepared feedstock was stored in plastic bags at 4$^{o}$C for later $H_2SO_4$ impregnation and steam pretreatment. The woody material composition is indicated in table 1.

### Impregnation and $H_2SO_4$-catalysed steam pretreatment

Several batches of *A. colubrina* were impregnated with 0.5-1.5 % $H_2SO_4$ (w/w), amount based on the water content of the woody material. The samples were wetted with sufficient sulphuric acid solution to give a liquid-to-solidratio of 2:1 (including the moisture content of the hardwood) in glass flasks for eight hours at room temperature. Following impregnation, the acidified hardwood was placed into a laboratory-scale hydrolysis reactor with a volume of 0.5-L, equipped with a flash collector tank and a steam generator. A batch of 7 g of dry wood was used in each experiment. The size of the material (1.2 mm) to be used and the charge in the reactor were tested in previous experiments. Dilute-acid hydrolysis was performed at temperature

Table 1. Composition of woody biomass (g $kg^{-1}$, dry basis)

| Component | *A. colubrina* [a] | Aspen [b] | *Salix* [c] |
|---|---|---|---|
| Oligosaccharides | | | |
| Glucan | 433 | 477 | 414 |
| Xylan | 156 | 158 | 150 |
| Arabinan | 21 | 5 | 12 |
| Mannan | 14 | 17 | 32 |
| Galactan | 21 | 6 | 23 |
| Lignin | | | |
| Klason lignin | 188 | 258 | 242 |
| Acid soluble lignin | 12 | 12 | 22 |
| Acetyl groups | N.A. | N.A. | 29 |
| Ash | 14 | 12 | 9 |
| Extractives | | | |
| Water | 126 | N.A. | N.A. |
| Ethanol | 17 | N.A. | N.A. |

[a] Current study; [b] source from De Bari et al. [17]; [c] source from Sassner et al. [20].

N.A. not analysed.

range of 180 to 220 °C with a residence time of 300 or 600 s. After hydrolysis, the slurries were cooled by flashing to atmospheric pressure and subsequently separated into two fractions, hydrolysate and fibre residue, by filtration. This procedure was repeated two times at each condition.

The temperature, residence time and catalyst concentration variables in steam pretreatment can be combined in single reaction typically reported as combined severity factor CS (Chum et al., 1990). CS is defined by the following equation

$$CS = log\left(t \times e^{\frac{T - T_{ref}}{14.75}}\right) - pH$$

where $t$ is residence time in minutes, $T$ is pretreatment temperature in °C, and $T_{ref}$ is a reference temperature set to 100 °C.

**Analytical methods**

The composition of *A. colubrina* with respect to carbohydrates, lignin, extractives and ash was determined at the *Instituto de Investigación y Desarrollo de Procesos Químicos* (IIDEPROQ) Laboratory, UMSA, La Paz, Bolivia. The oligomeric andmonomeric sugars of hardwood sawdust were determined according to standard procedure developed by NREL described in (Sluiter et al., 2008c). Extractives were determined by NREL method described in (Sluiter et al., 2008e). Ash was determined by a standard procedure NREL described in (Sluiter et al., 2008a).

**Oligosaccharides determination**

The water insoluble solids (WIS) were separated by filtration after hydrolysis, the filter cakes were washed thoroughly in hot water for 60 min, and the yieldof the fibrous material was determined. Moreover, the composition of the WIS pulp was determined according to NREL standard assay (Sluiter et al., 2008d). In addition, the liquid fractions were analysed for monomeric and oligomeric sugars, cellobiose and by-products (acetic acid, 5-hydroxymethyl furfural and furfural) using high-performance liquid chromatography (HPLC). Sugars and by-products were analysed according to NREL standard assay described in (Sluiter et al., 2008b). All hydrolysates were analysed in duplicate.

## HPLC analysis

All hydrolysates were centrifuged at 12100 x g for 5 min (Mini Spin Plus, Eppendorf, Germany) and filtered through 0.20 mm sterile filters before analysis by HPLC. Cellobiose, glucose, mannose, galactose, xylose and arabinose were analysed on an Aminex HPX-87P column (Bio-Rad laboratories, Hercules, CA, USA) at 85°C. MilliQ-water was used as eluent at a flow rate of 0.6 mL $min^{-1}$. Acetic acid, 5-hydroxymethyl furfural (HMF) and furfural were determined by Aminex HPX-87H column (Bio-Rad laboratories, Hercules, CA, USA) at 60°C eluted with 0.6 mL $min^{-1}$ of 5mM $H_2SO_4$. The analytical HPLC system was an Agilent 1100 (Santa Clara, CA, USA) equipped with a vacuum degasser G1379A (Santa Clara, CA, USA), an isocratic pump G1310A (Santa Clara, CA, USA), a refractive index (RI) detector G1362A (Santa Clara, CA, USA) and an UV-visible wavelength detector G1365B MWD (Santa Clara, CA, USA). All samples were quantified using a refractive index detector with the exception of acetic acid, HMF and furfural, which were quantified using a UV detector at 210 nm.

## Experimental design

In the experimental design, the effects of four variables, temperature (°C), residence time (min), percentage of $H_2SO_4$ (w/w), and moisture content (%), were investigated respect to two response variables, release of hemicellulose sugars (xylose and arabinose) by hydrolysis, and formation of by-products (furfural). Other possible variables, such as liquid-to-solid ratio or particle size, or responses, such as oligomer-to-monomer ratio were not included in the present study. The statistical experimental design was evaluated with Matlab software (V6.5, Mathworks Inc., Natick, MA, USA). The experiments were made in duplicates with a fully randomized run order. Thus, forty pretreatment experiments of *A. colubrina* were tested on two levels, according 24 factorial designs increasing the temperature from 180 to 220°C. The results were statistically analysed by ANOVA analysis for the response variables xylose yield, furfural yield and arabinose yield (expressed in g $100^{-1}$ $g^{-1}$). The conditions of the experiments are listed in table 2.

Table 2. Experimental design for pretreatment of *A. colubrina*

| Factor | Variable | Level | | | | |
|---|---|---|---|---|---|---|
| A* | Temperature (°C) | 180 | 190 | 200 | 210 | 220 |
| B | Time (min) | 5 | | 10 | | |
| C | H2SO4, % (w/w) | 0.5 | | 1.5 | | |
| D | Moisture content (%) | 40 | | 60 | | |

* Range considering in the experimental design: Low A [180-190°C]; moderate low A: [190-200°C]; moderate high A: [200-210°C]; high A: [210-220°C].

**Based on the characterization results, a dry metric ton of *A. colubrina* (as hardwood sawdust) would theoretically produce 329 liters of ethanol from hexoses and 128 liters of pentoses.**

## RESULTS AND DISCUSSION

### Chemical composition of the *A. colubrina*

The chemical composition of *A. colubrina* analysed in the present work is shown in table 1 together with reported values for aspen and *Salix*. The high carbohydrate content makes *A. colubrina* (hardwood) a potential feedstock for production of many other products like synthesis gas, ethanol, methanol, hydrogen and electricity. The glucan content is significantly presented in the woody material that than is comparable with common hardwoods previously investigated (De Bari et al., 2007; Sassner et al., 2008). The glucan fraction of yellow poplar (42.1%), birch (42.5%), willow (37.0%), and eucalyptus (36.0%) is much lower than *A. colubrina*, as is the total carbohydrate content (Eklund et al., 1995; IICA, 2007; Zhu & Pan, 2010; Vivekanand et al., 2013). The hemicellulose matrix in the hardwood is mainly made up and dominated by xylan. This material is to some extent similar to aspen and *Salix*, and it has a glucan content of about 43% and a xylan content of about 16%. Acetyl groups were not analysed but are known to constitute a minor contribution to the total content of hemicellulose (Delgobo et al., 1999). The material has a relatively low lignin content (20%) in comparison to e.g. aspen and *Salix*. The total lignin in aspen and *Salix* are 27.0 and 26.4%, respectively (De Bari et al., 2007; Sassner et al., 2008). The acid-insoluble lignin also constitutes a small part of total lignin value in *A. colubrina* than other hardwoods such as *Salix* and *Eucalyptus regnans* (Dekker, 1987; Sassner et al., 2008). The material contains large amounts of extractives as comparing previous studies of this wood specie (Mota et al., 2017). It is well-known that especially tropical wood contain significant amounts of extractives (Vassilev et al., 2012). Extractives analysis of *A. colubrina* tested show significant differences, where reported high values in contrast to commercial hardwoods (Grohmann et al., 1986). The very low ash of hardwood is also notable. Based on the values in table 1, one dry metric ton of *A. colubrina* would theoretically yield 329 litres ethanol from the hexose sugars and 128 litres from pentose sugars.

### Steam pretreatment of hardwood

The feedstock was then subjected to several steam explosion pretreatments in order to find the most suitable conditions giving a high level of hemicellulose hydrolysis with a small degradation of the cellulosic fraction.

Four factors, namely temperature, sulphuric acid concentration, residence time and moisture content were evaluated. The response measured was xylose and arabinose recovery and furfural formation. These sugars and furfural were selected as examples because the sugars are the most important pentoses and furfural is the main degradation product in hemicellulose pretreatment (Gairola & Smirnova, 2012). The significance of the effects was determined by ANOVA (table 4). All main factors showed a significant effect on the xylose and furfural yields from steam pretreatment of *A. colubrina*, whereas, the residence time did not come out as significant for the arabinose recovery. It was concluded that all variables are important factors to define the best conditions for pentose sugars recovery.

figure 1 shows the predicted relationship between temperature and residence time in the reactor charge (Carrasco, 2013). A significant portion of hemicellulose from *A. colubrina* wood became solubilized during the steam pretreatment. A large proportion of these sugars occurred as monomers rather than oligomers, due to the catalytic activity of the hydronium ions associated with low pH (the measured pH was in the range 1.6 to 2.5). From Fig. 1, the highest xylose yield achieved was around 12 g per 100 g DM at 200°C, i.e. 68 % of the theoretical maximum yield, at 1.5% (w/w) $H_2SO_4$ for 5 min. Overall, the xylose recovery in the current study appeared to be in the expected range for this kind of pretreatment (Ramos et al., 2000; Sassner et al., 2008). The hydrolysis of glucan was low at all conditions studied (table 3). Although not tested in the current study, the efficient removal of most of the hemicellulose is likely to give increased accessibility of the cellulose to the cellulase enzymes and thereby provide a material which can be hydrolysed (Horn & Eijsink, 2010). The major part of the weight loss during dilute acid pretreatment was caused by hydrolysis and solubilisation of hemicellulosic sugars, for averaged reaction times (5-10 min) and temperatures (190-200°C). Comparing to other hardwoods (e.g. poplar hardwood), slightly higher yields of pentose sugars have been obtained than for *A. colubrina* (cf. table 3) (Carrasco et al., 1994). It is noteworthy is that the high resistance to dilute acid hydrolysis exhibited by the pentose fraction of this feedstock biomass, despite the facts that it was subjected to the harshest conditions of hydrolysis. The formation of the by-products, furfural and 5-hydroxymethylfurfural, was low in all hydrolysates obtained (table 3). This indicates that the hydrolysates might not be very inhibitory for fermentation in ethanol production.

## CONCLUSIONS

Under the conditions tested in this study, pretreatment of $H_2SO_4$-impregnated *A. colubrina* for 5-10 min at 200-220°C resulted in pentose-rich hydrolysates. At such conditions, higher temperatures in the steam reactor seemed to impair higher severities to the hardwood material. This was apparent from the amount of glucose released in the liquid phase and lower hemicellulose recovery when pretreatment was carried out for higher severities of 220 °C. The best xylose recovery yield (nearly 70%) was obtained after pretreating 1.5% $H_2SO_4$-impregnated *A. colubrina* for 5 min at 200 °C.

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Table 3. Yield of sugars and by-products after $H_2SO_4$-steam pretreatment of *A. colubrina* (g/100 g of dry wood)

| Run | T | $H_2SO_4$ | τ | MC | CS | Xylose | | |
|---|---|---|---|---|---|---|---|---|
| | (ºC) | (%) | (min) | (%) | | Mono | Oligo | Total |
| 1 | 180 | 0.5 | 5 | 40 | 2.54 | 0.27 | 0.28 | 0.55 |
| 2 | 190 | 0.5 | 5 | 40 | 2.83 | 1.55 | 2.24 | 3.79 |
| 17 | 200 | 0.5 | 5 | 40 | 3.13 | 4.30 | 3.01 | 7.31 |
| 25 | 210 | 0.5 | 5 | 40 | 3.42 | 4.83 | 3.57 | 8.40 |
| 33 | 220 | 0.5 | 5 | 40 | 3.72 | 5.14 | 2.73 | 7.87 |
| | | | | | | | | |
| 3 | 180 | 0.5 | 10 | 40 | 2.84 | 0.06 | 3.07 | 3.13 |
| 4 | 190 | 0.5 | 10 | 40 | 3.13 | 1.13 | 5.44 | 6.57 |
| 18 | 200 | 0.5 | 10 | 40 | 3.43 | 4.63 | 4.51 | 9.14 |
| 26 | 210 | 0.5 | 10 | 40 | 3.72 | 6.66 | 3.84 | 10.50 |
| 34 | 220 | 0.5 | 10 | 40 | 4.02 | 5.88 | 4.56 | 10.44 |
| | | | | | | | | |
| 5 | 180 | 1.5 | 5 | 40 | 2.97 | 0.19 | 1.64 | 1.83 |
| 6 | 190 | 1.5 | 5 | 40 | 3.26 | 2.73 | 6.46 | 9.19 |
| 19 | 200 | 1.5 | 5 | 40 | 3.56 | 3.76 | 4.88 | 8.64 |
| 27 | 210 | 1.5 | 5 | 40 | 3.85 | 5.39 | 2.93 | 8.32 |
| 35 | 220 | 1.5 | 5 | 40 | 4.15 | 5.29 | 2.52 | 7.81 |
| | | | | | | | | |
| 7 | 180 | 1.5 | 10 | 40 | 3.27 | 3.32 | 3.11 | 6.43 |
| 8 | 190 | 1.5 | 10 | 40 | 3.57 | 6.06 | 3.59 | 9.65 |
| 20 | 200 | 1.5 | 10 | 40 | 3.86 | 7.19 | 1.90 | 9.08 |
| 28 | 210 | 1.5 | 10 | 40 | 4.15 | 7.09 | 1.51 | 8.61 |
| 36 | 220 | 1.5 | 10 | 40 | 4.45 | 6.53 | 1.38 | 7.91 |
| | | | | | | | | |
| 9 | 180 | 0.5 | 5 | 60 | 2.54 | 1.09 | 0.48 | 1.57 |
| 10 | 190 | 0.5 | 5 | 60 | 2.83 | 2.06 | 3.73 | 5.79 |
| 21 | 200 | 0.5 | 5 | 60 | 3.13 | 4.03 | 4.30 | 8.33 |
| 29 | 210 | 0.5 | 5 | 60 | 3.42 | 4.35 | 4.17 | 8.52 |
| 37 | 220 | 0.5 | 5 | 60 | 3.72 | 4.63 | 3.36 | 7.99 |
| | | | | | | | | |
| 11 | 180 | 0.5 | 10 | 60 | 2.84 | 2.49 | 3.39 | 5.89 |
| 12 | 190 | 0.5 | 10 | 60 | 3.13 | 6.02 | 4.29 | 10.31 |
| 22 | 200 | 0.5 | 10 | 60 | 3.43 | 5.04 | 3.49 | 8.53 |
| 30 | 210 | 0.5 | 10 | 60 | 3.72 | 5.13 | 3.11 | 8.24 |
| 38 | 220 | 0.5 | 10 | 60 | 4.02 | 4.89 | 2.61 | 7.50 |

| Arabinose | | | Glucose | | | HAc | Furf | HMF |
|---|---|---|---|---|---|---|---|---|
| Mono | Oligo | Total | Mono | Oligo | Total | | | |
| 0.01 | 0.02 | 0.03 | bdl | bdl | bdl | 0.02 | bdl | bdl |
| 0.22 | 0.30 | 0.52 | 0.19 | 0.27 | 0.46 | 0.25 | 0.06 | bdl |
| 0.65 | 0.09 | 0.74 | 0.53 | 0.09 | 0.62 | 0.84 | 0.17 | bdl |
| 0.64 | 0.20 | 0.84 | 0.42 | 0.58 | 1.00 | 1.01 | 0.23 | bdl |
| 0.57 | 0.09 | 0.66 | 0.20 | 0.91 | 1.11 | 2.13 | 0.05 | 0.01 |
| | | | | | | | | |
| 0.01 | 0.16 | 0.17 | 0.01 | 0.04 | 0.04 | 0.02 | 0.02 | bdl |
| 0.45 | 0.14 | 0.58 | 0.01 | 0.29 | 0.30 | 0.88 | 0.24 | bdl |
| 1.07 | 0.15 | 1.22 | 0.09 | 0.62 | 0.72 | 1.47 | 0.34 | 0.01 |
| 0.83 | 0.46 | 1.29 | 0.00 | 0.68 | 0.68 | 2.72 | 0.33 | 0.01 |
| 0.83 | 0.11 | 0.94 | 0.36 | 1.12 | 1.48 | 2.83 | 0.28 | 0.03 |
| | | | | | | | | |
| 0.08 | 0.37 | 0.45 | 0.03 | 0.07 | 0.10 | 0.02 | 0.05 | bdl |
| 0.79 | 0.18 | 0.97 | 0.16 | 0.17 | 0.33 | 0.43 | 0.29 | bdl |
| 0.74 | 0.19 | 0.93 | 0.44 | 0.28 | 0.72 | 0.59 | 0.23 | 0.01 |
| 0.63 | 0.13 | 0.75 | 0.49 | 0.43 | 0.91 | 1.67 | 0.16 | 0.01 |
| 0.30 | 0.35 | 0.65 | 1.62 | 1.07 | 2.69 | 2.60 | 0.11 | 0.03 |
| | | | | | | | | |
| 0.61 | 0.13 | 0.74 | 0.14 | 0.26 | 0.39 | bdl | 0.04 | bdl |
| 0.72 | 0.24 | 0.96 | 0.10 | 0.53 | 0.62 | 1.16 | 0.19 | 0.00 |
| 0.59 | 0.23 | 0.82 | 0.68 | 0.40 | 1.08 | 1.59 | 0.26 | 0.00 |
| 0.57 | 0.20 | 0.75 | 0.91 | 0.91 | 1.83 | 3.77 | 0.34 | 0.01 |
| 0.43 | 0.26 | 0.70 | 1.75 | 1.28 | 3.03 | 4.10 | 0.34 | 0.03 |
| | | | | | | | | |
| 0.60 | 0.13 | 0.74 | bdl | 0.30 | 0.30 | 0.72 | 0.06 | bdl |
| 0.95 | 0.15 | 1.11 | 0.05 | 0.25 | 0.30 | 0.99 | 0.12 | bdl |
| 0.68 | 0.24 | 0.92 | 0.00 | 1.01 | 1.01 | 2.00 | 0.18 | bdl |
| 0.64 | 0.19 | 0.82 | 0.12 | 0.82 | 0.94 | 2.50 | 0.19 | bdl |
| 0.48 | 0.26 | 0.74 | 0.36 | 1.06 | 1.41 | 2.53 | 0.05 | 0.01 |
| | | | | | | | | |
| 0.80 | 0.13 | 0.93 | 0.06 | 0.22 | 0.28 | 1.06 | 0.01 | bdl |
| 0.98 | 0.40 | 1.38 | 0.07 | 0.49 | 0.55 | 2.00 | 0.09 | bdl |
| 1.31 | 0.18 | 1.49 | 0.02 | 0.40 | 0.41 | 2.12 | 0.16 | bdl |
| 0.94 | 0.10 | 1.04 | 0.26 | 0.65 | 0.91 | 2.82 | 0.27 | 0.01 |
| 0.47 | 0.24 | 0.71 | 1.04 | 0.34 | 1.38 | 4.29 | 0.06 | 0.01 |

| Run | T | $H_2SO_4$ | τ | MC | CS | Xylose | | |
|---|---|---|---|---|---|---|---|---|
| | (ºC) | (%) | (min) | (%) | | Mono | Oligo | Total |
| 13 | 180 | 1.5 | 5 | 60 | 2.97 | 3.34 | 6.37 | 9.71 |
| 14 | 190 | 1.5 | 5 | 60 | 3.26 | 4.47 | 7.05 | 11.52 |
| 23 | 200 | 1.5 | 5 | 60 | 3.56 | 7.99 | 4.05 | 12.03 |
| 31 | 210 | 1.5 | 5 | 60 | 3.85 | 7.29 | 2.40 | 9.69 |
| 39 | 220 | 1.5 | 5 | 60 | 4.15 | 7.11 | 1.39 | 8.50 |
| | | | | | | | | |
| 15 | 180 | 1.5 | 10 | 60 | 3.27 | 4.14 | 3.99 | 8.14 |
| 16 | 190 | 1.5 | 10 | 60 | 3.57 | 4.93 | 5.43 | 10.36 |
| 24 | 200 | 1.5 | 10 | 60 | 3.86 | 7.32 | 3.91 | 11.23 |
| 32 | 210 | 1.5 | 10 | 60 | 4.15 | 7.47 | 2.87 | 10.34 |
| 40 | 220 | 1.5 | 10 | 60 | 4.45 | 7.34 | 2.09 | 9.43 |

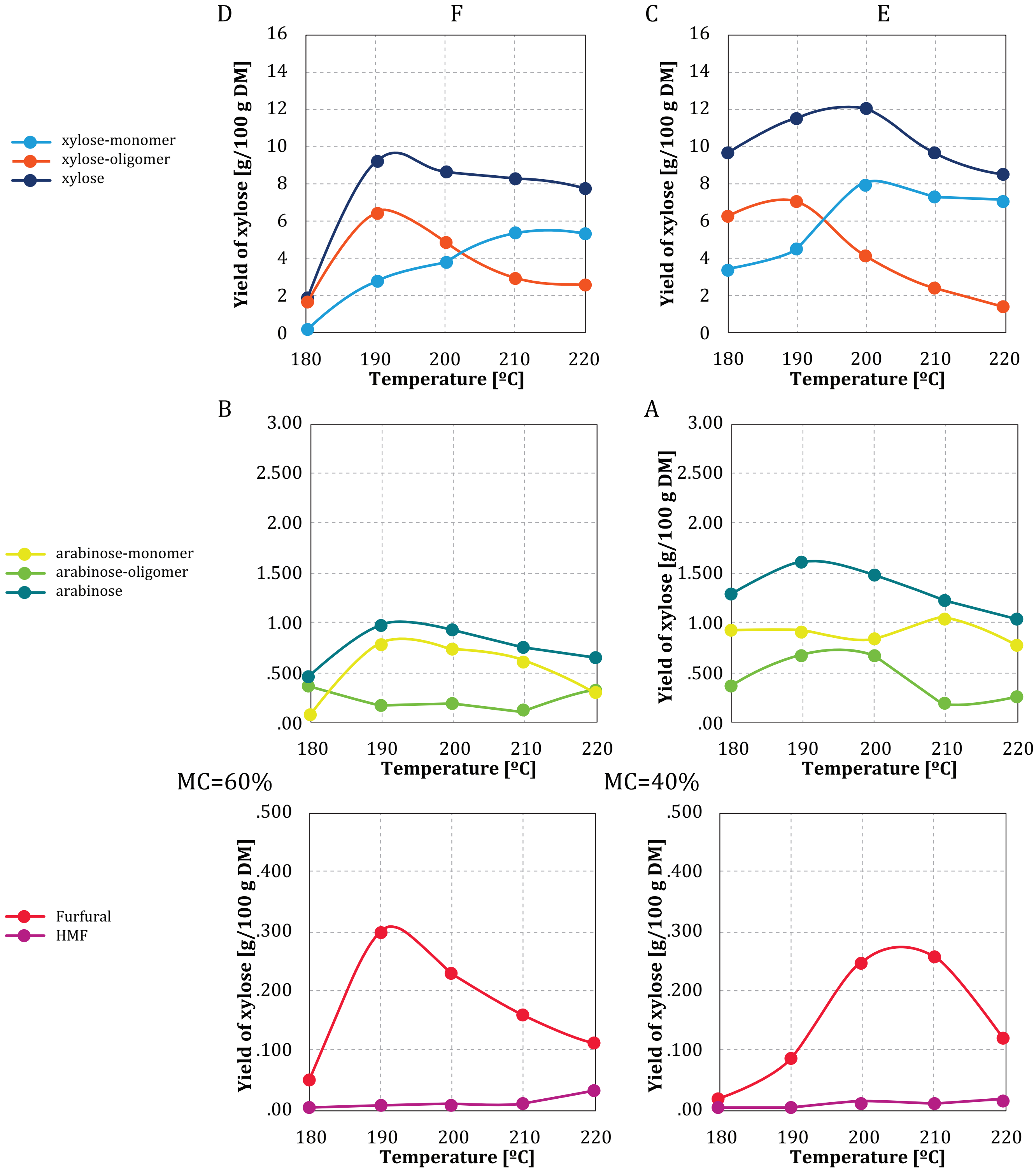
CS: combined severity factor; HAc: acetic acid; Fur: furfural; bdl: below detectable level.
The standard deviation was less than 5% based on duplicate experiments.

| Arabinose | | | Glucose | | | HAc | Furf | HMF |
|---|---|---|---|---|---|---|---|---|
| Mono | Oligo | Total | Mono | Oligo | Total | | | |
| 0.93 | 0.36 | 1.29 | 0.12 | 0.48 | 0.60 | 1.16 | 0.02 | bdl |
| 0.92 | 0.68 | 1.60 | 0.20 | 0.69 | 0.89 | 2.55 | 0.08 | bdl |
| 0.83 | 0.66 | 1.49 | 1.03 | 0.84 | 1.87 | 2.64 | 0.24 | 0.01 |
| 1.03 | 0.20 | 1.23 | 1.42 | 0.68 | 2.11 | 4.31 | 0.26 | 0.01 |
| 0.79 | 0.25 | 1.04 | 2.15 | 1.62 | 3.77 | 4.79 | 0.12 | 0.01 |
| | | | | | | | | |
| 0.56 | 0.37 | 0.93 | 0.10 | 0.56 | 0.66 | 1.12 | 0.01 | bdl |
| 0.81 | 0.38 | 1.19 | 0.11 | 0.72 | 0.83 | 2.76 | 0.19 | bdl |
| 0.80 | 0.16 | 0.96 | 1.09 | 0.56 | 1.64 | 4.26 | 0.33 | bdl |
| 0.62 | 0.29 | 0.91 | 1.29 | 1.17 | 2.46 | 4.53 | 0.33 | 0.02 |
| 0.49 | 0.38 | 0.88 | 2.40 | 1.70 | 4.11 | 4.83 | 0.14 | 0.07 |

Table 4. ANOVA for xylose and arabinose recovery, and furfural formation response from $H_2SO_4$-steam pretreatment

| Effect | Sum of squares | Degrees of Freedom | Mean Squares | F-ratio | P value |
|---|---|---|---|---|---|
| Xylose recovery | | | | | |
| Temperature (ºC) | 229.5665 | 4 | 57.3916 | 23.0627 | $2.7006\times10^{-12}$ |
| $H_2SO_4$, % (w/w) | 72.3240 | 1 | 72.3240 | 29.0632 | $8.4624\times10^{-7}$ |
| Time (min) | 28.9760 | 1 | 28.9760 | 11.6440 | 0.0011 |
| Moisture content (%) | 40.4589 | 1 | 40.4589 | 16.2583 | $1.3579\times10^{-4}$ |
| Error | 179.1723 | 72 | 2.4885 | | |
| Total | 550.4977 | 79 | | | |
| | | | | | |
| Arabinose recovery | | | | | |
| Temperature (ºC) | 1.8886 | 4 | 0.4722 | 8.4431 | $1.2113\times10^{-5}$ |
| $H_2SO_4$, % (w/w) | 0.3166 | 1 | 0.3166 | 5.6614 | 0.0200 |
| Time (min) | 0.0700 | 1 | 0.0700 | 1.2524 | 0.2668 |
| Moisture content (%) | 2.2152 | 1 | 2.2152 | 39.6128 | $2.1522\times10^{-8}$ |
| Error | 4.0264 | 72 | 0.0559 | | |
| Total | 8.5169 | 79 | | | |
| | | | | | |
| Furfural formation | | | | | |
| Temperature (ºC) | 0.5243 | 4 | 0.1311 | 31.7623 | $3.0219\times10^{-15}$ |
| $H_2SO_4$, % (w/w) | 0.0373 | 1 | 0.0373 | 9.0349 | 0.0036 |
| Time (min) | 0.0691 | 1 | 0.0691 | 16.7538 | $1.0982\times10^{-4}$ |
| Moisture content (%) | 0.0353 | 1 | 0.0353 | 8.5536 | 0.0046 |
| Error | 0.2971 | 72 | 0.0041 | | |
| Total | 0.9631 | 79 | | | |

Fig. 1 Pentose sugars and furans yields when A. colubrina hardwood is hydrolysed for different reaction temperatures. At 40% of moisture content: xylose in oligomeric and monomeric forms (A); arabinose in oligomeric and monomeric forms (C); furfural and HMF (E). At 60% of moisture content: xylose in oligomeric and monomeric forms (B); arabinose in oligomeric and monomeric forms (D); furfural and HMF (F).



## REFERENCES

Cao, X. 2003. Climate change and energy development: implications for developing countries. Resources Policy, 29(1–2), 61-67.

Carrasco, C. 2013. Bioethanol Production from Lignocellulosic Materials: Some studies on Sugarcane Bagasse, Paja Brava, Wheat Straw, Quinoa Stalks and Curupaú, Ph.D. thesis, Lund University.

Carrasco, C., Baudel, H.M., Sendelius, J., Modig, T., Roslander, C., Galbe, M., Hahn-Hägerdal, B., Zacchi, G., Lidén, G. 2010. SO2-catalyzed steam pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse. Enzyme and Microbial Technology, 46(2), 64-73.

Carrasco, J.E., Sáiz, M., Navarro, A., Soriano, P., Sáez, F., Martinez, J.M. 1994. Effects of dilute acid and steam explosion pretreatments on the cellulose structure and kinetics of cellulosic fraction hydrolysis by dilute acids in lignocellulosic materials. Applied Biochemistry and Biotechnology, 45-46(1), 23-34.

Chum, H., Johnson, D., Black, S., Overend, R. 1990. Pretreatment-Catalyst effects and the combined severity parameter. Applied Biochemistry and Biotechnology, 24-25(1), 1-14.

De Bari, I., Nanna, F., Braccio, G. 2007. SO2-Catalyzed Steam Fractionation of Aspen Chips for Bioethanol Production: Optimization of the Catalyst Impregnation. Industrial & Engineering Chemistry Research, 46(23), 7711-7720.

de Campos, C.P., Muylaert, M.S., Rosa, L.P. 2005. Historical CO2 emission and concentrations due to land use change of croplands and pastures by country. Science of The Total Environment, 346(1–3), 149-155.

Dekker, R.F.H. 1987. The Utilization Of Autohydrolysis-Exploded Hardwood (Eucalyptus Regnans) And Sofiwood (Pinus Radiata) Sawdust For The Production Of Cellulolytic Enzymes And Fermentable Substrates. Biocatalysis and Biotransformation, 1(1), 63-75.

Delgobo, C.L., Gorin, P.A.J., Jones, C., Iacomini, M. 1998. Gum heteropolysaccharide and free reducing mono- and oligosaccharides of Anadenanthera colubrina. Phytochemistry, 47(7), 1207-1214.

Delgobo, C.L., Gorin, P.A.J., Tischer, C.A., Iacomini, M. 1999. The free reducing oligosaccharides of angico branco (Anadenanthera colubrina) gum exudate: an aid for structural assignments in the heteropolysaccharide. Carbohydrate Research, 320(3–4), 167-175.

Ebringerová, A., Hromádková, Z., Heinze, T. 2005. Hemicellulose. in: Polysaccharides I, (Ed.) T. Heinze, Vol. 186, Springer Berlin Heidelberg, pp. 1-67.

ECLAC, FAO, IICA. 2013. The Outlook for Agriculture and Rural Development in the Americas: A perspective on Latin America and the Caribbean. IICA, Santiago, Chile.

Eklund, R., Galbe, M., Zacchi, G. 1995. The influence of SO2 and H2SO4 impregnation of willow prior to steam pretreatment. Bioresource Technology, 52(3), 225-229.

Emmel, A., Mathias, A.L., Wypych, F., Ramos, L.P. 2003. Fractionation of Eucalyptus grandis chips by dilute acid-catalysed steam explosion. Bioresource Technology, 86(2), 105-115.

Gairola, K., Smirnova, I. 2012. Hydrothermal pentose to furfural conversion and simultaneous extraction with SC-CO2 – Kinetics and application to biomass hydrolysates. Bioresource Technology, 123(0), 592-598.

Galbe, M., Zacchi, G. 2007. Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production. in: Biofuels, (Ed.) L. Olsson, Vol. 108, Springer Berlin Heidelberg, pp. 41-65.

Grohmann, K., Torget, R., Himmel, M. 1986. Optimization of dilute acid pretreatment of biomass. Biotechnol. Bioeng. Symp., 15(Copyright (C) 2013 American Chemical Society (ACS). All Rights Reserved.), 59-80.

Horn, S.J., Eijsink, V.G.H. 2010. Enzymatic Hydrolysis of Steam-Exploded Hardwood Using Short Processing Times. Biosci., Biotechnol., Biochem., 74(6), 1157-1163.

IICA. 2007. Agroenergy and biofuels Atlas of the Americas: I. Ethanol. Inter-American Institute for Cooperation on Agriculture ed. IICA, San Jose, Costa Rica.

Josefsson, T., Lennholm, H., Gellerstedt, G. 2002. Steam Explosion of Aspen Wood. Characterisation of Reaction Products. in: Holzforschung, Vol. 56, pp. 289.

Juslin, H., Hansen, E. 2002. Strategic marketing in the global forest industries. Authors Academic Press.

Mackie, K.L., Brownell, H.H., West, K.L., Saddler, J.N. 1985. Effect of Sulphur Dioxide and Sulphuric Acid on Steam Explosion of Aspenwood. Journal of Wood Chemistry and Technology, 5(3), 405-425.

McMillan, J.D. 1994. Pretreatment of Lignocellulosic Biomass. in: Enzymatic Conversion of Biomass for Fuels Production, Vol. 566, American Chemical Society, pp. 292-324.

Mota G.S., Sartori C.J., Miranda I., Quilhó T., Mori F.A., Pereira H. 2017. Bark anatomy, chemical composition and ethanol-water extract composition of Anadenanthera peregrina and Anadenanthera colubrina. PLoS ONE, 12(12).

Prado, D.E., Gibbs, P.E. 1993. Patterns of Species Distributions in the Dry Seasonal Forests of South America. Annals of the Missouri Botanical Garden, 80(4), 902-927.

Ramos, L.P., Carpes, S.T., Silva, F.T., Ganter, J.L.M. 2000. Comparison of the susceptibility of two hardwood species, Mimosa scabrella Benth and Eucalyptus viminalis labill, to steam explosion and enzymatic hydrolysis. Brazilian Archives of Biology and Technology, 43, 195-206.

Sassner, P., Mårtensson, C.G., Galbe, M., Zacchi, G. 2008. Steam pretreatment of H2SO4-impregnated Salix for the production of bioethanol. Bioresource Technology, 99(1), 137-145.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2008a. Determination of ash in biomass laboratory analytical procedure (LAP) : issue date, 7/17/2005, National Renewable Energy Laboratory. Golden, Colorado.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2008b. Determination of sugars, byproducts, and degradation products in liquid fraction process samples laboratory analytical procedure (LAP) : issue date, 12/08/2006, National Renewable Energy Laboratory. Golden, Colorado.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008c. Determination of structural carbohydrates and lignin in biomass laboratory analytical procedure (LAP) : issue date, 4/25/2008, National Renewable Energy Laboratory. Golden, Colorado.

Sluiter, A., Hyman, D., Payne, C., Wolfe, J. 2008d. Determination of insoluble solids in pretreated biomass material laboratory analytical procedure (LAP) : issue date, 03/21/2008, National Renewable Energy Laboratory. Golden, Colorado.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2008e. Determination of extractives in biomass laboratory analytical procedure (LAP) : issue date, 7/17/2005, National Renewable Energy Laboratory. Golden, Colorado.

Vassilev, S.V., Baxter, D., Andersen, L.K., Vassileva, C.G., Morgan, T.J. 2012. An overview of the organic and inorganic phase composition of biomass. Fuel, 94(0), 1-33.

Vivekanand, V., Olsen, E.F., Eijsink, V.G.H., Horn, S.J. 2013. Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. Bioresource Technology, 127(0), 343-349.

Zhan, F.B., Chen, X., Noon, C.E., Wu, G. 2005. A GIS-enabled comparison of fixed and discriminatory pricing strategies for potential switchgrass-to-ethanol conversion facilities in Alabama. Biomass and Bioenergy, 28(3), 295-306.

Zhu, J.Y., Pan, X.J. 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. Bioresource Technology, 101(13), 4992-5002.