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Evaluation of agave bagasse recalcitrance using AFEX™, autohydrolysis, and ionic liquid pretreatments

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HIGHLIGHTS

- First direct side-by-side comparative pretreatment assessment on agave bagasse.
- Ammonia fiber expansion (AFEX) completely preserves plant carbohydrates.
- Autohydrolysis solubilized 62% of xylan from untreated agave bagasse (AGB).
- Ionic liquid (IL) pretreatment removed 25% of lignin from untreated AGB.
- Syringyl and guaiacyl lignin ratios of pretreated biomass were determined.

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ABSTRACT

A comparative analysis of the response of agave bagasse (AGB) to pretreatment by ammonia fiber expansion (AFEX™), autohydrolysis (AH) and ionic liquid (IL) was performed using 2D nuclear magnetic resonance (NMR) spectroscopy, wet chemistry, enzymatic saccharification and mass balances. It has been found that AFEX pretreatment preserved all carbohydrates in the biomass, whereas AH removed 62.4% of xylan and IL extracted 25% of lignin into wash streams. Syringyl and guaiacyl lignin ratio of untreated AGB was 4.3, whereas for the pretreated biomass the ratios were 4.2, 5.0 and 4.7 for AFEX, AH and IL, respectively. Using NMR spectra, the intensity of β-aryl ether units in aliphatic, anomeric, and aromatic regions decreased in all three pretreated samples when compared to untreated biomass. Yields of glucose plus xylose in the major hydrolysate stream were 42.5, 39.7 and 26.9 kg per 100 kg of untreated AGB for AFEX, IL and AH, respectively.

1. Introduction

Agave bagasse (AGB) is a residual fiber left behind after the production of alcoholic beverages such as tequila or mezcal in Mexico, depending on the agave species used during the process. The annual agave consumption for tequila production in 2015 was around 8.09×10^5 tons. AGB represents usually ~40% of the processed agave on a dry weight basis (Davis et al., 2011; CRT, 2015). AGB is a sustainable feedstock for producing biofuels comparable to other lignocellulosic biomass feedstocks (e.g. corn stover or switchgrass). To overcome biomass recalcitrance, a pretreatment step is mandatory, and an efficient one must be economically viable, minimize degradation of carbohydrates to inhibitors, and should not inhibit the subsequent downstream processing steps (saccharification and fermentation).

Many different biomass pretreatments are currently being developed and evaluated, including ammonia fiber expansion (AFEX), autohydrolysis (AH) and ionic liquid (IL) pretreatments (da Costa Sousa et al., 2009). AFEX is currently undergoing scale up for potential commercialization and has been successfully demonstrated at the 1 ton per day level in pilot plant. All though three pretreatment methods are capable of producing high sugar

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yields after enzymatic hydrolysis, several challenges remain before these processes could be commercially scaled up. These challenges include adequate chemical for pretreating biomass, and their recovery, energy requirements, feedstock handling issues, high water requirements and downstream processing problems.

Autohydrolysis biomass pretreatment uses only hot liquid water or saturated steam. This process has two primary effects. First is the auto ionization of water into acid hydronium ions (H_3O^+) and the second is the hydration of the acetyl groups in hemicellulose leading to formation of acetic acid. The hydrogen ions from acetic acid act as catalysts in the process. Then the hemicellulose is depolymerized and solubilized in the liquid phase and a small portion of the lignin is also dissolved. Lignin is relocated to the surface of the pretreated solids due to the operating conditions applied. The dissolved hemicellulose exists mostly in the form of xylose oligomers, and requires additional hemicellulases or dilute acid hydrolysis to be converted into fermentable sugars. Some biomass feedstocks such as corn stover have the buffering capability to maintain the pH of the mixture at around 4; this feature helps to carry out pretreatment under milder conditions (140–180 °C) (Ruiz et al., 2013).

AFEX pretreatment uses about one kg of anhydrous ammonia per kg of biomass at moderate temperatures (e.g. 90–100 °C) and high pressures (e.g. 250–300 psi) for approximately 30 min followed by release of pressure resulting in biomass disruption (Balan et al., 2009). About 97% of the ammonia can be recovered in the gas phase and recycled. AFEX greatly increases the biomass internal porosity by solubilizing some of the lignin, hemicellulose and relocating these components to the surface of the biomass. Ammonolysis and hydrolysis are two competing reactions that take place during AFEX pretreatment process. The acetyl, feruloyl, coumaryl ester linkages present in biomass are converted into the corresponding amides and acids (Chundawat et al., 2010).

Certain ILs such as 1-ethyl-3-methylimidazolium acetate or [C_2C_1Im][OAc] have demonstrated great potential as efficient solvents for biomass processing, due to complete plant cell wall structure dissolution. Disrupting the hydrogen bond network of cellulose causes decrystallization (from cellulose I to cellulose II), and delignification which makes the biomass structure amenable for downstream processing (Singh et al., 2009). Typical IL pretreatment conditions employ a temperature range of 100–160 °C and relatively short residence times (up to 3 h) and is carried out at atmospheric pressure which can be highly recovered as well as employing cost effective IL recycle methods that will reduce overall processing costs (Papa et al., 2015). IL pretreatment has been shown to be effective in efficiently processing a wide range of feedstocks (hardwood, softwood and grasses) at high solids loading levels and does not appear to require finely milled material to achieve high sugar yields (Cruz et al., 2013; Li et al., 2013).

To date, there has not been a side-by-side comparison of AGB sugar conversion using leading pretreatment processes such as AFEX, AH and IL technologies. However, independent bioconversion studies have been performed on AGB where various pretreatments have been used to reduce its recalcitrance for subsequent saccharification, including alkali, dilute acid, organosolv and ILs (Ávila-Lara et al., 2015; Caspeta et al., 2014; Perez-Pimienta et al., 2013). Moreover, different approaches for the production of biofuels such as methane, n-butanol or ethanol using AGB have been reported (Arreola-Vargas et al., 2015; Corbin et al., 2015; Mielenz et al., 2015). It is very difficult to make any meaningful comparison between these studies, since they used different agave species, different pretreatment conditions, the source and activities of enzymes are different.

The main objective of this work is to perform the first detailed qualitative and quantitative comparison of three pretreatments (AFEX, AH and IL) for AGB sugar conversion (Fig. S1). To enable this comparative study, a single source of AGB was used as a feedstock for all three pretreatments, and one source of enzymes was applied to the pretreated biomass. A 2D ^{13}C–^{1}H heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectroscopy is employed in this study to chemically characterize the different linkages present in the untreated and pretreated cell wall of AGB. Syringyl (S) and guaiacyl (G) lignin substructures ratios are determined by HSQC-NMR. A comparison of process flowsheet and mass balances for the three pretreatments is used based on glucan and xylan conversion from saccharification experiments and compositional analysis.

2. Materials and methods

2.1. Sample preparation

Agave bagasse was donated by Tequila Corralejo based in Guanajuato, Mexico. This facility receives the central fruit (the stem or "pina") from defoliated agave plants aged 7–8 years. The stems were cooked for 18 h in an autoclave, then milled and compressed to separate the syrup from wet bagasse. Samples of the wet bagasse were collected, washed thoroughly with distilled water and dried in a convection oven at 40 °C. The dried AGB was milled with a Thomas-Wiley Mini Mill fitted with a 20 mesh screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA). This AGB was pretreated as follows: AH pretreatment was carried out at the Autonomous University of Coahuila, AFEX pretreatment was performed at Michigan State University (MSU), and IL pretreatment by done at the Autonomous University of Nayarit. After pretreatment, each biomass was dried in a convection oven at 40 °C for 3 days with an average moisture content below 5%. All pretreated materials were refrigerated at 4 °C in a sealed plastic bag until further analysis. Acid-insoluble lignin, and structural carbohydrates (glucan, xylan and arabinan) of agave bagasse before and after pretreatment were determined according to the standard analytical procedures of the National Renewable Energy Laboratory (NREL) by a two-step sulfuric acid hydrolysis (Sluiter et al., 2008). The biomass was extracted with water and ethanol prior to the acid hydrolysis step.

2.1.1. Chemicals

Ionic liquid, 1-ethyl-3-methylimidazolium acetate ([C_2C_1Im][OAc]), sulfuric acid, 3,5-dinitrosalicylic acid (DNS), dimethyl sulfoxide, anhydrous ammonia, glucose, xylose, citric acid, sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Biomass pretreatment

2.2.1. Autohydrolysis (AH)

Milled biomass was pretreated using a 1:10 mass ratio of biomass to water with DI water at 180 °C and 140 psi in a pressurized batch reactor with a PID (proportional–integral–derivative) controller for 30 min in an isothermal heating regime. These conditions were previously determined by Ruiz et al., 2013. After AH pretreatment, recovered biomass was vacuum filtered, thoroughly washed using 25 g of water per gram of AGB with DI water and dried to less than 10% water content.

2.2.2. Ammonia fiber expansion (AFEX)

AFEX pretreatment was carried out at Michigan State University as described previously (Balan et al., 2009). The pre-wetted AGB (40% moisture, dry weight basis) was loaded into a high pressure stainless steel Parr reactor and the reactor lid was closed. Anhydrous liquid ammonia was added to AGB in the ratio of 2:1 (dry weight basis), and the residence time was 30 min at 102 °C and

325 psi. AFEX is essentially a "dry to dry" process with no water washing after pretreatment. The pretreated biomass was kept in the hood until the residual ammonia had evaporated, then was bagged for further processing and analysis and stored in room temperature until further use.

2.2.3. Ionic liquid (IL)

A 10% (w/w) biomass solution was prepared by combining 1 g of AGB (dry basis) with 9 g of $[C_2C_1Im][OAc]$ in a 50 mL autoclave vial. The vials and the contents were heated to the desired temperature (120 °C) for 3 h. After pretreatment a washing step was performed as previously described (Perez-Pimienta et al., 2013). Briefly, 90 g of DI water per gram of AGB was slowly added into the biomass/IL slurry, immediately forming a precipitate. Solids were recovered after separating the supernatant containing IL with a centrifuge at 10,000*g* for 25 min.

2.3. Saccharification

2.3.1. Enzymes

Cellic® CTec2 (cellulase complex for degradation of cellulose) with 181 mg protein/mL and HTec2 (endoxylanase with high specificity toward soluble hemicellulose) with 180 mg protein/mL were a gift from Novozymes North America, Inc.

2.3.2. Enzymatic saccharification

Batch saccharification of untreated and pretreated AGB samples was carried out at 50 °C and 150 rpm in 50 mM citrate buffer (pH 4.8) in a rotary incubator with CTec2 and HTec2. All samples were diluted using a lower (5 g/L) and higher glucan content (20 g/L) in the biomass samples to understand the impact of each pretreatment on sugar production. Total batch liquid volume was 5 mL at the appropriate biomass loading in a 15 mL centrifuge tube. The loadings of CTec2 and HTec2 were set at 40 mg protein/g glucan and 4 mg protein/g xylan, respectively. These are not industrially relevant enzyme loadings; our goal was to examine the effects of pretreatment on biomass hydrolysis without enzymes as a limiting factor. All assays were performed in triplicate. Reactions were monitored by removing 100 μL samples of the supernatant at specific time intervals (0, 0.5, 1, 3, 6, 24, 48 and 72 h). All assays were performed in triplicate. Error bars show the standard deviation of triplicate measurements.

2.4. Sugar analysis

2.4.1. DNS assay

Reducing sugars were measured using the DNS assay in a DTX 880 Multimode Detector (Beckman Coulter, CA) at 550 nm on the saccharified samples which were centrifuged at 10,000*g* for 5 min (Li et al., 2010), using D-glucose solutions in water as calibration standards.

2.4.2. Glucose and xylose conversion

At the end of the saccharification period (72 h), samples were analyzed for glucose and xylose concentrations using a YSI 2700 Select Biochemistry Analyzer. This results in three concentration values that were averaged for each conversion data point, and also enables standard deviation calculations. The glucan conversion was calculated as follow:

$$\text{Glucan conversion } (\%) = \frac{\text{Glucose conc}\left(\frac{g}{mL}\right) \times \text{reaction vol (mL)}}{\text{Biomass } (g) \times \text{wt\% glucan in biomass}} \times \frac{162 \text{ (PM cellulose unit)}}{180 \text{ (PM glucose unit)}} \times 100 \quad (1)$$

Conversion is based on the total mass of each material prior to pretreatment, and thus represents an overall process conversion. The xylan conversion was calculated similarly as:

$$\text{Xylan conversion } (\%) = \frac{\text{Xylose conc}\left(\frac{g}{mL}\right) \times \text{reaction vol (mL)}}{\text{Biomass (g)} \times \text{wt\% xylan in biomass}} \times \frac{132 \text{ (PM xylan unit)}}{150 \text{ (PM xylose unit)}} \times 100 \quad (2)$$

and is based on the difference in molecular weight between xylan and the xylose unit (Shill et al., 2012).

2.5. Two-dimensional ^{13}C–^{1}H heteronuclear single quantum coherence (HSQC) NMR spectroscopy of AGB samples

Untreated and pretreated AGB cell walls were ball-milled, solubilized in DMSO-d_6, and then analyzed by 2D HSQC NMR as previously described (Sathitsuksanoh et al., 2014). The homogeneous solutions were transferred to NMR tubes. HSQC spectra were acquired at 25 °C using a Bruker Avance-600 MHz instrument equipped with a 5 mm inverse-gradient $^{1}H/^{13}C$ cryo-probe using a q_hsqcetgp pulse program (*ns* = 200, *ds* = 16, number of increments = 256, d_1 = 1.0 s) (Heikkinen et al., 2003). Gaussian apodization in F_2 (LB = −0.18, GB = 0.005) and squared cosine-bell in F_1 (LB = −0.10, GB = 0.001) was applied prior to 2D Fourier Transformation. Chemical shifts were referenced to the central DMSO peak at 39.5/2.5 ppm (δ_C/δ_H). Assignments of the HSQC spectra were described elsewhere (Sathitsuksanoh et al., 2014). A semi-quantitative analysis of the volume integrals of the HSQC correlation peaks was performed using Bruker's Topspin 3.1 processing software. Changes in lignin structure were determined based on volume integration of HSQC spectral contour correlations. The C_2–H_2 position of the guaiacyl unit and the $C_{2,6}$–$H_{2,6}$ positions in the syringyl unit were considered to be stable (Sette et al., 2011) and used as the internal standard that represents aromatic C_9 units in the lignin. Spectral integration was performed on the same contour level. All integrals displayed less than 10% error (based on the use of organosolv lignin in triplicate – data not shown), confirming the precision of the quantification from 2D HSQC spectra.

3. Results and discussion

3.1. Compositional analysis of untreated and pretreated agave bagasse samples

Table 1 summarizes the different pretreatment processes used to pretreat AGB and it composition in terms of major cell wall components before and after pretreatment. The composition of untreated AGB in dry basis was 31.2% glucan, 15.7% xylan, and 18.4% lignin, which is similar to other reported values in terms of xylan and lignin content (Ávila-Lara et al., 2015). However, the glucan content of untreated AGB is lower than that reported in other studies, namely 40% (Perez-Pimienta et al., 2013; Saucedo-Luna et al., 2011). This difference can potentially be attributed to environmental conditions of the biomass source, extraction, and post-harvest procedures, as well as process conditions during tequila production. Results show, as expected, the distinct chemistries of each pretreatment as demonstrated by differences in cell wall composition for the pretreated samples. For example, AFEX-pretreated AGB preserves all of the carbohydrates relative to the untreated samples. AFEX thus differs from the AH and IL pretreatments for which 52.6% and 69.5% is recovered after the wash step, and in which a large fraction of xylan and lignin are solubilized, respectively. A higher residual glucan level was obtained with AH–AGB (98.1%) than with both, AFEX and IL (31.2%, and 57.3%,

Table 1
Compositional analysis of untreated and pretreated agave bagasse (dry weight)[a,b].

Pretreatment	Recovered agave bagasse (%)	Glucan (%)	Xylan (%)	Arabinan (%)	Lignin (%)	Ash (%)
Untreated	100 ± 0.0 (100 kg)	31.2 ± 0.4 (31.2 kg)	15.7 ± 0.1 (15.7 kg)	0.5 ± 0.0 (0.5 kg)	18.4 ± 0.4 (18.4 kg)	6.4 ± 0.2 (6.4 kg)
AFEX	100 ± 0.0 (100 kg)	31.3 ± 0.0 (31.3 kg)	15.3 ± 0.3 (15.3 kg)	0.2 ± 0.2 (0.2 kg)	18.2 ± 0.4 (18.2 kg)	4.6 ± 0.1 (4.6 kg)
Autohydrolysis	52.6 ± 0.8 (52.6 kg)	51.6 ± 0.7 (27.1 kg)	5.9 ± 0.4 (3.1 kg)	0.0 ± 0.4 (0.0 kg)	34.3 ± 0.1 (18.0 kg)	2.6 ± 0.6 (1.4 kg)
Ionic liquid	69.5 ± 1.8 (69.5 kg)	39.8 ± 0.2 (27.7 kg)	14.6 ± 0.0 (10.2 kg)	0.1 ± 0.0 (0.1 kg)	13.8 ± 0.4 (9.6 kg)	3.3 ± 0.4 (2.3 kg)

[a] The inability to close the initial mass balance is likely due to the protein, and other sugars present in the biomass.
[b] Values in parentheses represent the amount of each component recovered from 100 kg total agave bagasse.

respectively), as calculated based on the weight of recovered product. Lignin was reduced by 25.0% using the IL pretreatment on AGB, which was higher than the delignification efficiency previously reported when similar process conditions were employed (120 °C for 3 h) (Perez-Pimienta et al., 2013). Furthermore, xylan content decreased by 62.4% and 7.0% with AH and IL pretreatments, respectively. However, there are not previous reports in the open literature in which AFEX and AH were used on AGB.

Compositional analysis also reveals the effects of each individual pretreatment chemistry on the principal components of AGB. AFEX pretreatment is a dry to dry process, where minimal carbohydrate degradation takes place, in addition xylan and lignin are partially solubilized, then gets relocated to the biomass surface during the process. This sequence of events leaves behind a highly porous AGB cell wall which is reflected in improved enzyme access to the embedded glucan and xylan (Chundawat et al., 2011). In contrast, AH pretreatment cleaves ester and ether linkages, producing a recovered material with higher levels of residual lignin (65.2%) when compared to the untreated AGB (18.4%). Also, the conditions employed favor the xylan to xylose conversion that can be used to obtain additional ethanol or furfural in certain conditions (Larsson et al., 1999). IL pretreatment effectively solubilizes plant cell wall components. After solubilization, most of the polysaccharides can be regenerated by adding an antisolvent (for example, water and/or ethanol). A significant portion of lignin remains in solution but toxic products such as furfural or formic acid are not formed as they are in organosolv or acid pretreatments. The changes in biomass composition of pretreated AGB are consistent with results from previous reports using agroindustrial residues (Kumar et al., 2009; Li et al., 2011; Ruiz et al., 2012).

3.2. Nuclear magnetic resonance studies of untreated and pretreated agave bagasse samples

Two dimensional HSQC-NMR was used to elucidate the structural changes in carbohydrates and lignins for untreated and pretreated biomass. The aliphatic regions from untreated and pretreated AGB are presented in Fig. S2. Assignments of the lignin ^{13}C–^{1}H correlation peaks in the 2D HSQC spectra of AGB cell wall are given in Table S1. Untreated AGB contains mainly β-aryl ether units, and methoxyls (MeO) with trace amounts of β-β and β-5 units. Compared to the untreated samples, X'_2 and X'_3 of AFEX-pretreated AGB disappear, indicating a removal of acetylated xylopyranosides, thus reducing steric hindrance and increasing cellulose accessibility (Singh et al., 2015). Consequently, the enzymatic hydrolysis of AFEX-pretreated AGB was higher than that of untreated AGB. This effect was not present in AH and IL pretreated AGB, where X'_2 and X'_3 remain. AH pretreatment deacetylates hemicelluloses, whereas IL pretreatment causes disappearance of β-aryl ether units from lignin, suggesting a higher degree of lignin de-polymerization by IL pretreatment.

The anomeric region correlation of untreated AGB shows some important polysaccharide anomeric units including (1-4)-linked β-D-glucopyranosyl units (β-D-Glc*p*, glucan) and (1-4)-linked β-D-xylopyranosyl units (β-D-Xyl*p*, xylan) (Supplementary information, Fig. S3). Noticeable differences in the pretreated plant cell walls are apparent and relate to the specific chemistry of each pretreatment. For example, AFEX-AGB showed a weaker 2-O-Ac-β-D-Xyl*p*(R), (1-4)-β-D-Glc*p*(NR) and 2,3-di-O-Ac-β-D-Xyl*p*(R) signal than those in AH and IL pretreated AGB which had a higher intensity. The aromatic regions of untreated and pretreated AGB are shown in the Supplementary information, Fig. S4. Agave bagasse lignin is a typical syringyl/guaiacyl (S/G) lignin as shown by the S/G substructure ratios of untreated and pretreated samples presented in Table 2. This the first time that S/G ratio of untreated agave *tequilana* is calculated. Untreated AGB had a S/G ratio of 4.3, considerably higher than biofuel feedstocks such as switchgrass (0.2), sugarcane bagasse (1.1) or corn stover (1.4) (Lupoi et al., 2015). However, this experimental S/G ratio for untreated AGB agrees with observations for other species of agave, e.g., S/G ratios from 3.4 to 3.6 for agave *sisalana* when compared to agave *tequilana* as with the present report (Lupoi et al., 2015). An increased S/G ratio is observed in AFEX and IL pretreated samples when compared to the untreated sample, but differences are not statistically significant compared to AH-pretreated AGB. Nevertheless, there is no clear trend as to whether or not a high S content results in increased monomeric sugar release during enzymatic saccharification. Even so, the S/G lignin ratios have proven to be an important parameter for gauging lignin recalcitrance (Lupoi et al., 2015).

3.3. Effect of pretreatment on enzymatic saccharification and sugar conversion

Untreated and pretreated samples were saccharified using commercial enzyme cocktails to compare their sugar production kinetics at lower (5 g/L) and higher glucan loading content (20 g/L) (Fig. 1). Pretreated samples substantially increase the sugar yields

Table 2
Comparison of syringyl (S) and guaiacyl (G) lignin substructures ratio determined by HSQC-NMR from untreated and pretreated agave bagasse.

Pretreatment	S/G ratio
Untreated – Agave *tequilana*	4.3
AFEX	5.0
AH	4.2
IL	4.7
Agave *sisalana*[a]	3.4
Agave *sisalana*[a]	3.6

[a] Lupoi et al. (2015).

under both approaches (lower and higher biomass loading) when compared to the untreated AGB. In all cases most sugars were produced during the first 12 h, after which the rate of sugar production diminished greatly. IL pretreated AGB was always higher than either AFEX or AH pretreated AGB in terms of sugar production, and this result occurred at both biomass loadings. At lower and higher AGB loadings, respectively, within 24 h using the IL-pretreated AGB, sugar yields were 6.8 and 25.5 g/L, AFEX-pretreated AGB gave 5.9 and 21.4 g/L and AH-pretreated AGB yielded 4.1 and 14.4 g/L. Untreated AGB produced 1.5 and 4.1 g/L, using lower and higher biomass loadings, respectively. The 72-h glucan and xylan conversions for all three pretreatments and the untreated AGB under lower and higher biomass loading are shown in Fig. 2.

Based on the washed, pretreated biomass (not washed in the case of AFEX), the IL-AGB gave the highest conversion yields of glucan and xylan to glucose and xylose, respectively; whereas AFEX-AGB produced the maximum overall sugar production (glucose plus xylose) of all three pretreatments during the enzymatic hydrolysis. The physicochemical properties and effects exerted by each pretreatment on the untreated biomass are responsible for the differences observed in glucan and xylan conversion. For example, the autohydrolysis pretreatment removes considerable xylan and modifies the lignin structures, resulting in improved cellulose accessibility and a glucan conversion above 80% using the two biomass loadings.

In the other hand, AFEX pretreatment disrupts lignin-carbohydrates linkages and increases biomass porosity without forming many inhibitors that affect downstream biological processes (Alvira et al., 2010). Hence, AFEX-AGB also enabled a glucan conversion above 80%. IL pretreatment modifies cellulose structure from a crystalline to a more amorphous one (cellulose I to cellulose II), accompanied by significant lignin removal, to increase sugar conversion (Cheng et al., 2011) and achieve ~95% glucan and ~83% xylan conversion for the washed, pretreated biomass. When the initial biomass loading during saccharification decreased from

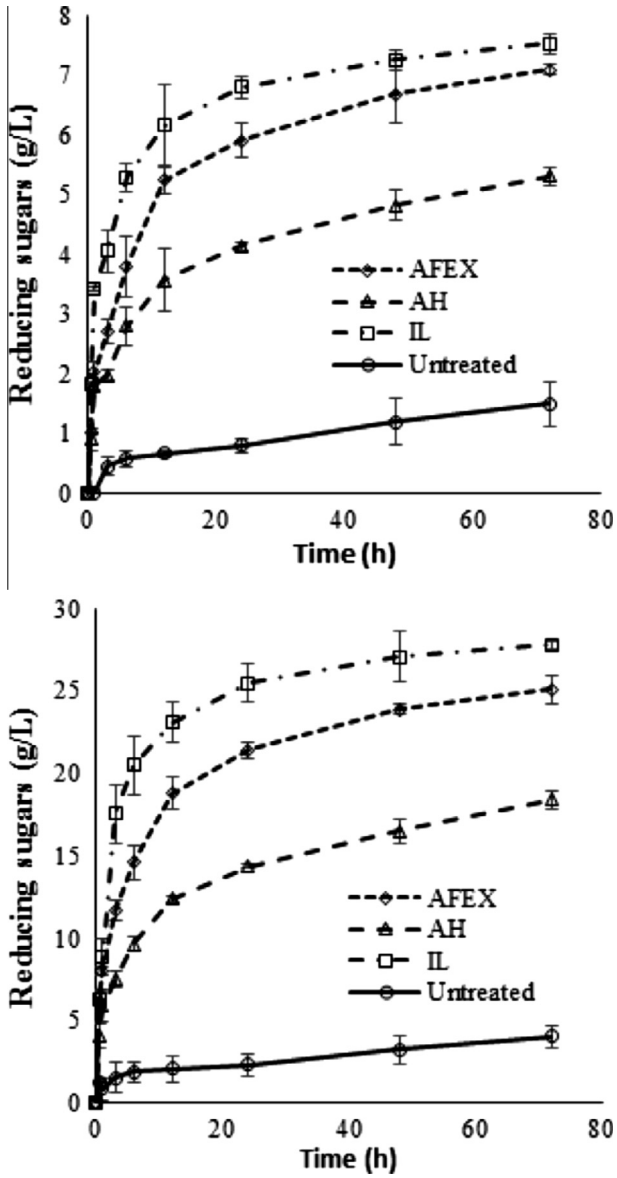

Fig. 1. Enzymatic saccharification of untreated and pretreated agave bagasse using a lower (top) and higher biomass loading (bottom). AH and IL samples were washed prior to enzymatic saccharification. Error bars show the standard deviation of triplicate measurements.

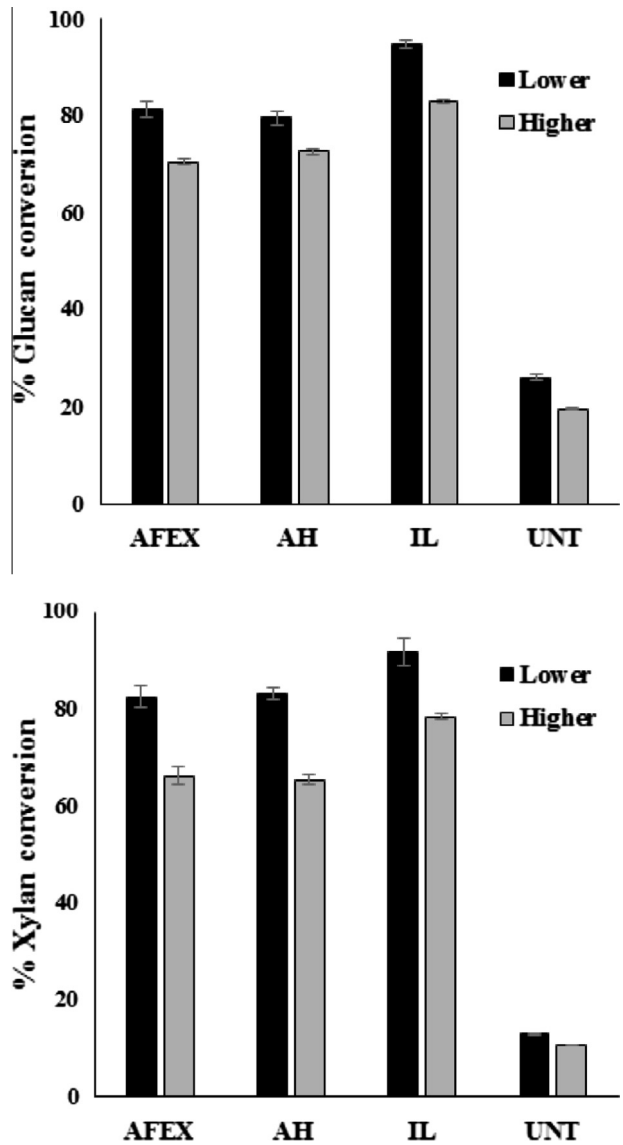

Fig. 2. Glucan (top) and xylan (bottom) conversion of untreated and pretreated agave bagasse at 72 h of saccharification (72 h) using a lower (5 g glucan/L) and higher (20 g glucan/L) biomass loading. AH and IL materials were washed prior to enzymatic hydrolysis. Error bars show the standard deviation of triplicate measurements.

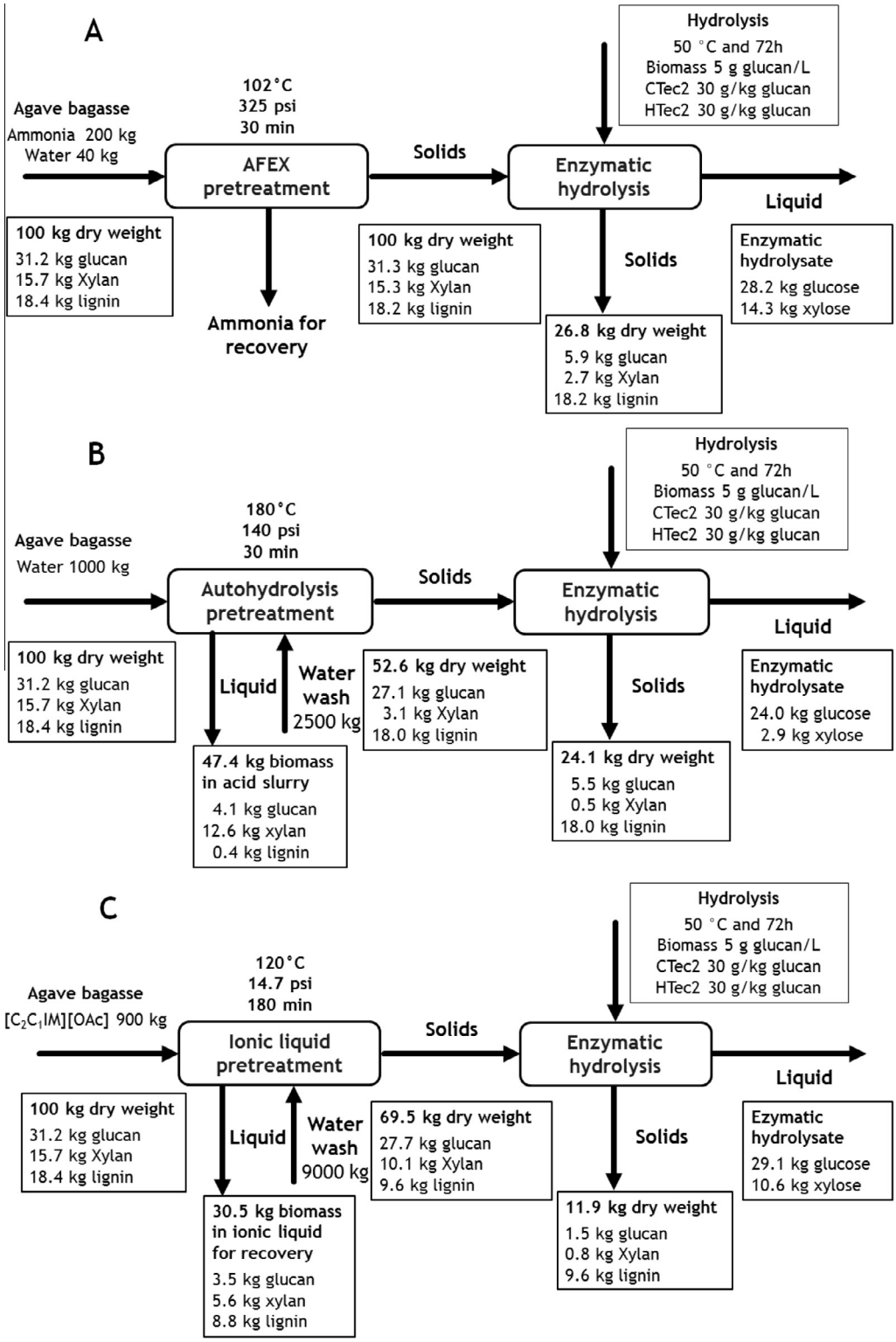

Fig. 3. Mass balance flow diagram on a 100 kg basis of agave bagasse for three pretreatments. (A) AFEX, (B) AH and (C) IL pretreatments and enzymatic hydrolysis.

20 to 5 g glucan/L, both the glucan and xylan conversions increased. For example, glucan conversions increased by 1.15, 1.09 and 1.14-fold for AFEX, AH and IL pretreated AGB, respectively, when going from low to high biomass loadings. These results agree with previous reports where similar conditions achieved good glucan and xylan conversions for agro-industrial feedstocks such as corn stover or wheat straw, using as catalysts: water in AH, ammonia in AFEX and [C_2C_1Im][OAc] in IL pretreatment (Li et al., 2011; Ruiz et al., 2012).

3.4. Process mass balances

To understand the impact of each of the three individual pretreatments analyzed (AFEX, AH, and IL) in AGB, compositional

analysis and subsequent enzymatic hydrolysis were used to develop individual mass balances for the pretreatments. All process yields were normalized on the basis of 100 kg of dry, untreated AGB, including the weight gain due to hydrolysis water (Fig. 3).

Catalyst (ammonia and [C_2C_1Im][OAc]) recycle was not investigated in this study. When properly detoxified, the liquid hydrolyate resulting from AH is xylose-rich and might be further transformed into ethanol or other products (Ruiz et al., 2013). The recovered biomass after AH pretreatment was 52.6% of the untreated AGB, primarily due to high xylan removal during the process. Currently, 2500 kg of water is required after pretreatment to wash 100 kg of the biomass. For the IL pretreatment, residual IL is removed by a series of washing steps in which 9000 kg of DI water was used per 100 kg of initial AGB. The IL removed in the wash stream can potentially be recycled and reused in further pretreatments. The washed and recovered solid stream from IL pretreatment was 69.5% by mass of the initial untreated biomass. It is this stream that was subjected to enzymatic hydrolysis. Two different enzymatic hydrolysis schemes using a lower and higher biomass loading were performed on the pretreated AGB, however, only the low biomass loading at 5 g glucan/L and the glucan/xylan conversion at 72 h were used for the mass balances reported here.

For AFEX pretreated AGB, 28.2 kg of glucose and 14.3 kg of xylose, or a total of 42.5 kg of monomeric sugars, were produced during the enzymatic hydrolysis per 100 kg of initial biomass. This is the highest combined sugar production of the three pretreatments studied in this paper. The residual, unhydrolysed solid fraction stream is composed of 8.6 kg of polymeric recalcitrant carbohydrates and 18.2 kg of lignin per 100 kg of initial biomass.

Enzymatic hydrolysis of AH pretreated AGB achieved 24.0 kg of glucose and 2.9 kg of xylose per 100 kg of initial biomass, or glucan and xylan conversions of 79.6% and 83.1%, respectively. Like AFEX pretreatment, a solid fraction of 26.9 kg of solids per 100 kg of inlet biomass remained after the saccharification step. These solids might be used to generate electricity in a biorefinery scheme. Finally, IL pretreated AGB achieved 29.1 kg of glucose and 10.6 kg of xylose per 100 of initial biomass. Likewise, a solid fraction of 39.7 kg of total solids per 100 kg of initial biomass was obtained after saccharification.

Uppugundla et al. (2014) reported that AFEX pretreated corn stover gave 26.0 kg of glucose and 13.0 kg of xylose; while, IL pretreatment gave 34.4 kg of glucose and 17.0 of xylose per 100 kg of corn stover. The glucan and xylan contents for the AGB studied here are lower that other literature values of 40% and 20%, respectively (Saucedo-Luna et al., 2010).

All three pretreatment technologies significantly improved biomass saccharification for AGB. In a biorefinery scenario, an AH-based process should use the xylose-rich wash stream generated after pretreatment (12.6 kg xylan per 100 of initial AGB) thereby increasing the final fermentation yield. Pretreated biomass resulting from the dry-to-dry AFEX process can be used at higher concentrations as a dry-to-dry process, thereby achieving potentially higher sugar and ethanol concentrations (Uppugundla et al., 2014). An IL pretreatment-based system can potentially utilize the xylan and lignin components extracted from the liquid wash stream. These extracted components can possibly be further upgraded into valuable products, including ionic liquids for biomass pretreatment, hence proving process economics (Socha et al., 2014).

4. Conclusions

Compositional analysis of AFEX pretreated AGB did not show a significant differences when compared to untreated AGB. In contrast, about 62.4% of xylan was solubilized with AH pretreatment, and 25% of delignification occurred after IL pretreatment. All three pretreatments enhanced sugar production in enzymatic hydrolysis. Yields of glucose plus xylose were 42.5, 39.7 and 26.9 kg per 100 kg of untreated AGB in the major hydrolysate stream for AFEX, IL and AH, respectively. We believe that these comparative analyses will contribute to a better understanding of AGB recalcitrance and enable more rational selection of biorefinery configurations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.03.103.

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