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Sugarcane bagasse mild alkaline fractionation and production of purified fractions by pulse chromatography with water

Vincent Oriez^{a,*}, Marlène Beyerle^b, Pierre-Yves Pontalier^{a,*}, Jérôme Peydecastaing^a

^a Laboratoire de Chimie Agro-Industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

^b Novasep Process, 5 chemin du Pilon, 01700 St-Maurice de Beynost, France

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ABSTRACT

Sugarcane bagasse (SCB) was treated under mild alkaline conditions (solid:liquid ratio of 1:20 (w/v), 1.5% NaOH (w/v), 60 °C, 6h) to fractionate the lignocellulose in order to produce a typical mild alkaline extract from a lignocellulosic biomass. The solid residue was enriched in cellulose, while the SCB alkaline extract contained lignin and hemicelluloses, but also inorganic salts, five phenolic monomers and acetic acid. After concentration of the alkaline extract by evaporation, low pressure chromatography with water as eluent was performed to produce purified fractions. Two different strong acid cation (SAC) exchange resins were tested: one gel-type resin and one macroporous-type resin. The lignin and hemicelluloses were separated from the inorganic salts by the gel-type SAC exchange resin. On this resin, the phenolic monomers were partitioned regarding the presence or absence in their structure of a carboxyl group. On the macroporous-type SAC exchange resin, the largest sugar oligomers and lignin oligomers were obtained in a fraction free of inorganic salts, phenolic monomers and acetic acid.

1. Introduction

Sugarcane was the most produced crop in the world in 2013 with 1.9 billion tons (FAO, 2015). Sugarcane bagasse (SCB) is a lignocellulosic by-product of the sugar and alcohol industry from sugarcane, and is nowadays mainly burnt to produce electricity. However, in the last decade, SCB has been widely studied as a substrate to produce ethanol by fermentation of the glucose coming from the cellulose, or of the other C6 and C5 sugars coming from the hemicelluloses (Cardona et al., 2010). The pretreatment of the incoming lignocellulosic material into the second generation ethanol biorefinery, consisting in the separation of the three main components, cellulose, hemicelluloses and lignin, is a key step for economic viability and environmental efficiency in the overall process (Mosier et al., 2005; Yang and Wyman, 2008). Acidic conditions for the pretreatment were extensively studied and have been applied industrially for twenty years, and present the advantage of obtaining monomeric sugars in a single step process (Farone and Cuzens, 1997; Mosier et al., 2005).

Chromatography was investigated to purify the monomeric sugars resulting from lignocellulosic biomass treatment by concentrated acid (usually H₂SO₄ at 70–75%), using gel type SAC exchange resin under H⁺ form and water as eluent (Neuman et al., 1987; Hester et al., 1995). Monomeric sugars can be separated from sulfuric acid and other

impurities such as acetic acid, furfural and hydroxymethylfurfural (HMF) (Heinonen and Sainio, 2010). Once the acid and other impurities are removed, the mixture of sugars can be purified by another chromatographic step with water as eluent. When the monomeric sugars are a mixture of glucose, xylose and arabinose, gel type SAC resin with Ca²⁺ as counter ion was found to be the most efficient resin for their separation (Caruel et al., 1991; Lei et al., 2010; Chen et al., 2018). However, prior to this second chromatographic step, the extract had to undergo decationization through ion exchange and neutralization with the addition of NaOH inducing extra economic and environmental cost to the process (Lodi et al., 2017).

Inspired from pulp and paper processes, alkaline pretreatment is gaining importance in the second generation ethanol biorefinery (Hayes, 2009) due to improved overall ethanol yields (Saha and Cotta, 2007; Kim et al., 2016), mild reaction conditions and possible valorization of the solubilized lignin and hemicelluloses (Cardona et al., 2010; Kim et al., 2016). Among the different alkaline pretreatments mentioned in the literature, mild sodium hydroxide conditions appear to lead to the highest lignin and hemicelluloses extraction yields at reasonable costs (Peng et al., 2012; Kim et al., 2016). These conditions also induce the hydrolysis of ester bonds between hemicelluloses and lignin, phenolic monomers and lignin, and acetate groups from hemicelluloses (Xiao et al., 2001; Chen et al., 2012). Purifying the alkaline

* Corresponding authors.

E-mail addresses: vincentoriez@yahoo.fr (V. Oriez), pierre Yves.pontalier@ensiacet.fr (P.-Y. Pontalier).

extract components to enable their further valorization is of major importance to give value to the whole process of lignocellulosic ethanol production after alkaline pretreatment (Ragauskas et al., 2014).

Recovery of lignin or hemicelluloses from lignocellulosic alkaline extracts (black liquors in the pulp and paper industry) have been investigated by acid precipitation (Uloth and Wearing, 1989; Sun and Tomkinson, 2001), ethanol precipitation (Peng et al., 2009; Bian et al., 2012) and membrane filtration (Uloth and Wearing, 1989; Wallberg et al., 2003). However, purification through precipitation led to high chemical consumption, while membrane filtration generated fractions of mediocre purity due to difficult salt removal. Resin adsorption process has also been investigated either for the production of pure phenolic compounds such as *p* coumaric acid (*p* CA) (Ou et al., 2009) and ferulic acid (FA) (Ou et al., 2007), or for hemicelluloses purification (Zeitoun et al., 2010). These operations can lead to high economic and environmental costs through a significant consumption of chemicals and numerous process steps loading, rinsing, desorption, regeneration, equilibration and so far, no industrial development has been reported.

Chromatography is an interesting alternative purification technique, implying both size exclusion and ionic repulsion phenomena. It presents the advantage of using only one eluent and an easier process set up loading, elution both for batch (pulse chromatography) and continuous process (Sequential Moving Bed). However, unlike for lignocellulosic acid extracts, very few studies can be found on chromatography to purify lignocellulosic alkaline extracts. In the case of liquors from soda anthraquinone pulping process, separation was not performed directly on the alkaline extract. The media was first treated with acid until pH 1.2 to precipitate the lignin, then by chromatography on SAC exchange resin with water as eluent at 65 °C to specifically separate aliphatic carboxylic acids from sodium sulfate (Alén et al., 1991). More recently, chromatography was tested on a corn stover alkaline extract, but mesoporous silica materials were used as stationary phase, acidic water or organic solvent as mobile phase and the goal was to specifically separate monomeric C5 sugars from monomeric C6 sugars (Modenbach, 2013).

This paper focuses on the purification of raw SCB extract, obtained under mild alkaline conditions, to give a higher value to the overall biorefinery scheme. Pulse chromatography, using water as mobile phase and SAC exchange resins as adsorbents was studied in order to produce purified fractions from the SCB alkaline extract, composed mainly of lignin oligomers, hemicelluloses, acetic acid, phenolic monomers and inorganic salts.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide ($\geq 98.5\%$), sulfuric acid 72% for analytical hydrolysis, sulfuric acid 95% and acetonitrile ($\geq 99.9\%$) to prepare HPLC eluents, and methanol ($\geq 99.8\%$) used as a tracer for column void volume, were purchased from VWR. Calcium carbonate ($\geq 98.5\%$) was purchased from Merck. HPLC standards: D (+) cellobiose ($\geq 98\%$), D (+) glucose ($\geq 99.5\%$), D (+) galactose, L (+) arabinose (99%), D (+) xylose (99%), D (+) mannose ($\geq 99\%$), fructose ($\geq 99\%$), acetic acid ($\geq 99\%$), furfural (99%), 5 hydroxymethyl 2 furfuraldehyde (99%), gallic acid (97%), 4 hydroxybenzoic acid ($\geq 99\%$), caffeic acid ($\geq 98\%$), vanillic acid (97%), syringic acid ($\geq 95\%$), 4 hydroxybenzaldehyde (98%), vanillin (99%), *p* coumaric acid ($\geq 98\%$), syringaldehyde (99%), *trans* ferulic acid ($\geq 99\%$), sinapic acid ($\geq 98\%$), *trans* 3 hydroxycinnamic acid (99%), were all purchased from Sigma Aldrich. Blue Dextran, 2,000,000 Da molecular weight, came from Sigma Aldrich too. Both SAC exchange resins, XA2004 30Na⁺ and XA2054Na⁺ (Table 1) were provided by Novasep Process, France.

Table 1

Characteristics of the resins.

	XA2004-30Na ⁺	XA2054Na ⁺
Nature	SAC	SAC
Matrix	Styrene + DVB	Styrene + DVB
Type	Gel (pore size: 3 nm)	Macroporous (max pore size: 20–50 nm)
Active site	-SO ₃	-SO ₃
Capacity	1.4 Eq/L	1.1 Eq/L

2.2. Alkaline extraction

Dry SCB was provided by eRcane (La Réunion, France) and ground on a 2 mm mesh by a knife mill (Mill F6 N V, Electra). The alkaline extraction conditions are based on Sun et al., (1995) to optimize the extraction yield of lignin and hemicelluloses (Sun et al., 1995). The conditions were the following: 150 g of SCB in 3 L of sodium hydroxide solution at 1.5% (w/v) in a 4 L jacketed glass reactor, leading to a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 0.3:1 (w/w), under continuous stirring (200 rpm) for 6 h at 60 °C. The SCB solid residue was removed from the alkaline extract on Whatman filters grade 3 (150 mm diameter) on a Büchner filtration device, then dried at 50 °C for 48 h and finally ground by a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analysis. The filtrated alkaline extract was concentrated by Rotavap at 55 °C under 100 mbar. A dry solid content of at least 20% is generally required to reach a good productivity on the chromatographic purification step and make it economically viable at industrial scale.

2.3. Pulse tests

Pulse tests were run on 500 mL resin packed in a 1 m high and 26 mm diameter jacketed glass column. Two resins were tested, their characteristics are indicated in Table 1. Both resins are under Na⁺ form as the main cation in the alkaline extract is Na⁺ due to the sodium hydroxide introduced during the extraction step. The resin was mixed with water at 60 °C for degassing and packed from the top of the column. The upper piston was brought as close as possible to the top of resin to minimize the dead volume. A Y valve successively enabled the injection of 5 mL feed (Blue Dextran, methanol, synthetic solutions or concentrated alkaline extract) or eluent (distilled water) on top of the column. The eluent was circulated from the top to the bottom of the column thanks to a peristaltic pump and its volume was accounted as resin Bed Volume (BV). The temperature of the column was maintained at 40 °C thanks to a water bath. At the outlet of the column, a fraction collector (GradiFrac, from Pharmacia Biotech) was set to collect 15 mL fractions representing 0.03 BV. The collected samples were analyzed after the run was completed. Blue Dextran (Sigma Aldrich, France) at 0.1% (w/v), was used to determine the void volume of the resin bed (i.e., inter particles porosity), as it cannot enter the pores of the resins (2,000 kDa molecular weight) or interact with the resin matrix (Ladisch, 2001). A pulse test was also run with methanol at 5% (v/v) in order to determine the total void volume of the resin bed (i.e., inter and intra particles porosity), since methanol, a small uncharged molecule, can penetrate all the pores of the resins without adsorbing on the styrene DVB matrix of the resins thanks to its polarity (Lodi et al., 2017).

2.4. Analytical methods

2.4.1. Dry solid and ash

Dry solid (DS) content was gravimetrically determined at 103 °C for 12 h and ash content at 500 °C for 12 h. The conductivity (mS/cm) was measured for every fractions of the pulse tests and converted into ash concentration (g/L) from a linear relationship with a coefficient of 0.443.

2.4.2. Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008), Acid Insoluble Lignin (AIL) was gravimetrically quantified and Acid Soluble Lignin (ASL) was determined at 240 nm using an absorptivity constant of 25 L/g/cm. High Performance Liquid Chromatography (HPLC) on a Rezex RPM Monosaccharide Pb⁺² 300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RPM Monosaccharide Pb⁺² 50 x 7.8 mm guard column (Phenomenex) was used to quantify cellobiose, glucose, xylose, galactose, arabinose, mannose and fructose coming from the acidic hydrolysis of cellulose (yields glucose and potentially cellobiose if the hydrolysis is not complete), hemicelluloses (yields all C5 and C6 sugars) or residual sucrose. Prior to the injection, the samples were filtered on SPE cartridge ABW (Phenomenex) to remove the salts and avoid interference with the sugar peaks. Isocratic conditions were used with Milli Q water at 0.6 mL/min, the injection volume was 20 µL, the column was maintained at 80 °C and the RI detector at 50 °C. For the alkaline extract and the purified samples, HPLC on a Rezex RHM Monosaccharide H⁺ 300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RHM Monosaccharide H⁺ 50 x 7.8 mm guard column (Phenomenex) was used to quantify glucose, xylose, arabinose, acetic acid, furfural and HMF (Sluiter et al., 2006). Unlike with the RPM column, salts did not interfere with sugar peaks on the RHM column, therefore no desalting of the samples was required prior to the injection. Isocratic conditions were applied with 5 mmol/L H₂SO₄ at 0.6 mL/min, the injection volume was 50 µL, the column was maintained at 65 °C and the RI detector at 50 °C. Since solid residue, alkaline extract and purified fractions were very alkaline, the NREL protocol was adapted to ensure total hydrolysis of the sugar oligomers under acidic conditions. 150 mg of solid residue were analyzed instead of 300 mg (Sluiter et al., 2008) and liquid samples (Sluiter et al., 2006) were diluted by 4 with distilled water before acid hydrolysis.

2.4.3. Monomeric sugars and hemicelluloses acetyl groups

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of HPLC eluent with the RHM column, then the extract was analyzed on the RHM column without running NREL protocol (Sluiter et al., 2006). pH adjusted samples directly injected on HPLC enabled the quantification of monomeric sugars and free acetic acid, whereas samples analyzed through NREL protocol gave the total amount of sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

2.4.4. Phenolic monomers

Quantification of twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) gallic acid, 4 hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4 hydroxybenzaldehyde (4HBA), vanillin, *p* CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid was studied by HPLC on an OmniSpher 3 C18 100 × 4.6 column (Agilent Technologies). The gradient was as follow: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min and the column was equilibrated for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10 µL and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) prior to injection.

2.4.5. Pulse chromatography tracers

The Blue Dextran concentration was monitored by UV vis at 620 nm. The methanol concentration was followed by HPLC on Rezex RHM column under isocratic conditions with 5 mmol/L H₂SO₄ at 0.6 mL/min, the injection volume was 50 µL, the column was maintained at 60 °C and the RI detector at 50 °C.

Table 2a

Retention time and response factor of identified components in the sugarcane bagasse on Rezex RPM column with RI detector.

Component	Retention time (min)	Response factor
Glucose	13.5	4.339
Xylose	14.4	4.086
Arabinose	16.4	4.128

3. Results and discussion

3.1. Alkaline extraction

HPLC analysis of raw SCB on the Rezex RPM column revealed the presence of three main sugars: glucose, xylose and arabinose. Traces of galactose and mannose were detected but their concentrations were too low to enable a reliable follow up at the extraction and purification stages. In other studies on SCB from China, galactose and mannose have been quantified in hemicelluloses at levels of about 2 to 3% and traces to 1%, respectively (Sun et al., 2004; Cheng et al., 2008). Fructose was not found in the raw SCB. The retention time and response factor of all the detected components on the Rezex RPM and RHM HPLC columns are presented in Table 2a 2b.

For every compound, the mass balance between the inlet (SCB) and the outlet (solid residue and alkaline extract) was close to 100% (Table 3). Glucan was almost fully recovered in the solid residue (95%) and its purity increased from 35.9 to 43.6%. Under the alkaline extraction conditions employed in this work, 22% of the xylan, 50% of the arabinan and 46% of the lignin were recovered in the alkaline extract. SCB hemicelluloses are composed of a xylan backbone on which arabinosyl substituents are bond (Sun et al., 2004), the difference between xylan and arabinan yields found in our work suggested that the branched portions of hemicelluloses were preferably extracted compared to the xylose backbone. Besides, no significant quantity of furfural and HMF were detected in the alkaline extract, confirming that the alkaline extraction conditions did not produce these sugar degradation products. Indeed, furfural and HMF come from C5 and C6 sugars degradation, respectively, mainly under acidic conditions (Mosier et al., 2005). Most importantly, unlike in lignocellulosic acid extracts, no sugar monomers were found in the mild alkaline extract. In this extract, glucose, xylose and arabinose were under oligomeric form: glucan, xylan and arabinan, respectively. The same amount of free acetic acid and total acetic acid was found in the extract, indicating that all the acetate groups have been released from the solubilized hemicelluloses. Once released from the hemicelluloses in the alkaline extract, acetic acid concentration (8.1 g/L) was about 40% of the xylan concentration (19.4 g/L) and higher than the concentration of glucan (3.2 g/L) and arabinan (5.3 g/L). We could also consider that under the alkaline conditions used in this work, ester bonds linking *p* CA and FA to hemicelluloses were all broken (Sun et al., 2004).

Out of the twelve phenolic monomers tested, five of them (VA, 4HBA, vanillin, *p* CA, FA) were present in detectable quantities in the alkaline extract. The retention time and response factor of the detected phenolic monomers are presented in Table 2c. They accounted for 4.1%

Table 2b

Retention time and response factor of identified components in the sugarcane bagasse alkaline extract on Rezex RHM column with RI detector.

Component	Retention time (min)	Response factor
Glucose	10.8	11.357
Xylose	11.5	11.027
Arabinose	12.4	11.749
Acetic acid	16.5	8.023
HMF	30.6	13.422
Furfural	42.8	13.101

Table 2c

Retention time and response factor of identified components in the sugarcane bagasse alkaline extract on Omnispher 3 C18 column with UV detector at 280 nm.

Component	Retention time (min)	Response factor
VA	6.5	0.515
4HBA	8.2	2.187
Vanillin	11.2	1.228
<i>p</i> -CA	14.2	1.445
FA	19.0	0.852

of the DS of the alkaline extract, *p* CA being the main phenolic monomer with 3.6% of the DS (Table 3).

After concentration of the mild alkaline extract on Rotavap from 2.6% to 19.2% DS content at 55 °C, no significant difference was observed in the composition of the alkaline extract and the concentrate (Table 3) and no degradation products were observed in the HPLC chromatograms. A progressive increase of pH by 0.75, at 55 °C did not hydrolyze sugars and phenolic oligomers nor degrade phenolic monomers. Concentrated SCB alkaline extract was composed of five major pools of molecules: 92.4 g/L salts, 38.5 g/L AIL, 27.9 g/L oligomeric sugars (19.4 g/L xylan, 5.3 g/L arabinan, 3.2 g/L glucan), 8.6 g/L phenolic monomers and 8.1 g/L acetic acid. Different concentration of these five same groups of molecules is unlikely to change their separation behavior by chromatography as demonstrated with pulse test run on SAC resin with lignocellulosic acid hydrolysates (Chen et al., 2018).

Hence, this SCB alkaline extract constituted a model lignocellulosic alkaline extract in order to study the separation of the solubilized molecules by low pressure chromatography.

3.2. Pulse tests

The mass balance was established between the inlet of the column (feed) and the outlet (the sum of the purified fractions) for every compound of every pulse test. All mass balances ranged between 90 and 110% indicating that there is no significant adsorption of any compound on the resins. Only ASL mass balance was largely over 100%, because the polystyrene divinylbenzene backbone of the resins released compounds absorbing at 240 nm, so ASL was not presented in the results.

Blue Dextran pulse test showed a column void volume (i.e., inter particle porosity) of about 0.38 BV for both resins, with symmetric peaks, the front of the peak appearing at 0.33 BV due to diffusion

phenomena (Fig. 1). During the different pulse tests, all the molecules exiting the column at this BV were excluded from the resin pores either due to size exclusion or ionic repulsion. The pulse tests with methanol showed that the total void volume (i.e., inter and intra particle porosity) was 0.71 BV for the gel type resin and 0.83 BV for the macroporous type resin. The molecules eluting from 0.38 to 0.71 BV and from 0.38 to 0.83 BV for the gel type and the macroporous type resins, respectively, can penetrate from a partial to a full extent into the resin pores.

3.2.1. Molecules separation

Sugar oligomers, AIL and phenolic monomers with a carboxyl group (VA, *p* CA and FA) were eluted at 0.38 BV and can be separated from salts, acetic acid and phenolic monomers with no carboxyl group (vanillin and 4HBA), on the XA2004 30Na⁺ resin with an eluent velocity of 2.26 m/h (Fig. 2). In the fraction eluted before 0.42 BV, 78% of the xylan, 72% of the arabinan, 75% of the glucan and 80% of the AIL were recovered while only 21% of the inorganic salts (i.e., ash) were recovered in this fraction. The phenolic monomers, VA, *p* CA and FA were mainly recovered before 0.42 BV with yields of 76, 73 and 71%, respectively, whereas 4HBA and vanillin were mostly recovered in the fraction eluted after 0.42 BV with 78 and 74%, respectively. FA molecule size is 1.01 nm (Kim et al., 2013) and the other phenolic monomers might be approximately at that size based on their molecular weights (between 122 and 194 g/mol) and structure (presence of a phenol group), thus they can potentially diffuse into the resin pores (3 nm). However, phenolic monomers with a carboxyl group (VA, *p* CA and FA with pKa of 4.53, 4.64 and 4.58, respectively) were excluded from the resin pores, certainly due to ionic repulsion as they were negatively charged at pH 13.7 in the concentrated alkaline extract and the SAC resin carried negatively charged sulfonate groups. Phenolic monomers with no carboxyl group (vanillin and 4HBA) exited the column later, they were able to penetrate into the resin pores as they are uncharged. This behavior difference between phenolic monomers with a carboxyl group and those without a carboxyl group is particularly marked with vanillin and vanillic acid as they differ by only 16 g/mol (O atom) and a functional group (aldehyde for vanillin and carboxyl for vanillic acid). Acetic acid was partitioned in the two fractions, with 43% yield in the fraction eluted before 0.42 BV. Acetic acid carried a negative charge at alkaline pH (pKa = 4.76), but exited the column later (0.42 BV) than charged phenolic monomers, showing its ability to penetrate at least partially into the resin pores. Acetic acid is smaller (0.44 nm molecular diameter) than the phenolic monomers (Marcus, 1998), which could explain that it was able to enter some of the resin pores even if negatively charged. Inorganic salts (mainly sodium hydroxide) eluted at

Table 3

SCB, solid residue, alkaline extract and concentrated alkaline extract composition and extraction yields.

Components	SCB	Solid Residue		Alkaline Extract		Concentrate
	Composition	Composition	Yield	Composition	Yield	Composition
DS	92.5	96.4	59	2.6	35	19.2
Ash	9.9	19.7	36	56.1	61	53.6
Glucan	35.9	43.6	95	1.5	2	1.5
Xylan	19.4	16.6	67	9.4	22	9.0
Arabinan	2.3	1.6	53	2.5	50	2.5
AIL	21.6	13.8	50	21.1	45	18.0
ASL	5.5	3.8	54	6.2	52	5.2
Total Lignin	27.1	17.7	51	27.3	46	23.2
VA				traces		traces
4HBA				0.1		0.1
Vanillin				0.1		0.1
<i>p</i> -CA				3.6		3.4
FA				0.4		0.4
Total phenolic monomers				4.1		4.0
Mass Closure	95.2	99.1		98.9		92.4

All the values are calculated based on percentage of dry solid. All the analyses were run in duplicate, standard deviation was at most 1%.

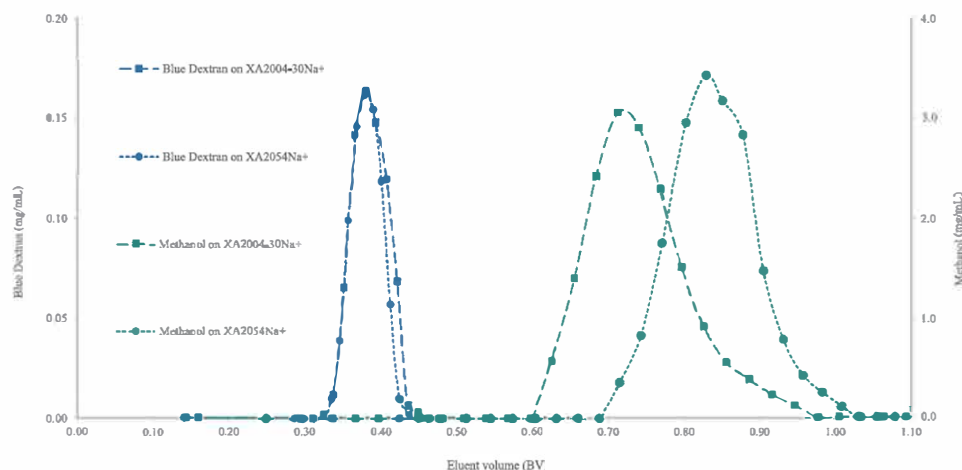


Fig. 1. Blue Dextran and methanol pulse tests at 2.26 m/h on the gel-type (XA2004-30Na+) and the macroporous-type (XA2054Na+) SAC exchange resins.

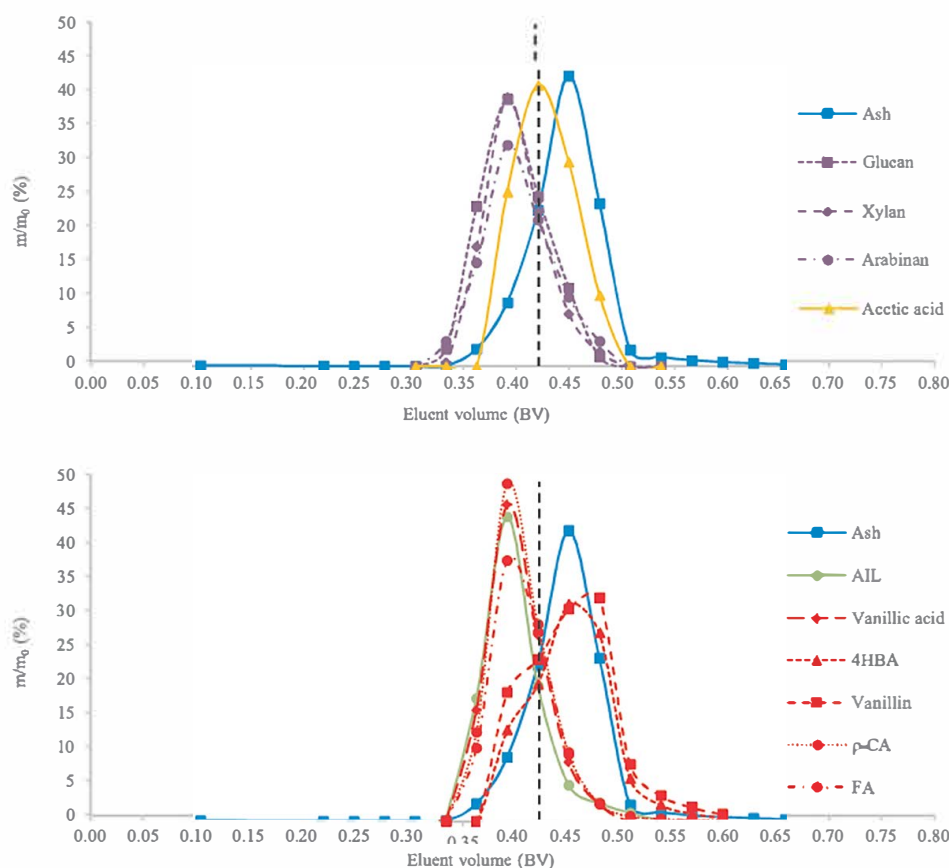


Fig. 2. Concentrated alkaline extract pulse test on XA2004-30Na+ with water as eluent at 2.26 m/h, with m the mass of a given compound in the fraction and m_0 its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV.

0.45 BV. The form of the peak is asymmetric with a dispersive front; it indicates that the resin was saturated with salts due to their high concentration in the alkaline extract (Ladisch, 2001), the same phenomenon was observed on synthetic solutions (Figs. 3 and 4B).

In order to confirm the behavior of the phenolic monomers, several pulse tests were run with synthetic solutions. With a solution of p -CA at 4.79 g/L and NaOH added to adjust the pH to 13.7 (pH of the concentrated alkaline extract), both compounds had the same retention volume and peak shape as in the alkaline extract (Fig. 3). This result confirmed that the behavior of p -CA was not due to interactions with other compounds of the concentrated alkaline extract. To study the influence of the charge of the phenolic monomers with a carboxyl

group, pulse tests were run at lower pH. Due to the very limited solubility of p -CA under acidic conditions, the next pulse tests were run with FA. A synthetic solution of FA at 200 mg/L (Fig. 4A) was eluted on the column. The fractions exiting the column had a very low conductivity (below 10 μ S/cm), leading to an unreliable pH measurement. FA peak exited the column at 0.38 BV indicating that FA was excluded from the resin pores, it must have been negatively charged. Therefore, a synthetic solution was prepared with FA at 200 mg/L, NaCl at 21.9 g/L in order to have a Na^+ concentration close to its concentration in the concentrated alkaline extract, and HCl to adjust the pH to 2.0 (Fig. 4B). At a pH more than 2 points below its pKa, FA was protonated and was eluted at about 0.42 BV. This retardation was caused by the ability of

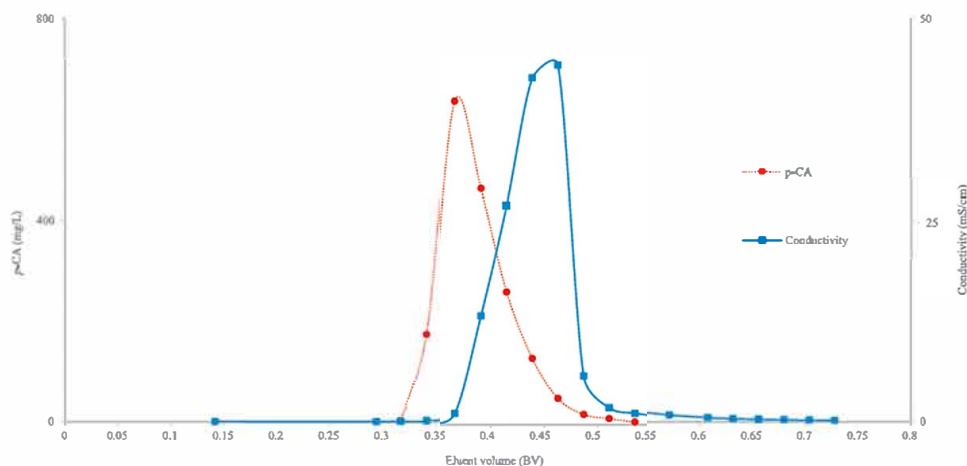


Fig. 3. p-CA synthetic solution at 4.79 g/L at pH = 13.7 adjusted with NaOH, pulse test on XA2004-30Na + with water as eluent at 2.26 m/h.

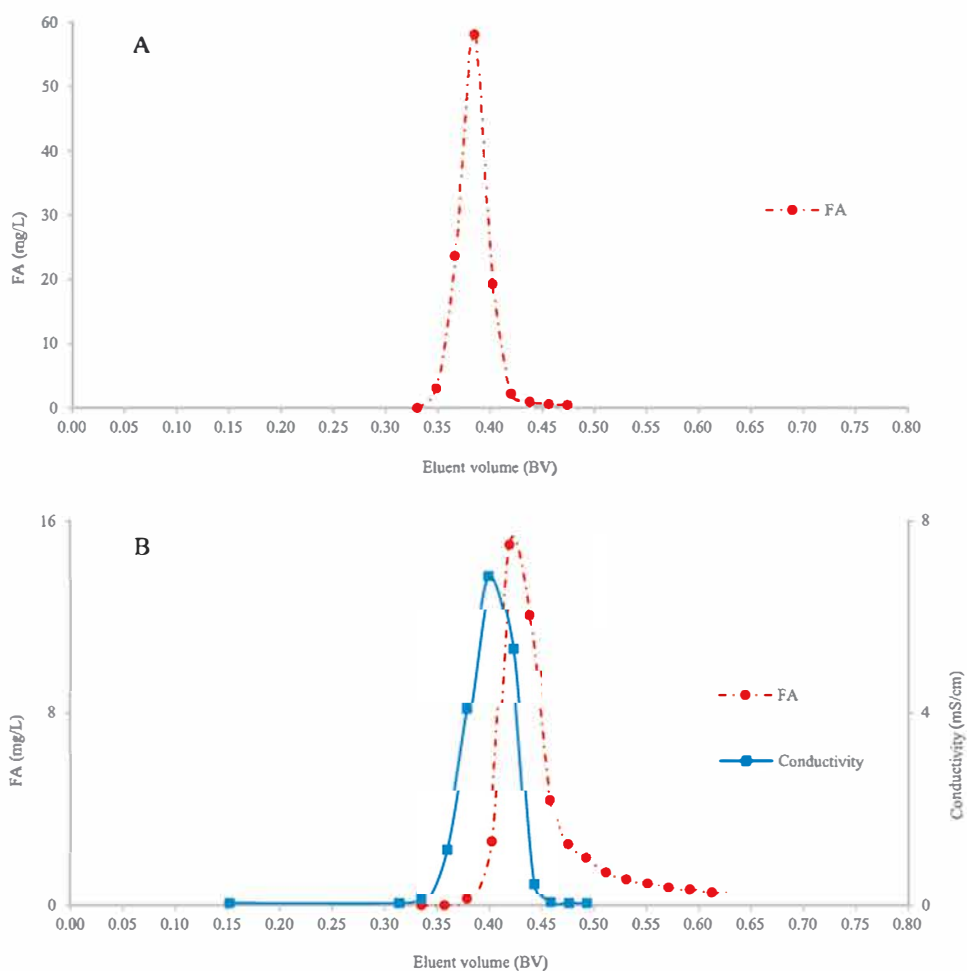


Fig. 4. FA synthetic solutions pulse tests on XA2004-30Na + with water as eluent at 2.26 m/h. (A) FA at 200 mg/L at pH = 3.73. (B) FA at 113 mg/L, NaCl at 22.3 g/L and pH adjusted to 2.0 with HCl.

uncharged FA to penetrate partially into the resin pores. These pulse tests confirmed that despite having approximately the same size and structure, VA, 4HBA, vanillin, p CA and FA did not present the same behavior on gel type SAC exchange resin under alkaline conditions. Phenolic monomers with a carboxyl group can be negatively charged when the pH is over their pKa and ionic repulsion prevented them from diffusing inside the pores of the gel type resin. Unexpectedly, conductivity, mainly due to NaCl (Fig. 4B), exited the column at 0.38 BV,

showing that NaCl was totally rejected by ionic repulsion from the resin pores, whereas NaOH was eluted at 0.45 BV. Different salts were not excluded to the same extent of the resin pores.

3.2.2. Flow rate influence

The flow rate was increased from 20 mL/min to 40 mL/min in order to define the influence of the hydrodynamic flow on the separation. On the XA2004 30Na⁺ resin with an eluent velocity of 4.52 m/h, the BVs

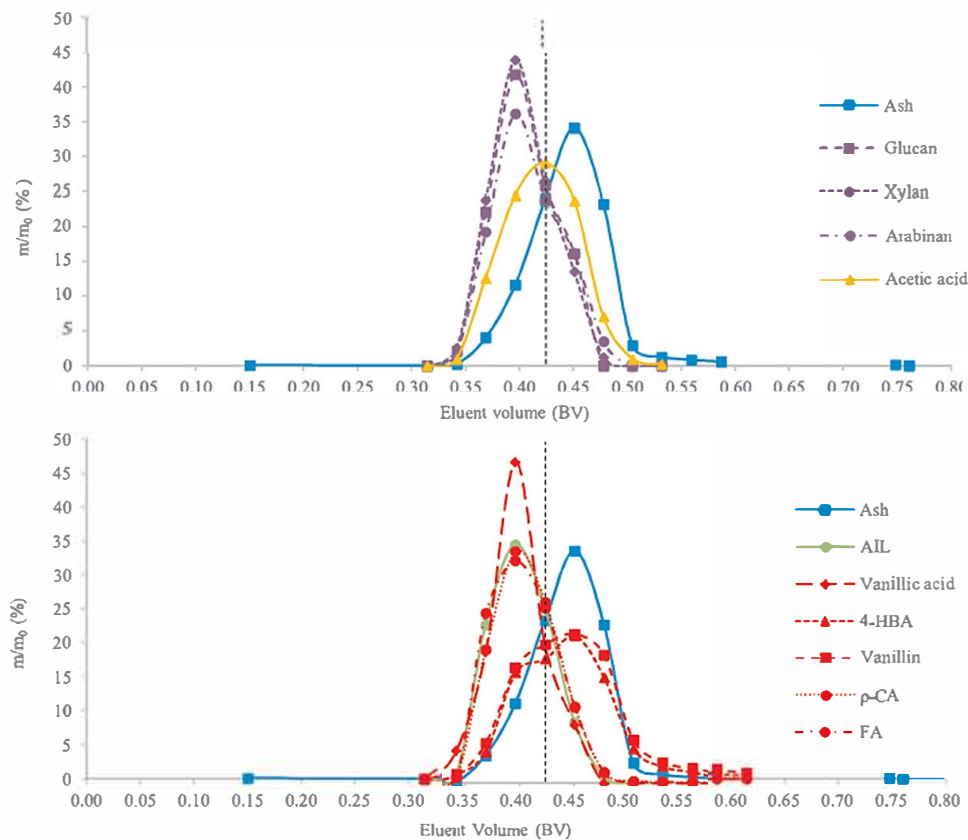


Fig. 5. Concentrated alkaline extract pulse test on XA2004-30Na⁺ with water as eluent at 4.52 m/h, with m the mass of a given compound in the fraction and m_0 its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV.

of the different compounds were the same as at 2.26 m/h, but the separation was less efficient, due to the broadening of the peaks (Fig. 5). This can be quantified by the resolution (R_s) given by Eq. (1) (Kromidas and Kuss, 2009):

$$R_s = 1.18 \frac{(t_{R2} - t_{R1})}{(W_{h1} + W_{h2})} \quad (1)$$

With t_{R1} and t_{R2} the retention times of compounds 1 and 2, W_{h1} and W_{h2} the widths of the peaks at half height.

For instance, at 20 mL/min the resolution for the xylose/ash separation was 0.024, whereas at 40 mL/min the resolution decreased to 0.016. Increasing the eluent velocity led to a lower peak resolution, showing that the optimal velocity for this system is below 4.52 m/h according to the Van Deemter equation (Ladisch, 2001). Increasing the flow rate induced an increase of the dispersion due to hydrodynamic hindering and thus the enlargement of the peaks, but did not reduce the influence of the electrostatic forces on the rejection of the small ionized molecules. However, the loss of resolution is counter balanced by the gain in productivity due to a higher eluent flow rate.

3.2.3. Pore size influence

The influence of the size of the resin pores on the separation of the different compounds of the alkaline extract was studied by running another pulse test on the macroporous type XA2054 Na⁺ resin. This resin presents wider pores 20 to 50 nm and a more heterogeneous pore size distribution, but otherwise it has similar properties as the gel type XA2004 30Na⁺ resin (Table 1). The SCB alkaline extract elution on the XA2054 Na⁺ resin showed that a pool of molecules constituted of sugar and AIL oligomers eluted at 0.40 BV (Fig. 6). Different sugar peaks were observed between 0.40 and 0.77 BV probably representing different size groups of sugar oligomers well separated from each other. On the gel type and macroporous type resins, xylan, arabinan and

glucan profiles exhibited the same tendency, suggesting that xylose, arabinose and glucose are linked and part of the same oligomer structure. AIL presented a single massive peak from 0.45 to 0.77 BV suggesting a more homogeneous size dispersion of AIL oligomers compared to sugar oligomers. We can consider the lignin fragments as dissociated from each other and their rejection representative of their size and not of their aggregation. Indeed, the pH of the collected samples after 0.33 BV ranged between 10.8 and 12.9, so acidic groups in lignin were dissociated yielding polyelectrolytes formation and preventing the aggregation of lignin molecules that is observed at pH below 10.5 (Wong and de Jong, 1996). Phenolic monomers with a carboxyl group (VA, p-CA and FA) were recovered after 0.47 BV like the uncharged phenolic monomers (vanillin and 4HBA). Acetic acid and salts were also eluted later than on the gel type resin. This was due to a larger intra particular volume on the macroporous type resin. The resin pores were too wide to prevent charged molecules from penetrating into the pores because of ionic repulsion. It confirmed the observation made with acetic acid and salts on XA2004 30Na⁺ gel type resin.

If the limit between the two fractions was set at 0.47 BV (Fig. 6), recoveries in the fraction before 0.47 BV for xylan, arabinan, glucan and AIL were 32, 26, 20 and 14%, respectively. They can be completely separated from smaller sugar oligomers, phenolic oligomers, all phenolic monomers, acetic acid and ash. Recoveries of all phenolic monomers, acetic acid and ash were all over 99% in the fraction after 0.47 BV, leading to a conductivity in the fraction before 0.47 BV below 100 μ S/cm. The use of macroporous type SAC resin enabled to produce a pure polymer fraction containing only the largest oligomers of lignin and hemicelluloses.

Soft alkaline extraction conditions helped producing large oligomers (Sun et al., 2004; El Mansouri and Salvadó, 2006) that can be better purified in a single economical step by low pressure chromatography on macroporous type SAC cation resin. These polymers could be

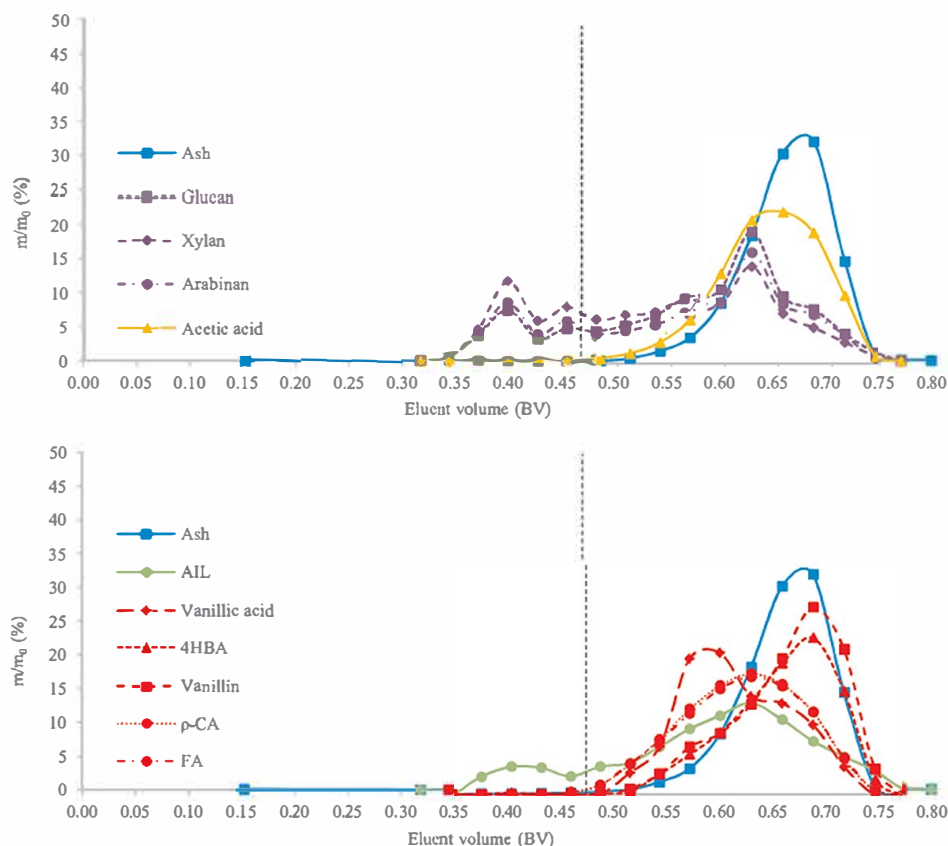


Fig. 6. Concentrated alkaline extract pulse test on XA2054-Na + with water as eluent at 2.26 m/h, with m the mass of a given compound in the fraction and m_0 its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV.

interesting for some applications where long chain of lignin or hemicelluloses are looked for, as non exhaustively in coatings, surfactants, adhesives and cosmetics applications. (Werpy et al., 2004; Holladay et al., 2007).

4. Conclusion

Alkaline pretreatment of lignocellulosic biomass produced original pools of molecules oligomeric lignin, oligomeric hemicelluloses, phenolic monomers, acetic acid and inorganic salts compare to the acid pretreatment.

For the first time, purification of a lignocellulosic alkaline extract was studied by chromatography on SAC exchange resins with water as eluent, without chemical addition. SCB alkaline extract elution on a gel type SAC exchange resin enabled the separation of sugar oligomers, AIL and phenolic monomers with a carboxyl group from ash and neutral phenolic monomers. However, acetic acid was partitioned between these two pools of molecules. This procedure could be used for the demineralization of the sugars and lignin or for the separation between phenolic monomers with a carboxyl group from the other phenolic monomers or salts.

A pure fraction of the biggest AIL and sugar oligomers was obtained using macroporous type SAC resin, while all the other components of the SCB alkaline extract were recovered in another fraction. Playing on the porosity of the SAC resin can help to adjust the fractionation of the different pools of molecules.

This purification technique could be part of an integrated process, with other purification techniques such as precipitation, membrane filtration or crystallization, whose goal would be to obtain pure molecules from a lignocellulosic alkaline extract. Lignin and hemicelluloses obtained on macroporous type resin could be separated by precipitation with alcohol for instance as mentioned previously, to keep the high

purity of both lignin and hemicelluloses.

The transfer from pulse chromatography to simulated moving bed (SMB) would improve the selectivity of the process. These pulse tests gave enough information to consider that an efficient separation will be performed with a SMB, but yield, purity, productivity and utilities consumption at pilot scale are to be investigated.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: <https://doi.org/10.1016/j.indcrop.2018.09.019>.

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*Title:	Supplementary data on the analyses of raw sugarcane bagasse and associated alkaline extract by HPLC on Rezez RPM, Rezex RHM and OmniSpher 3 C18 columns
Authors:	Vincent Oriez ^{a,} , Marlène Beyerle ^b , Pierre-Yves Pontalier ^{a,*} , Jérôme Peydecastaing ^a
*Affiliations:	^a Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France 4 allée Emile Monso, 31 030 Toulouse cedex 4, France ^b Novasep Process, 5 chemin du Pilon, St-Maurice de Beynost, 01708 Miribel, France
*Contact email:	vincentoriez@yahoo.fr , vincent.oriez@ensiacet.fr (V. Oriez), pierreyves.pontalier@ensiacet.fr (P.Y. Pontalier)
*Co-authors:	
*CATEGORY:	Analytical chemistry

Data Article

Title:

Supplementary data on the analyses of raw sugarcane bagasse and associated alkaline extract by HPLC on Rezez RPM, Rezex RHM and OmniSpher 3 C18 columns

Authors:

Vincent Oriez^{a,*}, Marlène Beyerle^b, Pierre-Yves Pontalier^{a,*}, Jérôme Peydecastaing^a

Affiliations:

^a Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France 4 allée Emile Monso, 31 030 Toulouse cedex 4, France

^b Novasep Process, 5 chemin du Pilon, St-Maurice de Beynost, 01708 Miribel, France

Contact email:

vincentoriez@yahoo.fr, vincent.oriez@ensiacet.fr (V. Oriez), pierreyves.pontalier@ensiacet.fr (P.Y. Pontalier)

Abstract

Raw sugarcane bagasse from eRcane, La Réunion, was analyzed following National Renewable Energy Laboratory (NREL) procedure for carbohydrates and lignin determination and the HPLC chromatograms

on Rezez RPM and Rezex RHM columns are presented. The influence of Soxhlet extraction with water and ethanol prior to the acid hydrolysis steps of the NREL procedure was presented as well as the salts removal through SPE cartridge Strata ABW. The free acetic acid content compared to bound acetate groups on hemicelluloses as well as the proportion of monomeric sugar content compared to total sugar content in sugarcane bagasse mild alkaline extract was also determined. The main phenolic monomers constitutive of the sugarcane bagasse mild alkaline extract were also identified.

Specifications Table

Subject area	<i>Analytical chemistry</i>
More specific subject area	<i>Characterization of raw sugarcane bagasse and sugarcane bagasse mild alkaline extract</i>
Type of data	<i>figures</i>
How data was acquired	<i>High-Performance Liquid Chromatography</i>
Data format	<i>Raw chromatograms</i>
Experimental factors	<i>Sugarcane bagasse was dried at 50°C for 24h and ground on a 2 mm mesh</i>
Experimental features	<i>National Renewable Energy Laboratory protocol for the characterization of carbohydrates and lignin applied to raw sugarcane bagasse and sugarcane bagasse mild alkaline extract associated with HPLC on Rezex RPM and RHM columns. HPLC on OmniSpher 3 C18 column for the follow up of phenolic monomers.</i>
Data source location	<i>Sugarcane bagasse from eRcane, La Réunion</i>
Data accessibility	<i>Data is with this article</i>
Related research article	<i>This is a direct submission to Data in Brief, the most relevant research article is "Sugarcane bagasse mild alkaline fractionation and production of purified fractions by pulse chromatography with water"</i>

Value of the Data

- Influence on HPLC chromatograms of Soxhlet extraction with water and ethanol on sugarcane bagasse characterization prior to acid hydrolysis steps of NREL protocol on Rezex RPM column
- Influence on HPLC chromatograms of salts removal by SPE cartridge Strata ABW prior to analysis on Rezex RPM columns
- HPLC chromatogram on Rezex RHM column following NREL protocol on raw sugarcane bagasse
- Determination of free acetic acid compared to bound acetate to hemicelluloses and the determination of monomeric sugar compared to total sugar
- Determination of the main phenolic monomers in sugarcane bagasse alkaline extract

Data

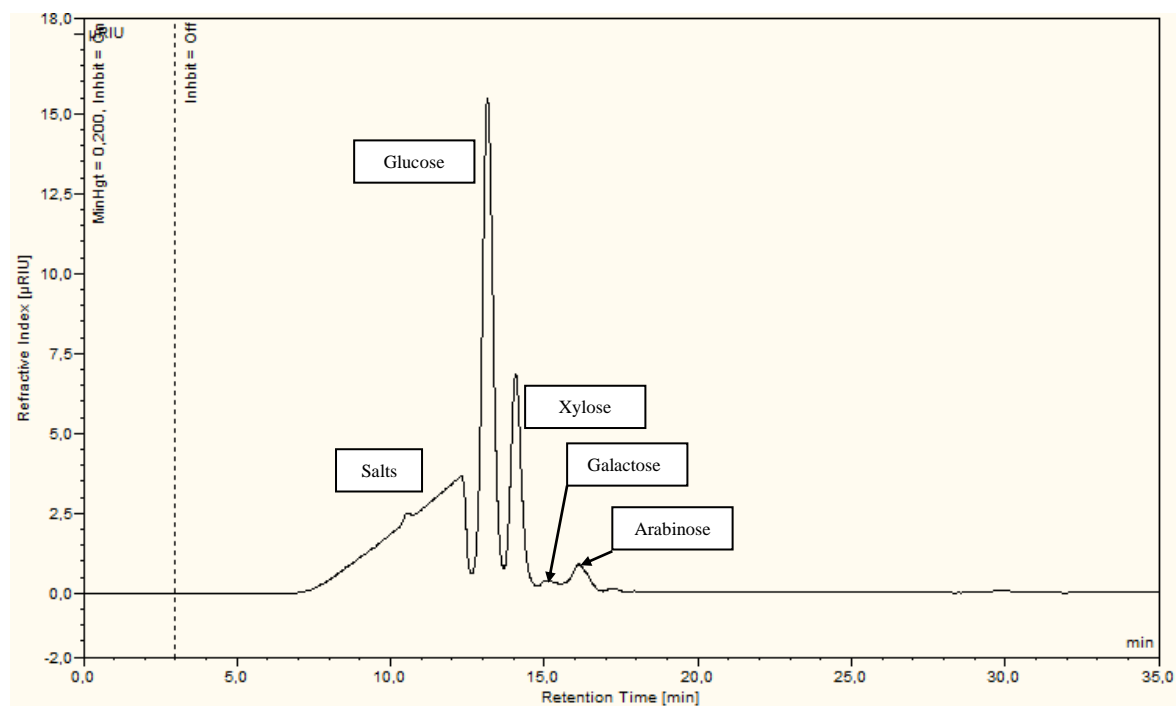


Fig. 1. HPLC chromatogram on Rezex RPM column of SCB raw material analyzed following NREL protocol (including Soxhlet extractions with water and ethanol).

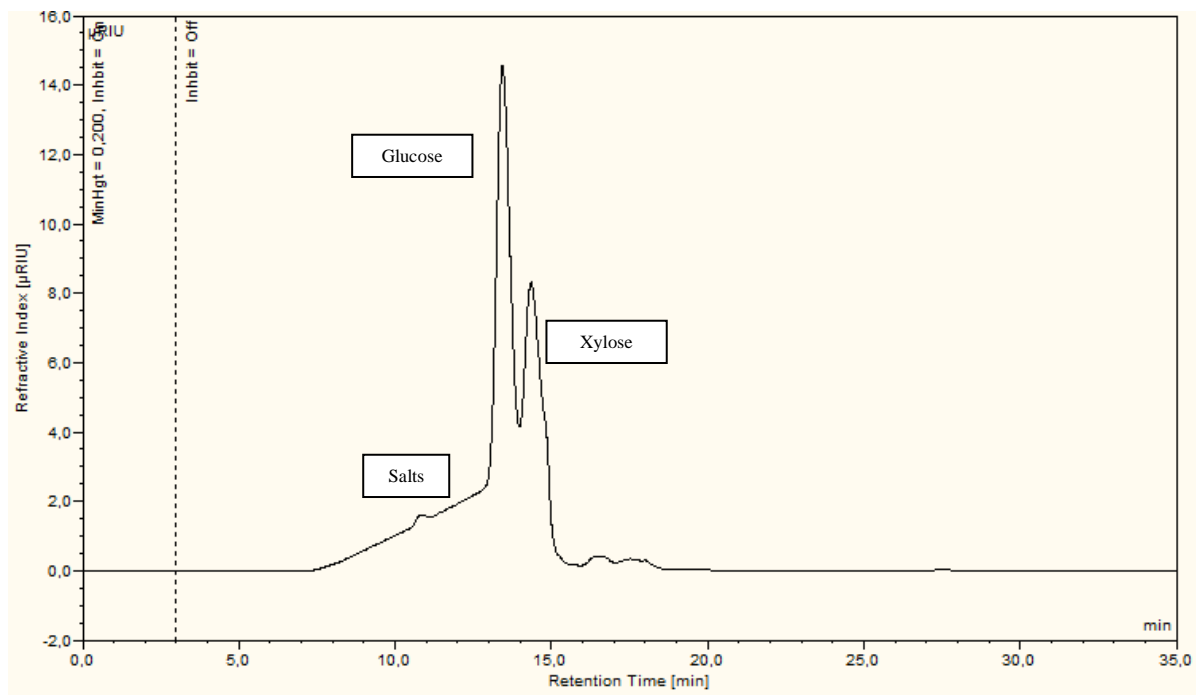


Fig. 2. HPLC chromatogram on Rezex RPM column of SCB raw material analyzed by NREL protocol (without Soxhlet extractions with water and ethanol). Salt peak interfered with the sugars peaks.

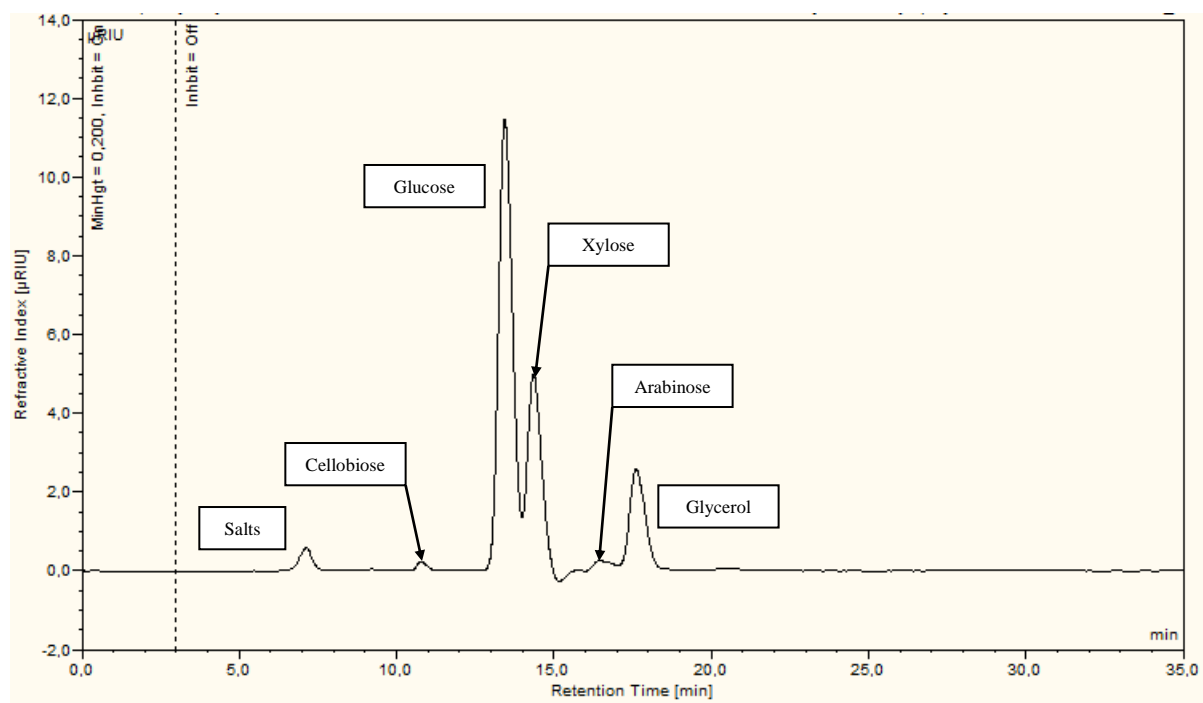


Fig. 3. HPLC chromatogram on Rezex RPM column of SCB raw material analyzed following NREL protocol (without Soxhlet extraction with water and ethanol) and filtration on SPE cartridge Strata ABW. Glycerol appeared as a preservative solution of the cartridge.

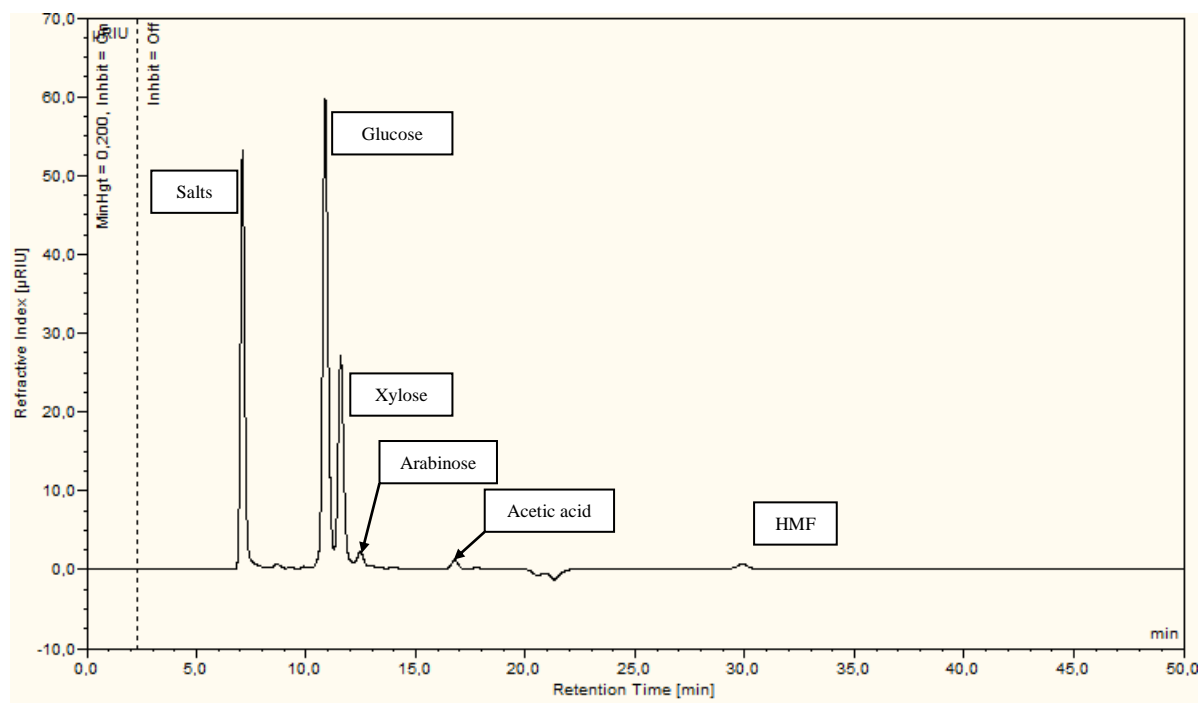


Fig. 4. HPLC chromatogram on Rezex RHM column of SCB raw material analyzed following NREL protocol (without Soxhlet extractions)

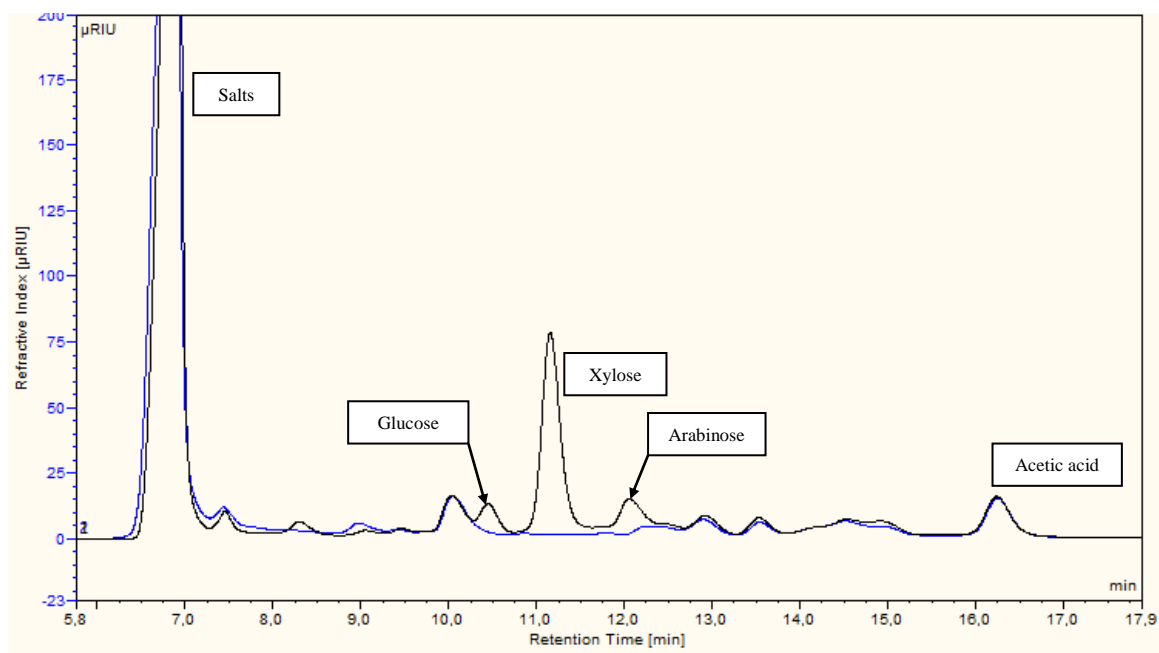


Fig. 5. HPLC chromatograms on Rezez RHM column of SCB concentrated mild alkaline extracts, analyzed after only a pH adjustment to 2 (blue line) and analyzed by NREL protocol on liquid fractions (black line). It showed that no sugar monomers were found in the SCB mild alkaline extract and that all acetic acid is under its free form (not bound to hemicelluloses).

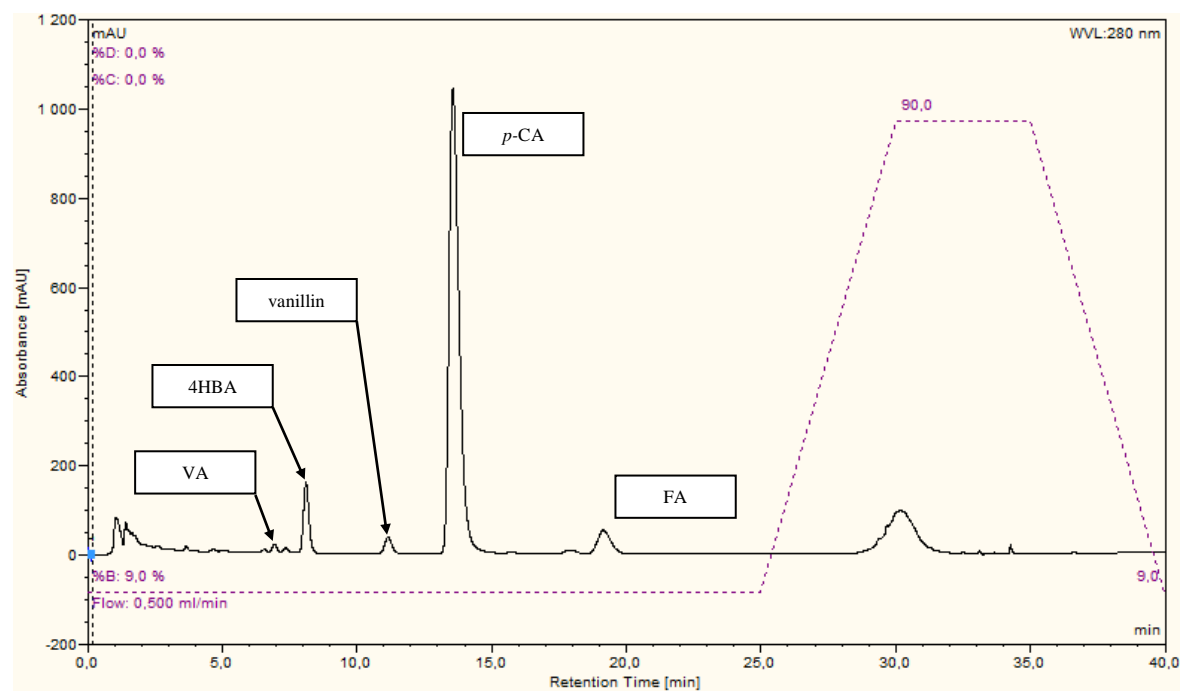


Fig. 6. HPLC chromatogram on OmniSpher 3 C18 column of SCB concentrated mild alkaline

Experimental Design, Materials, and Methods

1. Carbohydrates and lignin analyses

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008), High-Performance Liquid Chromatography (HPLC) on a Rezex RPM-Monosaccharide Pb⁺² 300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RPM-Monosaccharide Pb⁺² 50 x 7.8 mm guard column (Phenomenex) was used to quantify cellobiose, glucose, xylose, galactose, arabinose, mannose and fructose coming from the acidic hydrolysis of cellulose (yields glucose and potentially cellobiose if the hydrolysis is not complete), hemicelluloses (yields all C5 and C6 sugars) or residual sucrose. Prior to the injection, the samples were filtered on SPE cartridge ABW (Phenomenex) to remove the salts and avoid interference with the sugar peaks. Isocratic conditions were used with Milli-Q water at 0.6 mL/min, the injection volume was 20 µL, the column was maintained at 80°C and the RI detector at 50°C. For alkaline extract and purified samples, HPLC on a Rezex RHM-Monosaccharide H⁺ 300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RHM-Monosaccharide H⁺ 50 x 7.8 mm guard column (Phenomenex) was used to quantify glucose, xylose, arabinose, acetic acid, furfural and HMF (Sluiter et al., 2006). Unlike with the RPM column, salts did not interfere with sugar peaks on the RHM column, therefore no desalting of the samples was required prior to the injection. Isocratic conditions were applied with 5 mmol/L H₂SO₄ at 0.6 mL/min, the injection volume was 50 µL, the column was maintained at 65°C and the RI detector at 50°C.

2. Monomeric sugars and hemicelluloses acetyl groups analyses

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of HPLC eluent with RHM column, then the extract was analyzed on RHM column without running NREL protocol (Sluiter et al., 2006). pH adjusted samples directly injected on HPLC enabled the quantification of monomeric sugars and free acetic acid, whereas samples analyzed through NREL protocol gave the total amount of sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

3. Phenolic monomers analyses

Quantification of twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) - gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid - was studied by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follow: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min and the column was equilibrated for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10 µL and the column temperature was maintained at 25°C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) prior to injection.

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