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Deconstructing sugarcane bagasse lignocellulose by acid-based deep eutectic solvents to enhance enzymatic digestibility

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ABSTRACT

Biorefinery with deep eutectic solvent (DES) is an emerging processing technology to overcome the shortcomings of conventional biomass pretreatments. This work evaluates the biorefinery of sugarcane bagasse (SCB) with DES formulated with choline chloride as hydrogen bond acceptor and three hydrogen bond donors: lactic acid, citric acid, and acetic acid. Acetic acid showed unique ionic properties responsible for the selective removal of lignin and the deconstruction of cellulose to improve the digestibility of up to 97.61 % of glucan and 63.95 % of xylan during enzymatic hydrolysis. In addition, the structural characteristics of the polysaccharide-rich material (PRM) were analyzed by X-rays, ATR-FTIR, SEM, and enzymatic hydrolysis, and compared with the original material sample, for a comprehensive understanding of biomass deconstruction using different hydrogen bond donors (HBD) as DES pretreatment.

1. Introduction

Lignocellulosic biomass is attributed great potential for the continuous and sustainable supply of energy in the form of biofuels and value bioproducts (Kumar et al., 2020).

Sugarcane baggasse (SCB) is a biomass from agriculture and industrial processing with the highest production among agricultural residues (1044.8 million tons) (Chourasia et al., 2021). Various studies have shown the ability of SCB to produce various value-added products (Chandel et al., 2012), principally due to its composition rich in cellulose (35–45 %), hemicellulose (26–35 %), lignin (11–25 %), and other extracts (3–14 %) (Morán-Aguilar et al., 2021; Ravindra et al., 2021).

However, the main limitation for the use of lignocellulosic biomass is attributed to the recalcitrance of the cell-wall to biochemical and biological decomposition, conferred by the heterogeneous polyphenolic structure of lignin linked to polysaccharides by ester bonds (lignin-polysaccharide complex), which prevent easy access of enzymes to cellulose. Therefore, it is necessary to apply pretreatments that promote an alteration in the lignocellulose structure, through the deconstruction of the lignin-polysaccharide complex (LPC) in order to improve the accessibility of the enzymes by the substrate during the enzymatic hydrolysis that enriches the use of biomass in biorefinery processes (Zoghlami & Paës, 2019).

Promising technologies for the biorefinery of lignocellulosic biomass have recently emerged with the use of deep eutectic solvents (DES) as pretreatment for biomass fractionation (Shen et al., 2020). DES are generally composed of a hydrogen bond acceptor (HBA) as choline chloride ([ChCl]) and a hydrogen bond donor (HBD) (including amines, amides, alcohols or carboxylic acids). When they are mixed the resulting DES can degrade the physical structure of the biomass with a minimal energy consumption during pretreatment (Shen et al., 2020).

The DES mechanism could consist in the formation of hydrogen bonds between Cl^- from [ChCl] and hydroxyl groups (^-OH) in LPC, which leads to a feeble interaction between the hydrogen bonds and the

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complex LPC. Subsequently, the presence of acidic protons provided by HBD promotes the incision of ester bonds, which could allow a selective removal of lignin and hemicellulose (Morais et al., 2020).

Therefore, the intermolecular interactions generated by the formation or breaking of hydrogen bonds play a crucial role in the particular fractionation of biomass, which deserves an improved analysis and study.

Pretreatments with DES have demonstrated the capacity to fractionate lignin and xylan, as well as to reduce the degree of polymerization of cellulose on various agricultural residues (Lin et al., 2020; Loow et al., 2018). In their way, Shen et al. (2019) employing [ChCl] and lactic acid to deconstruct *Eucalyptus camaldulensis* for further cellulose enzymatic hydrolysis and lignin valorization achieved saccharification yields nearby 94.3 % for glucan. Similarly, Kohli et al. (2020) pretreated birch wood using [ChCl]-acetic acid and [ChCl]-lactic acid achieving delignification percentages between 20 and 70 %, respectively. Nevertheless, Tian et al. (2020) using formic, lactic and acetic acid, as HBD in poplar wood pretreatment demonstrated the need for deeper analysis on the behavior of acid DES since their efficiency varies from the effective removal of lignin to the solubilization/degradation of polysaccharides under mild operational conditions.

On the other hand, in order to achieve viable processes preserving a green concept, it is necessary the use of non-toxic and moderate acidity acids as HBDs, that provide an efficient yield of polysaccharide digestibility, without compromising the severe degradation/solubilization of cellulose and hemicellulose, since the reduction of hemicellulose degradation and its harnessing would improve the economic viability of DES pretreatment and the associated biorefinery (Chen et al., 2022).

In light of these findings, this study aimed to evaluate the physicochemical modifications generated in the structure of SCB after pretreatment with DES based on [ChCl] as HBA and different HBDs: lactic acid (LA), citric acid (CA) and acetic acid (AA) in order to select an optimal HBD for bagasse digestibility during the enzymatic hydrolysis stage. In addition, analysis techniques such as X-ray diffraction (X-ray), Attenuated Total Refrectance Fourier-Transform Infrared Spectrometry (ATR-FTIR), Scanning Electron Microscopy (SEM), and enzymatic digestibility by enzymatic hydrolysis were employed to explain in detail the effect of HBD in the polysaccharide-rich material (PRM) obtained after pretreatment.

2. Materials and methods

2.1. Materials

SCB was supplied by the National Institute of Silviculture, Agriculture and Livestock Research (INIFAP) (Veracruz, Mexico).

[ChCl] was obtained from Alfa Aesar (purity > 98 %), acetic acid from the brand Panreac (purity > 96 %), citric acid from the brand Carlo ERBA (purity 99 %), and lactic acid (purity 90 %) from Ultimate Fluka. [ChCl] was kept in a desiccator to avoid moisture absorption.

2.2. Methods

2.2.1. DES synthesis

The [ChCl] was mixed with the HBD: LA, AA, CA, with a molar ratio 1:4, 1:4, and 1:1 (mol/mol), respectively. The [ChCl]:LA and [ChCl]:AA was stirred for 30 min at 50 °C until a colorless liquid was formed.

However, due to the high viscosity of [ChCl]:CA (131 mPa·s) the addition of water as a low cost and efficient strategy to reduce the viscosity was employed. According to New et al. (2019) water tends to promote the formation of hydrogen bonds between DES and the substrate, which enhances the fractionation of lignocellulose components. Therefore, 30 % (*w/w*) water was added after mixing [ChCl]:CA components for 1 h at 80 °C (Tan et al., 2019). Finally, all DES were stored at room temperature (25 °C) until use.

2.2.2. DES pretreatment

The DES pretreatment was carried out with a liquid-solid ratio (LSR) of 15:1 (*v/w*) for 90 min at 130 °C in a sand bath with orbital shaking (120 rpm). Once the reaction was completed, DES was recovered, adding an antisolvent constituted by CH_3COCH_3 (purity of 99.8 %) and distilled water with a 1:1 (*v/v*) ratio, in a LSR of 25:1 (*v/w*). The mixture was stirred at 250 rpm for 30 min in orbital shakers (Optic Ivymen System, Comecta S.A., distributed by Scharlab, Madrid, Spain) causing the precipitation of delignified PRM. Finally, PRMs were washed with distilled water (LSR of 50:1 (*v/w*)) and dried for 24 h at 50 °C in an oven (Celsius 2007, Memmert, Schwabach, Germany).

2.2.3. Polysaccharides and lignin content

The composition of native and SCB pretreated were tested according to National Renewable Energy Laboratory (NREL) Technical Report (Sluiter et al., 2011). The quantification of polysaccharides was carried out by HPLC system (Agilent model 1200, Palo Alto, CA, USA). A refractive index detector and an Aminex HPX-87H ion exclusion column (Bio Rad 300 × 7.8 mm, 9 μ particles) with guard column were used. Samples were eluted with 0.3 g/L sulfuric acid at 0.6 mL/min and 50 °C. Total lignin was quantified involving acid soluble lignin (ASL) and Klason lignin (KL). The percentage of lignin removed was calculated according to (Eq. (1)):

$$Delignification\ (\%) = \left[1 - \left[\frac{Total\ lignin\ in\ pretreated\ SCB}{Total\ lignin\ in\ native\ SCB}\right] * S\right] * 100\% \quad (1)$$

where S = Solid recovered (g).

2.2.4. Physicochemical composition analysis

SEM analysis was employed to observe the morphological changes in SCB and PRMs using a JEOL JSM6010LA Scanning Electron Microscope (SEM). ATR-FTIR measurements were conducted with a Thermo Nicolet 6700 FTIR Spectrometer (Thermo Fisher Scientific Inc., Madison, WI, USA), and attenuated total reflection ATR accessory equipped with a diamond crystal (Smart Orbit Diamond ATR, Thermo Fisher, USA). PRMs were recorded without preparation in the range 4000 to 400 cm^{-1} at 4 cm^{-1} resolution and 20 scans using a deuterated triglycine sulfate (DTGS) KBr detector.

Cellulose crystallinity alterations were evaluated by the expression of the Lateral Order Index (LOI) (Eq. (2)) using the absorbance obtained in each sample (Kljun et al., 2011).

$$LOI = \frac{A_{1437\ cm^{-1}}}{A_{898 cm^{-1}}} \quad (2)$$

The X-ray spectroscopy (Siemens D500) was used to measure the crystallinity of SCB and treatment with DES employing diffraction angles ranging from 2θ = 2–45°, with a step size of 0.02° and a step time of 0.5 s. The crystalline index (CrI) was calculated as reported by Outeiriño et al. (2021) using the following expression:

$$CrI = \left[\frac{I_{cry} - I_{am}}{I_{cry}}\right] 100 \quad (3)$$

where I_{cry} is the intensity of the crystalline region at 2θ = 22.35 and I_{am} is the intensity in the amorphous region at 2θ = 16.17.

2.3. Enzymatic saccharification of PRM

The saccharification was performed using Cellic CTec2 (Cellic CTec2-SAE0020) commercial enzyme from Sigma-Aldrich. Cellulase and cellobiase activities were quantified employing the methodology described by Ghose (1987) and xylanase activity, acording to Bailey et al. (1992). The enzyme activity was assessed to be 254.50 ± 4.53 FPU/mL (cellulase activity), 89.53 ± 0.43 U/mL (cellobiase activity) and 12,084.88 ± 169.33 U/mL (xylanase activity).

The saccharification was carried out using 100 mg of PRM and an enzyme load of 4 FPU/100 mg in sodium citrate buffer pH 4.8 in a LSR 30:1 (*v/w*) at 150 rpm for 72 h (Chourasia et al., 2021). At the end of the hydrolysis the enzyme was denatured in a water bath at 100 °C for 5 min. All the tests were carried out in triplicate, likewise, the sugars in the aliquots were determined by HPLC to calculate the glucan and xylan digestibility as follows:

$$Glucan\ digestibility\ (\%) = \left[\frac{Glucose\ amount\ in\ enzymatic\ hydrolyzate*0.9}{Glucan\ amount\ in\ substrate}\right]*100 \quad (4)$$

$$Xylan\ digestibility\ (\%) = \left[\frac{Xylose\ amount\ in\ enzymatic\ hydrolyzate*0.88}{Xylan\ amount\ in\ substrate}\right]*100 \quad (5)$$

2.4. Statistical analysis

The statistical analysis of lignocellulosic composition, sugars released, saccharification yield and lignin rate after DES pretreatments were performed using an analysis of variance (ANOVA) and the statistical software Minitab 17 (version 17.1.0, Minitab Inc.). The comparison of means was established by the Tukey test at 95 % confidence. In this study, each value in the graphs was expressed as the mean ± standard deviation of three independent experiments.

3. Results and discussion

3.1. Effect of HBD in DES pretreatment

3.1.1. Lignocellulosic composition analysis

The chemical composition of native SCB by dry weight (%) was comprised of glucan (34.49 ± 0.30), xylan (28.64 ± 0.51), arabinan (4.57 ± 0.19), and total lignin (23.63 ± 0.52). Total lignin is constituted by ASL (4.45 ± 0.35) and KL (19.18 ± 0.68). These values are consistent with the extensive literature available for the composition of SCB (Liu et al., 2021; Sharma et al., 2021).

Table 1 indicates a change in the lignocellulosic composition after DES pretreatment in SCB, with an enriched glucan content of 1.70, 1.80 and 1.10 fold-times than native SCB and the removal of total lignin until 54.53, 39.61, and 2.74 % for [ChCl]:LA, [ChCl]:AA and [ChCl]:CA, respectively, and xylan removal of 60.30 % and 19.58 % employing [ChCl]:CA and [ChCl]:AA. According to Morán-Aguilar et al. (2022), DES performances as a mild acid-base catalytic solution that breaks the β-O-4 aryl ester bonds between LPC, as well as ester linkages between lignin and 4-*O*-methylglucuronic acid xylan chains. Therefore, a major fraction of cellulose is promoted in the PRM.

In addition, lignin removal in SCB can differ according to DES mixture applied, the type of biomass as well as the operating conditions worked. Liu et al. (2021) reports lignin removal (~89 %) using TEBAC: LA at 120 °C and 4 h, while Chourasia et al. (2021) reported between a 60–80 % of lignin removal using [ChCl]:LA (1:5) for 12 h at 80 °C.

Tan et al. (2019) discussed that the effectiveness of DES pretreatment is affected by various factors such as functional groups, due to the $^{-}$OH and $^{-}$COOH groups in HBD are beneficial for lignin dissolution, but more than one $^{-}$COOH group declines the lignin dissolution caused by increased hydrogen bonding and extensive dimer chains that significantly augmented viscosity and decreases mass transfer between biomass and DES pretreatment (Yu et al., 2022). The aforementioned coincides with the results obtained for SCB pretreated with [ChCl]:CA since it has a high viscosity (131.00 Pa·s at 25 °C) and surface tension (41.04 mN/m), which could interfere with the efficient solubilization of lignin (Shafie et al., 2019).

3.1.2. Physicochemical modifications study

3.1.2.1. Morphological analysis. The morphological alterations on the pretreated SCB surface are visible in Fig. 1. Picture of native sample revealed a smooth, intact, and ordered fibril surface, while SEM analysis of the pretreated samples showed structural differences, with a rough and exposed structural morphology.

Micrographs applying [ChCl]:LA exhibited the appearance of a smooth and consistent surface, mostly indicating the presence of crystalline cellulose. These results are consistent with the compositional analysis in Table 1, by means of increasing LOI and XRD values, indicating a higher degree of crystallinity and a more ordered cellulose structure than the native sample (Corgié et al., 2011; Poletto et al., 2014). This suggests the removal of amorphous compounds as lignin and hemicellulose after the [ChCl]:LA pretreatment (Chen et al., 2018). Otherwise, the image of [ChCl]:CA pretreated biomass denotes porous structures with flats and the heterogeneous surfaces formed by various fibril debris. Finally, picture of SCB pretreated with [ChCl]:AA indicates a deformed structure with wide cracks and holes along with other modifications. These morphological alterations were more relevant in the last pretreatment with an improved deformation with loss of fibers and increment in the porous surface. According to Lin et al. (2020) mild acidic DES pretreatment improves cellulose reactivity through cellulose deconstruction/swelling process, by removing lignin and hemicellulose (mainly in the form of xylan) to better expose the innermost cellulosic component of biomass for the accessibility of enzymes. This result is consistent with those reported by Tian et al. (2020) using poplar wood and [ChCl]:AA to evaluate the potential for chemical conversion of cellulose obtained after a DES pretreatment. In this case, the quantification of the staining value of Simon (47.6 mg/g) showed that pretreatment with [ChCl]:AA was effective in increasing the available cellulose area and porosity at the molecular level.

3.1.2.2. ATR-FTIR analysis. The ATR-FTIR analysis was carried out to evaluate the alterations in the functional groups of SCB pretreatment with DES (Fig. 2a). Wide adsorption bands of approximately 3334 cm^{-1}

Table 1
Chemical composition of PRMs after DES pretreatment with different HBD at 130 °C and 90 min.

Pretreatment	Polysaccharides (%)			Lignin (%)		Recovery (%)		Removal (%)		Crystallinity (%)	
	Glucan	Xylan	Arabinan	ASL	KL	Solid yield	Glucan	Xylan	Total lignin	CrI	LOI
Native	34.49 ± 0.30	28.64 ± 0.51	4.57 ± 0.19	4.45 ± 0.35	19.18 ± 0.68	100	–	–	–	41.01	1.43
[ChCl]:LA	57.83 ± 3.36	30.34 ± 2.19	N.D.	5.03 ± 0.08	5.72 ± 0.28	22.93 ± 2.67	31.83 ± 2.15	–	54.53 ± 1.33	53.52	2.25
[ChCl]:CA	38.00 ± 2.37	11.37 ± 1.43	N.D.	3.37 ± 0.20	19.61 ± 0.72	44.80 ± 0.25	49.36 ± 0.28	60.30 ± 0.33	2.74 ± 0.89	46.07	1.38
[ChCl]:AA	62.09 ± 0.54	23.03 ± 1.59	N.D.	2.00 ± 0.04	12.27 ± 0.84	37.15 ± 0.45	66.89 ± 0.82	19.58 ± 0.18	39.61 ± 0.45	54.09	1.62

[ChCl]:LA: choline chloride and lactic acid; [ChCl]:CA: choline chloride and citric acid; [ChCl]:AA: choline chloride and acetic acid; ASL:acid soluble lignin; KL: Klason lignin; LOI: Lateral Order Index; CrI: Crystalline Index; N.D.: Not detected; Solid yield recovery in dry weight after DES pretreatment.

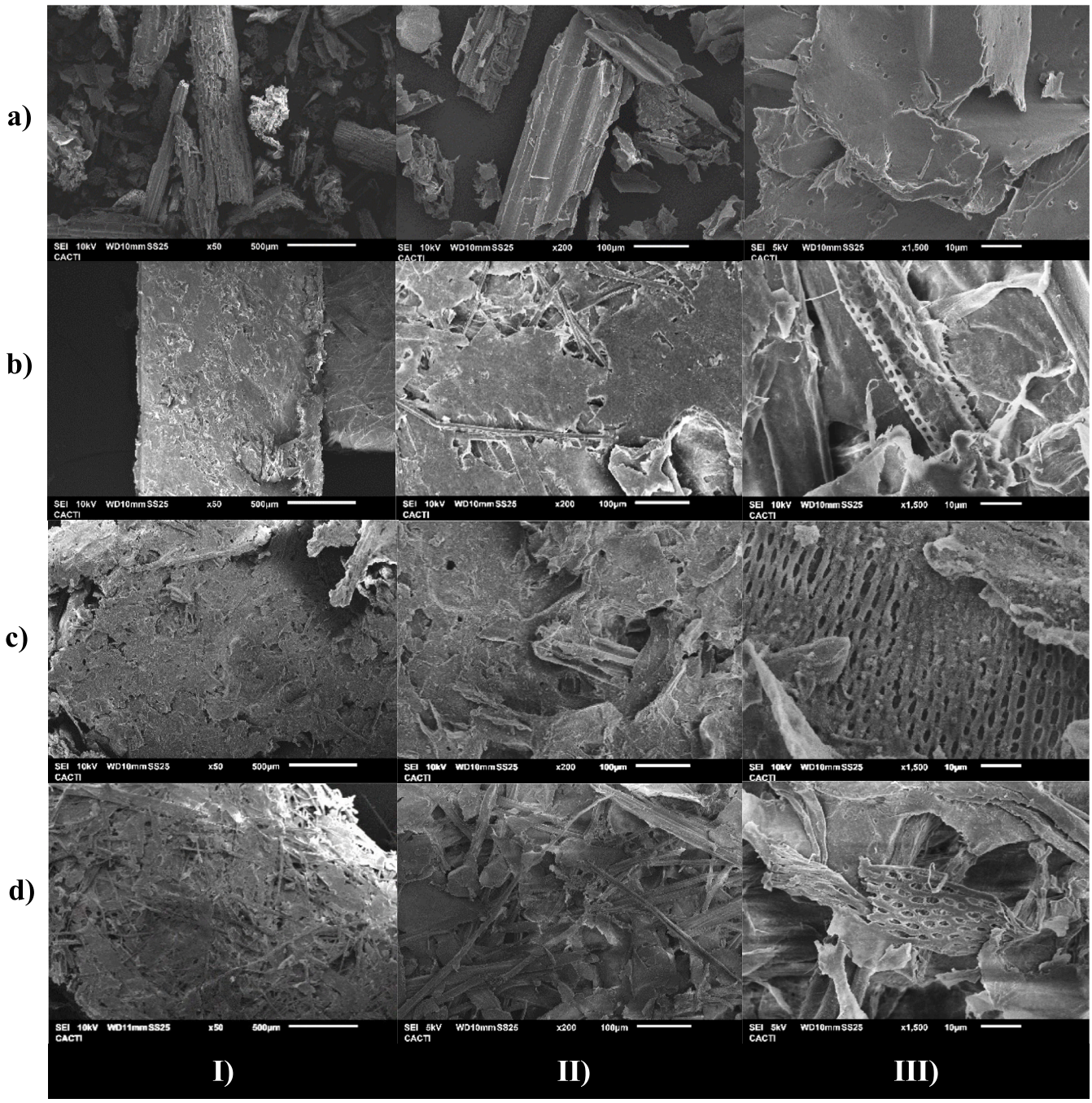

Fig. 1. SEM images of the native (a) and SCB pretreated with different HBD: [ChCl]:LA (b), [ChCl]:CA (c) and [ChCl]:AA (d). Micrographs were taken with variable magnification: I) ×50; II) ×200; III) ×1500.

(OH group intramolecular hydrogen bonds), 2896 cm^{-1} (CH_3 and CH_2), 1030 cm^{-1} (Stretching C—O) assigned to cellulose, were observed mainly after pretreatment with [ChCl]:AA. These results indicated an enrichment in the percentage of cellulose after DESs pretreatments (Sai & Lee, 2019). In addition, an increase in band at 897 cm^{-1} (stretching C-O-C at β-(1,4) glycosidic linkage in cellulose component) was observed mainly for [ChCl]:AA and [ChCl]:CA. This indicates that AA and CA as HBD were more efficient in the deconstruction of cellulose through the formation of a greater number of amorphous zones in SCB biomass. However, [ChCl]:LA pretreatment generates a decrease in this peak, this possibly indicates a major content in crystalline cellulose after pretreatment.

Representative peaks indicate the presence of hemicellulose mainly due to the xylan content through the stretching in C—O and CH_3 (1323 and 1370 cm^{-1}) (Li et al., 2021).

The characteristic absorption peaks of the aromatic biopolymer lignin can be observed at 1099 cm^{-1} assigned to plane deformation C—H, in this case an increase is observed for LA > CA > AA. The peak at 1256 cm^{-1} corresponding to stretching C—O in guaiacyl unit disappeared for LA and CA and only decreased for AA. This could be related to the breakage of β-O-4-aryl ether bonds, which are cleaved in acidic environments (Sturgeon et al., 2014). However, after pretreatment with LA, an increase in band at 1515 and 1607 cm^{-1} can be observed assigned to vibration C═C guaiacyl aromatic skeletons and stretching C═O in the conjugated carboxyl (Azizan et al., 2022).

On the other hand, a band at 1725 cm^{-1} was perceived to a greater

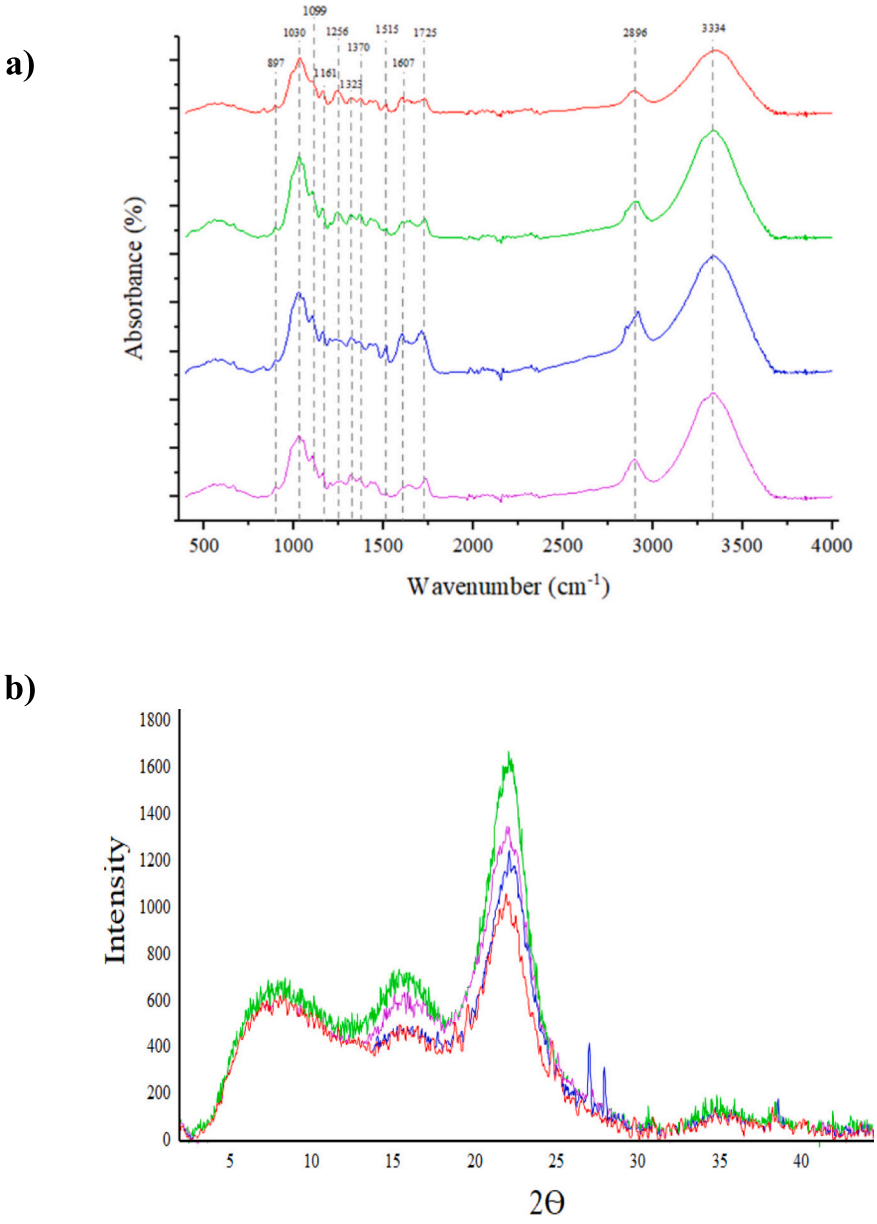

Fig. 2. Chemical modification in SCB after DES pretreatment at 130 °C and 90 min. a) FTIR spectra and b) XRD diffractograms of native SCB (line red) and DES pretreatment with [ChCl]:CA (line purple), [ChCl]:AA (line green) and [ChCl]:LA (line dark blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extent for LA > CA > AA allocated to C═O stretching of carboxylic acid (Azizan et al., 2016). This could suggest the remains of minor amounts of HBD after DES pretreatment.

Likewise, the LOI values were determined to interpret the qualitative changes in crystallinity of cellulose structure due to the action of DES pretreatments in SCB. The LOI values were obtained from the absorbance value at 1437 cm^{-1} (associated with crystalline cellulose), and from values at 898 cm^{-1} (related to amorphous cellulose) (Kljun et al., 2011) (Table 1). The LOI values increased after DES pretreatments, 2.25 % and 1.62 % for [ChCl]:LA and [ChCl]:AA, respectively. Meanwhile, the value for [ChCl]:CA was unchanged compared to native SCB which could indicate a decrease in crystallinity but an increase in amorphous cellulose (Kljun et al., 2011; Yue et al., 2015). This could be related to the severity of the pretreatment caused by this type of HBD, that might modify the viscosity, interaction forces, and free volume of DES on the biomass (Shafie et al., 2019). However, it also largely depends on the type of biomass and the type of pretreatment involved, since the decrease in LOI value has been reported from brewery spent grain and wheat straw using ionic liquids such as cholinium glycinate and imidazoles pretreatment (Morais et al., 2016).

3.1.2.3. X-ray analysis. Crystallinity has been widely discussed as one of the factors that indicates the degree of transformation in biomass pretreatment, as well as it has been involved in the efficiencies obtained during enzymatic saccharification (Zhao et al., 2018).

Therefore, diffractogram was obtained from the XRD analysis of the native SCB and after DES pretreatment (Fig. 2b) exhibiting prominent signals of 2θ at 16° corresponding to amorphous regions of the biomass mainly for pretreatments with AA and CA as HBD, this also corresponds with the increase in the area of the valley to 18° associated with the amorphous region of disordered cellulose, hemicellulose, and lignin (Morais et al., 2016).

Consequently, the calculation of the CrI was carried out for each sample (Table 1). The CrI value increased after DES pretreatment, particularly using [ChCl]:LA (53.52 %) and [ChCl]:AA (54.09 %) compared to the native SCB (41.01 %). The crystallinity of the cellulose can be modified using biomass pretreatment technologies, but also as a consequence of the elimination of amorphous compounds after pretreatment (Zhao et al., 2018). However, a reduction in CrI value can be noted using [ChCl]:CA. This, according to (Shafie et al., 2019), can be attributed to a swelling and dissolution of cellulose (glucan) and hemicellulose (xylan and arabinan) in biomass residues.

It must be pointed that these results are consistent with those reported in Table 1 concerning the alterations in the chemical composition, since the higher contents of glucan and removal of lignin were observed after pretreatment with [ChCl]:LA and [ChCl]:AA. It is worth mentioning that these results are similar to those reported by Chourasia et al. (2021) using different eutectic mixtures on SCB. In that study, CrI values increased after pretreatments with [ChCl]:lactic acid (88.7 %), [ChCl]:glycerol (82.1 %) and [ChCl]:malic acid (62.8 %), compared with the native SCB (56.2 %).

Therefore, according to physicochemical analysis, the pretreatments with [ChCl]:AA and [ChCl]:LA transformed the most morphological and chemical structure of SCB, removing a large amount of lignin (40–55 %), increasing the polysaccharide content and improving the contact area to favor a higher efficiency during enzymatic hydrolysis.

3.1.3. Enzymatic saccharification

Fig. 3a illustrates the release of sugars mainly by [ChCl]:AA (25.86 g/L) > [ChCl]:LA (16.77 g/L) > [ChCl]:CA (8.58 g/L). These results are near to 6, 5, and 2-fold times higher than those obtained for native SCB. Therefore, DES pretreatments are crucial to improve the surface accessibility of biomass to enzymatic attack. Also a similar tendency was observed regarding the yield percentages obtained after enzymatic hydrolysis (Fig. 3b), since the maximum saccharification yields of glucan (97.61 ± 0.72) and xylan (63.95 ± 0.68) were attained after [ChCl]:AA treatment.

However, the maximum saccharification yield does not coincide with the highest lignin removal reported in Table 1. This discrepancy could be related to the level of cellulose alteration after DES pretreatment, which corresponds with the SEM images, ATR-FTIR and X-ray results demonstrating an increase in the amorphous zones of the cellulose mainly after [ChCl]:AA pretreatment.

Therefore, according with ATR-FTIR and X-ray results the additional OH groups in [ChCl]:LA could improve its ability to donate hydrogen bonds not only with lignin but also between the amorphous zones of the cellulose, generating a pretreated biomass rich in crystalline cellulose that does not allow direct access of the enzymes through the substrate. In addition, Ling et al. (2021) explained that the more severe operational condition generates an interaction with $^{-}$OH groups of lignin and amorphous cellulose with HBD of DES pretreatment, forming

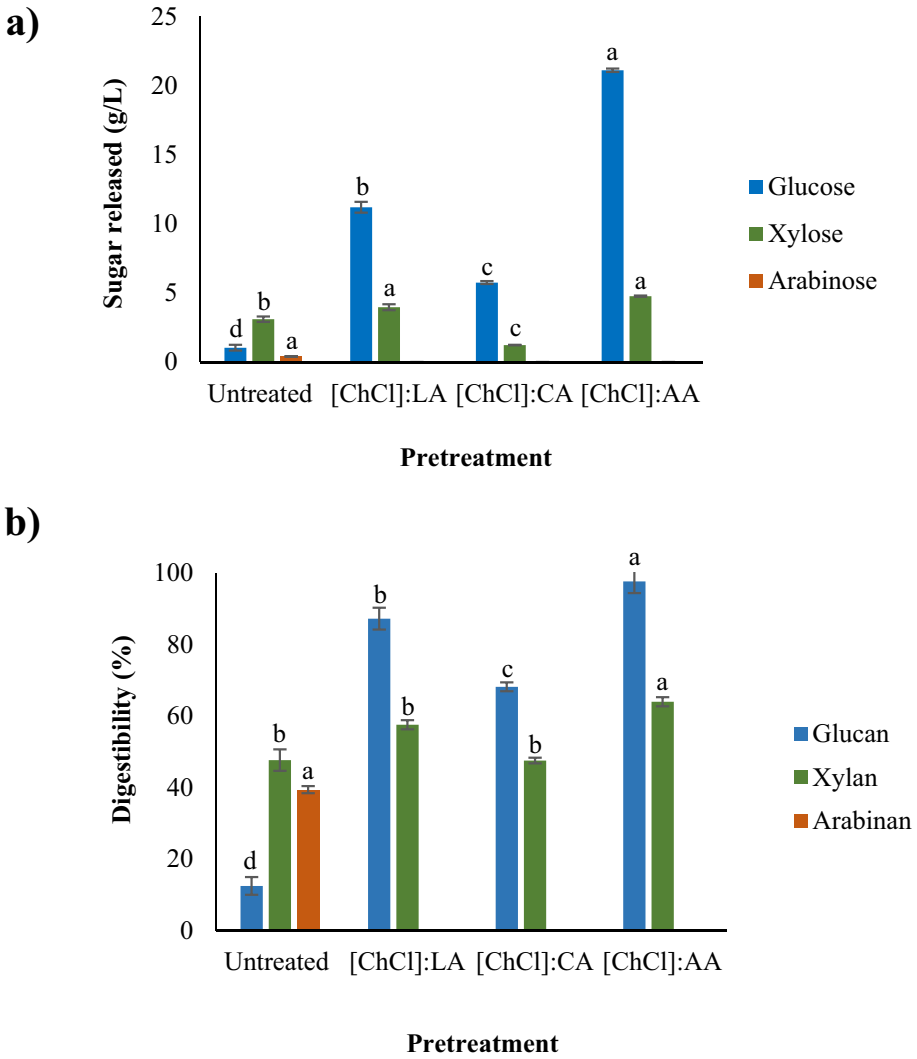

Fig. 3. Sugars released (a) and percentage of digestibility (b) obtained after enzymatic hydrolysis of SCB native and pretreated with different HBD at 90 min and 130 °C. Different letters represent statistically significant differences (one-way ANOVA, Tukey's test; $P < 0.05$).

conglomerations that prevent a greater interaction among the enzymes and polysaccharides reducing saccharification yield. This can be verified by the absorption peak (1725 cm^{-1}) corresponding to carboxylic acid, being most prevalent for [ChCl]:LA.

On the other hand, a low release of fermentable sugars and saccharification yields were observed using the pretreatment with [ChCl]:CA, this could be associated to the individual properties of the eutectic mixture conferred according to its composition (interaction between the [ChCl] and the HBD, the number of hydroxyl groups, carboxyl groups and viscosity) (Shafie et al., 2019). In addition, the existence of additional OH^+ in CA causes more intermolecular interactions between the Cl^- of [ChCl] and the OH^+ groups of CA, leading to a higher formation of hydrogen bonds, increasing the attraction force and decreasing the free volume of DES, as well as the interaction between SCB and DES. Moreover, compared to AA ($C_2H_4O_2$), LA ($C_3H_6O_3$) the additional groups in CA ($C_6H_8O_7$) result in a larger molecule size that increases viscosity and steric hindrance that reduces lignin removal (Zhao et al., 2018). According to Xu et al. (2020), DES constituted by a monocarboxylic HBD are more efficient in lignin deconstruction than a dicarboxylic acid. First, the carboxyl group ^-COOH confers a polar character to acids, which induces the formation of hydrogen bonds between the carboxylic acid molecule and the [ChCl] molecule. Secondly, the higher the polarity of the HBD, the greater the acidity of the HBD with a low pKa value, which allows to easily donate an H^+ cation and generate higher solvent-solute interactions (Teles et al., 2017), while increasing the number of carboxyl groups could reduce the solubility of lignin (Soares et al., 2017).

The above could justify the efficiency of the results obtained with the pretreatments [ChCl]:LA and [ChCl]:AA concerning [ChCl]:CA, since the first two HBD have a monocarboxylic group while citric acid has three, a factor that could interfere in the interaction during deprotonation of the phenolic hydroxyl group of lignin (Suopajärvi et al., 2020).

In summary, it was observed that the effect of different acid DES pretreatment in SCB generated a selective dissolution of lignin and the deconstruction/swelling of cellulose. In addition, several literature about acid-based DES pretreatment mentioned that higher acidity achieved better yields in the lignin extraction and therefore during the saccharification of different biomass. However, during this work AA with a moderate acidity as HBD presented a high potential for its application in biorefinery processes since yields are exposed to high levels of saccharification for glucan and xylan as well as the application of simple processes with mild operating conditions.

4. Conclusion

Sugarcane baggasse (SCB) can be pretreated with deep eutectic solvent (DES) with different hydrogen bond donors (HBD) as a key point to perform a simple, environmental and effective process that minimizes production costs in biorefinery processes. This study presents an exhaustive analysis of the effect of different HBDs on the composition of SCB, demonstrating the importance of electing an adequate HBD to generate a selective deconstruction of biomass that allows an efficient release of sugars during saccharification, without generating the degradation of polysaccharides. It is shown that the use of [ChCl]:AA under mild operating conditions generates the best digestibility of glucans and xylans during enzymatic hydrolysis, since this pretreatment provides a swollen and deconstruction structure disposed to greater enzymatic attack. Therefore, it can be highlighted that it is not necessary to generate a biomass with the lowest lignin content to achieve the highest release of sugars.

CRediT authorship contribution statement

María Guadalupe Morán-Aguilar: Investigation, Methodology, Visualization. **Montserrat Calderón-Santoyo:** Writing – review & editing. **Ricardo Pinheiro de Souza Oliveira:** Writing – review & editing. **María Guadalupe Aguilar-Uscanga:** Writing – review & editing. **José Manuel Domínguez:** Conceptualization, Resources, Project administration, Supervision, Writing – original draft.

Declaration of competing interest

Authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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