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Title

Xylo-oligosaccharides, fermentable sugars, and bioenergy production from sugarcane straw using steam explosion pretreatment at pilot-scale

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Abstract

This study investigated the production of xylo-oligosaccharides (XOS) from sugarcane straw (SCS) using steam explosion (SE) pretreatment at pilot-scale, as well as co-production of other fermentable sugars and lignin-rich residues for bioethanol and bioenergy, respectively. SE conditions 200 °C; 15 bar; 10 min led to 1) soluble XOS yields of up to 30% (w/w) of initial xylan with ~50% of the recovered XOS corresponding to xylobiose and xylotriose, considered the most valuable sugars for prebiotic applications; 2) fermentable glucose yields from the enzymatic hydrolysis of SE-pretreated SCS of up to ~78%; 3) increase in the energy content of saccharified SCS residues (16%) compared to the untreated material. From an integrated biorefinery perspective, it demonstrated the potential use of SCS for the production of value-added XOS ingredients as well as liquid and solid biofuel products.

1. Introduction

Sugarcane straw (SCS), a residual by-product of the sugarcane industry, is gaining attention as an abundant and low-cost lignocellulosic resource to produce biofuels and high-value products for sugarcane biorefineries (Aguiar et al., 2021). This is partly because the practice of burning sugarcane plantations to remove the straw before harvesting is being prohibited due to health and environmental concerns. Moreover, the utilization of SCS yields additional lignocellulosic material without expanding feedstock cultivation areas, hence maximizing the productivity and competitiveness of the existing sugarcane sugar and bioethanol production platforms (Cherubin et al., 2021). For example, in Brazil, which is the leading producer of sugarcane in the world (657 million metric tons in the crop year 2020/2021), at least 20 million tonnes of SCS biomass could be harvested for biorefinery applications without impacting soil quality (Carvalho et al., 2017; USDA 2021, 2021).The composition of SCS, typically consisting of dry leaves (~60%) from the stalk and green leaves on the top (~40%), is primarily glucan (~30 to 45% w/w), xylan (~25 to 30% w/w) and lignin (~20 to 30% w/w).

Its chemical composition can vary widely depending on the plant development stage, variety, and collection method from site to site (Aguiar et al., 2021). From a biorefinery perspective, the physical and chemical complexities of the lignocellulosic components require deconstruction to be converted into biofuels and biochemicals. In this context, physical (milling and grinding), chemical (alkaline, acid, hydrothermal, and ionic liquids), physicochemical (ammonia fiber expansion – AFEX, and steam explosion – SE), or biological (enzymatic and microorganisms) pretreatment methods are an essential part of the processing steps to overcome the recalcitrance of biomass and in turn, can dictate the efficiency of production as well as the economic and environmental impacts of bio-based products. Several pretreatment strategies for the bioconversion of SCS into value-added bio-products have been explored and are summarized elsewhere (Aguiar et al., 2021). Among these pretreatments, SE is generally considered an environmentally friendly, efficient, chemical-free, economic, mild and fast treatment process suitable for industrial applications and various lignocellulosic feedstocks (Auxenfans et al., 2017). During SE, lignocellulose is saturated with high-pressure (7 to 48 bar) steam at high temperatures (160 to 240 °C) for several minutes (5 to 15 min) and then suddenly de-pressurized, causing the lignocellulosic biomass to undergo an explosive decompression (Yu et al., 2022). Under the high temperature combined with pressure, the hydronium ions formed from water dissociation together with the acetic acid released through the hydrolysis of acetyl groups linked to the xylan backbone act as *in situ* catalysts. As a result, disruption of the glucan-xylan-lignin complex occurs, leading to the depolymerization and removal of the hemicellulose xylan and mixed-linkage glucan, with the limited dissolution of glucan and partial degradation of lignin (Auxenfans et al., 2017; Bhatia et al., 2020b). However, there are also certain disadvantages, such as the incomplete deconstruction of the lignin-carbohydrate complex or production of inhibitors and degradation products depending on the SE severity conditions (Biswas et al.,

2015). For a comprehensive review on the impacts of a steam explosion step enhancing the physical and chemical properties of the biomass feedstock and the different subsequent conversion processes of biomass including densification for solid pellets (heating value) and enzymatic hydrolysis (cellulose accessibility) for fermentation to liquid biofuels, see Yu et al., 2022.

Recently, there has been a growing interest in producing xylo-oligosaccharides (XOS) from xylan-rich plants, including sugarcane (Prenexus Health, USA) and corncob (Shangdong Longlive Biotechnology, China) (Kumar et al., 2021). With a degree of polymerization (DP) from 2 up to 14 units of xylose, XOS can exhibit multiple properties such as pH (2.5 to 8) and temperature (up to 100°C) stability, low-calorie sweetening potency for sugar and fat alternatives, moisture retention capabilities and prebiotic properties beneficial for health (Álvarez et al., 2020; Amorim et al., 2019). The market price of XOS ranges from 25 $/kg to 50 $/kg depending on purity level (70 to 95%), and the global market is expected to achieve ~$130 million by 2025 (Santibáñez et al., 2021; Singh et al., 2018). Hence, XOS sugars have gained commercial interest as animal feed, pharmaceutical, food, and beverage ingredients (Pinales-Márquez et al., 2021).

XOS production methods using chemical, enzymatic, hydrothermal, and SE pretreatments for sugarcane residues have been employed at different scales. However, XOS from SCS has been less extensively studied relative to sugarcane bagasse (SCB), an abundant sugarcane agro-industrial by-product (Carvalho et al., 2018). A particular challenge for these pretreatment processes is to achieve high XOS yields in the recommended XOS ranges (DP 2 to 6) for prebiotic action while minimizing the production of undesirable impurities (monosaccharides and sugar degradation products) that compromise XOS purity for commercial exploitation (Santibáñez et al., 2021). Nevertheless, the production of value-added XOS products from lignocellulosic residues and wastes could contribute to the

sustainability, and economic viability of a commercial sugarcane-based biorefinery, primarily since the production of XOS and a variety of bio-based products from SCS has not previously been investigated.

There is a knowledge gap in pilot-scale SE pretreatment conditions for SCS biomass that seeks maximum hemicellulose solubilization into XOS, which may be advantageous to overcome reports of low XOS conversion yields and/or multi-step downstream processes (Milessi et al., 2021). The primary focus of the study is the production of XOS from SCS using an economical single step SE pretreatment process without the cost and use of additional catalytic chemicals and expensive enzymatic conversions. Important SE parameters to achieve high XOS yields with low DP and low by-products and sugar degradation products were identified, and enzymatic hydrolysis of SE pretreated SCS was proposed for the co-production of fermentable sugars and lignin-rich residues for bioethanol and bioenergy, respectively.

2. Materials and methods

2.1. Feedstock preparation

SCS, provided in bales by Usina Ferrari (São Paulo, Brazil), was unpacked, crushed, screened, air-dried to ~10% (w/w) moisture content (MC), hammer-milled and de-ashed in a Disintegrator DM 540 (IRBI, São Paulo, Brazil). The resulting SCS with ~7% (w/w) MC and particle size in the range from 0.1 to 2.4 mm (see supplementary material) was stored in an air-tight sealable polyethylene bag at room temperature until further use.

2.2. Steam explosion pretreatment

SCS (0.25 kg) were suspended in deionized water at 10:1 water/solid ratio (g/g) and soaked for 2 h at 20 ± 5 °C. The excess liquid of the mixture was drained using a muslin cloth. Aliquots of the recovered liquid were analyzed for total sugar content according to NREL/TP-510-42623 procedure (Sluiter et al., 2008). The strained SCS (~80% MC) was

loaded into a 30 L pilot-scale reactor Cambi SE rig (Cambi, Norway), and pretreatments were carried out at temperatures of 180 °C (9 bar), 200 °C (15 bar), and 210 °C (20 bar) with residence times of 5, 10 and 15 min. For each pretreatment condition, a minimum of two batches was processed. After each pretreatment, the reactor was discharged, and the material was collected in a 10 L bucket to cool down. Deionized water was added to the slurry (0.5 L) and strained using a muslin cloth to separate the liquid from the pretreated fraction. In addition, non-soaked (0 h) and soaked SCS at 70 °C for 2 h were also pretreated as a strategy to increase XOS yields. The pretreated solid material was stored at -20 °C, and the liquid fractions were stored at 4 ºC until further use. Biomass recovered (%) was estimated as DM pretreated solids obtained after pretreatment per 100 g DM of untreated solids. The severity factor (SF) (Equation 1) was calculated according to (Overend and Chornet, 1987).

$$SF = \log10 [t * \exp [(T-100)\ 14.75^{-1}]] \quad (1)$$

Where: t is residence time (min), T is temperature (°C) and 14.75 is activation energy value.

2.3. Chemical characterization of pretreated solids and hydrolysates

Compositional analysis of untreated and SE-pretreated SCS was determined according to the NREL/TP-510-42618 procedure (Sluiter et al., 2012). All SE-pretreated biomass was thoroughly washed with deionized water to ensure the complete removal of residual hydrolysate before compositional analysis. Compositional analysis of the hydrolysates (mono- and oligosaccharides) was determined according to the NREL/TP-510-42623 procedure (Sluiter et al., 2008).

2.4. Analysis of xylo-oligosaccharides, monosaccharides, degradation products, and by-products

XOS was quantified by High-Performance Anion Exchange Chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) (Thermofisher ICS-5000), using a Dionex CarboPac PA200 guard (3 × 50 mm) and analytical (3 × 250 mm) columns at 30 °C,

flow rate of 0.3 mL/min and 25 µL injection volume. The elution program was described previously (Bhatia et al., 2020b). Xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), and xylohexaose (X6) purchased from Megazyme were used to construct a calibration curve ranging from 1.25 to 20 µg/mL.

Monosaccharides were quantified by HPAEC-PAD (Thermofisher ICS-5000) using a Dionex CarboPac SA10 guard (4×50 mm) and analytical (4×250 mm) columns at 45 °C, a flow rate of 1.5 mL/min with 1 mM KOH as eluent and 25 µL injection volume. Glucose, xylose, arabinose, galactose, mannose, fructose, sucrose, cellobiose, and fucose were run as calibration standards from 1.25 to 20 µg/mL. By-products and degradation products were analysed by High-performance liquid chromatography (HPLC) equipped with a refractive index detector using an Aminex HPX-87H column (Bio-Rad) at 55 °C, a flow rate of 0.6 mL/min with 5 mM H_2SO_4 as eluent according to NREL's standard procedure (Sluiter et al., 2008).

2.5. Enzymatic hydrolysis of pretreated solids

Enzymatic hydrolysis was carried out in triplicate at a solid biomass loading of 1% (w/v) with 0.05 M sodium acetate buffer (pH 5) at 50 °C using the commercial cocktail Cellic® CTec2 (Novozymes A/S, Denmark) in a total volume of 5.0 mL. The total cellulase activity against filter paper (FPU) measured by the standard IUPAC method was previously reported to be 133 FPU/mL (Kontogianni et al., 2019). The hydrolysis experiment was conducted in 15 mL falcon tubes in a rotary shaker set at 150 rpm. Dosage response curve experiments were carried out with enzyme loadings of 5, 10, and 20 mg protein/ g glucan, and samples were withdrawn after 4, 24, 48, and 72 h. The enzymatic hydrolysis was ended by boiling samples at 100 °C for 10 min. After centrifugation (10 min, 10,000 × *g*), the supernatants were analyzed for glucose and xylose yields by HPAEC-PAD.

175 *2.6. Determination of hemicellulose/holocellulose and lignin/holocellulose using attenuated*
176 *total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy*
177 Pretreated SCS was milled using an MF 10 microfine grinder (IKA® Werke GmbH & Co.
178 KG, Germany) to obtain particles that passed through a 0.5 mm sieve. The IR spectra of the
179 samples were collected five times using a Nicolet 6700 FT-IR, Pike Technologies GladiATR
180 diamond spectrometer (Thermo Scientific, Waltham, MA, USA) at 25 °C. The spectral range
181 included was 4000–600 $cm^{-1,}$ and spectra were obtained using 64 scans (128 for the
182 background) and a resolution of 4.0 cm^{-1}. After subtraction of a separate linear background
183 for each peak, the peak area ratio 1732/1160 cm^{-1} (ranges 1675-1750 and 1142-1182 cm^{-1},
184 respectively) was used for hemicellulose/holocellulose estimation, while the ratio 1512/1160
185 cm^{-1} (ranges 1483-1531 and 1142-1182 cm^{-1}, respectively) was used for lignin/holocellulose
186 estimation (Lupoi et al., 2014).
187 *2.7. Thermal analysis*
188 Lignin was isolated from raw SCS using sodium hydroxide (10%, 100 °C, 3 h), recovered by
189 adding concentrated sulfuric acid until pH 2 and dried in an oven at 30 °C until the MC was
190 below 5%. The thermal stability was evaluated in a TGA/DSC analyzer (SDT Q600, TA
191 Instruments). 5 mg of lignin was placed in air-tight aluminum support with a heating rate of
192 10 °C min^{-1}, from 25 to 800 °C under a synthetic air atmosphere.
193 *2.8. Energy content measurement*
194 Energy content measurements of SCS before and after pretreatment and lignin-rich residues
195 remaining after enzymatic hydrolysis were performed in a standard bomb calorimeter (Parr™
196 6400 Automatic Isoperibol Calorimeter). All samples were dried in an oven at 30 °C until the
197 MC was below 5%, milled to less than 0.5 mm, and then compressed into pellets using a
198 hydraulic pelletizer before being weighed (~1.5 g of sample was used). Heat content was

199 determined in a sealed steel bomb by burning the samples with an excess of oxygen at a
200 pressure of 430 psi (30 bar).
201 *2.9. Statistical analysis*
202 Statistical analysis was performed using Statistica for 12.0 (StatSoft, Poland). One-way
203 analysis of variance (ANOVA), followed by a post hoc Tukey test at $P<0.05$, was used to
204 compare the data between SE treatment conditions.
205 **3. Results and Discussion**
206 *3.1. Sugarcane straw composition*
207 SCS was initially milled and de-ashed to homogenize the SCS particle size and to remove
208 extraneous sand and clay residues prior to SE pretreatment. A reduction in ash content was
209 observed from ~18% to 5% (w/w). The chemical composition of the SCS used in this study,
210 consisting of a mix of ground tops and leaves, is shown in Table 1. The untreated SCS
211 material was mainly composed of glucan (~39%), xylan (~24%), lignin (20%), and small
212 amounts of arabinan (~4%) and galactan (~1%) (w/w) on a dry matter (DM) basis,
213 corresponding to values previously reported (Brenelli et al., 2020). The initial xylan content,
214 which is of particular interest for the production of XOS, was slightly higher for SCS (~24%)
215 in this study than reported for SCB (~22%) (w/w) (Carvalho et al., 2018; Zhang et al., 2018).
216 The SCS xylan also had ~3% (w/w) of acetyl substituents (Table 1), a critical catalytic source
217 (acetic acid) during autohydrolysis reactions that is required to promote acidic conditions and
218 the depolymerization of the xylan backbone into XOS (Brenelli et al., 2020; Zhang et al.,
219 2018). Hence, SCS could represent another suitable and alternative xylan-rich biomass
220 candidate to produce value-added XOS products for sugarcane biorefineries compared to
221 SCB. The initial SCS glucan (~39%) and lignin (~20%) content was lower (Table 1) when
222 compared to that of SCB glucan (~42 to 44%), and lignin (~23 to 25%) reported previously
223 (Carvalho et al., 2018; Silveira et al., 2018; Zhang et al., 2018). It should be noted that a

224 lower SCS lignin content in this study may be beneficial as it could increase the release of
225 xylan-based XOS from the raw SCS material, thus exposing the accessibility of glucan to
226 cellulolytic enzymes for improved fermentable glucose yields (Bhatia et al., 2020a).
227 Nonetheless, there is a marked natural variation in the biochemical composition of sugarcane
228 residues (straw and bagasse) due to plant variety and developmental period, including
229 collection site and weather conditions, which must be understood for the biomass to be
230 effectively utilized and processed to produce bioproducts and biomaterials for biorefineries
231 (Aguiar et al., 2021).
232 *3.2. Steam explosion pretreatment to produce XOS*
233 The SCS was submitted to pilot-scale SE pretreatment, and the conditions (SF 3.05 to 4.41)
234 tested were based on previous work on pilot-scale SE pretreatment for XOS production from
235 other biomass feedstocks (Bhatia et al., 2020b; Silveira et al., 2018).
236 The SE conditions 200 °C; 15 bar; 10 min (SF =3.94) resulted in XOS yields of up to ~31%
237 (w/w) of initial DM xylan, corresponding to 8% (w/w) of initial DM biomass, and a low yield
238 of xylose ~7% (w/w) (Table 2a). The percentage recovery of XOS (~31% w/w) was
239 relatively low compared to SE pretreatment of alkali-impregnated sugarcane harvesting
240 residues (sugarcane trash, SCT) (~51% w/w) under similar SE conditions (204 °C, 10 min)
241 (Mihiretu et al., 2019). This higher XOS yield with little or no formation of monomeric
242 xylose from SCT can be attributed to the alkali agents/conditions that preserved the xylan
243 extracts in their oligomeric and polymeric form and that led to significant removal of lignin
244 (up to 70%) due to cleavage of the ester bonds linking lignin with xylan (Mihiretu et al.,
245 2019). Nonetheless, similar to this study, comparable XOS yields (~33%) were attained but
246 with the low formation of xylose (1%) and co-solubilization of lignin (27%) (w/w) for alkali
247 deacetylated SCS subjected to hydrothermal pretreatment (Brenelli et al., 2020). From such
248 alkaline pretreatment studies, it was also inferred that the acetyl side groups cleaved from

249 xylan and released as acetic acid could neutralize and counter-balance the alkalinity of
250 extraction conditions and thus affect the degree of xylan and lignin solubilization. In this
251 context, the lower XOS (~31% w/w) yields (Table 2a) may also have been achieved during
252 SE by means of buffering of the released acetic acid through the high ash content in SCS (~
253 5% w/w) compared to deacetylated SCS (~ 3% w/w) (Brenelli et al., 2020). It should also be
254 noted that a different SE condition (210 °C; 20 bar; 5 min) with a similar SF (3.94) to the
255 optimal condition (200 °C; 15 bar; 10 min) showed a lower XOS yield (~24%) (Table 2a),
256 suggesting that temperature and residence time and not SF *per se*, are the important
257 parameters influencing the final properties of the biomass substrate (Yu et al., 2022).
258 Although SE pretreatment of untreated SCS resulted in relatively high xylose yields (~7%
259 w/w) (Table 2a) compared to deacetylated SCS (1% w/w), nanofiltration membranes or yeast
260 fermentation can be applied to effectively remove and minimize the undesired monomeric
261 xylose sugars to meet the purity requirements of commercial XOS (75 to 95%) (Huang et al.,
262 2019; Wijaya et al., 2020). Regarding the amount and DP of XOS, SE-pretreatment (200 °C;
263 15 bar; 10 min) produced ~337.0 g of XOS per kg of initial xylan, with more than 50%
264 comprised of X2 and X3 (~29 and ~25% respectively), ~18% as X4, ~15% as X5, ~9% as
265 X6, and only ~4% as XOS with DP >6 (Table 2b). The effect of the pre-soaking step for SCS
266 under optimal SE conditions for the highest XOS production was also evaluated with similar
267 results to (Bhatia et al., 2020b) (Table 2b) showing that XOS recovery in the hydrolysate did
268 not significantly change with pre-soaking conditions. Moreover, the XOS produced did not
269 undergo significant changes in the distribution of DP 2 to 6 XOS (Table 2b). Certainly,
270 acetyl-assisted autohydrolysis of SCS has the advantage of releasing predominantly XOS
271 with DP 2 to 6 under acidic conditions, whereas alkaline extraction conditions for SCS can
272 lead to xylan solubilization into long-chain XOS with DP ≥5 (~85%) (Brenelli et al., 2020).
273 The fact that SCS hemicellulose fragments released during SE pretreatment are partially

acetylated is a positive aspect because acetylation contributes to the high solubility of the extracted XOS in water (Arai et al., 2019). It is well known that XOS with DP from 2 to 10 have prebiotic properties (de Freitas et al., 2021; Ho et al., 2018; Huang et al., 2019). Particularly XOS with a low DP, i.e. xylobiose and xylotriose, present strong prebiotic activity among the xylose oligomers, hence being more suitable in the field of functional foods and pharmaceutical applications (Moura et al., 2007). On the other hand, XOS with DP>4 can enhance physicochemical properties such as elasticity, firmness, and moisture content when incorporated into food products (Ferrão et al., 2018). SE pretreatment of SCS represents an efficient procedure for producing XOS with mainly DP 2 to 6 range and consequently may involve fewer processing steps for end-use applications such as adding a hydrolysis treatment with *endo*-xylanases. Nonetheless, further studies will be necessary to evaluate the prebiotic activities of the low-DP (2–6) SCS XOS, and removal of degradation products (e.g., furfural, HMF, phenolics) might be required prior to testing. To isolate XOS from the crude SE liquor, a two-step membrane filtration followed by anion-exchange could be used. These membrane filtration steps assist in removing carbohydrate-based degradation compounds such as HMF and furfural as well as reduce potential fouling effects on the ion exchange resins, enabling a highly refined XOS product (with DP 3-10) eligible as a prebiotic food or feed ingredient (Míguez et al., 2021).

3.3. Chemical composition of pretreatment solids, oligomers, and liquid fraction

The effect of SE pretreatment at different severities on the composition of SCS-pretreated solids and the mass balance of biomass components were evaluated (Table 1). Glucan content ranged from ~38 to 52% whereas xylan varied considerably from ~2 to 24% (w/w). Lignin ranged from ~20 to 28% and acetyl-residues from ~2 to 0.2% (w/w). Arabinan was also detected in the pretreated SCS (Table 1). As expected, SE pretreatment selectively promoted xylan solubilization with acetyl groups and generated solids enriched with glucan and lignin

299 compared to the untreated SCS. Thus, glucan recovered in the SE-pretreated solids was on
300 average ~90%, although the SF = 4.12 appeared to expose more fractions of the glucan
301 thereby influencing the dissolution properties of glucan and leading to reduced glucan
302 recovery down to 80%.
303 An increase in glucan and lignin content and reduction in xylan has been observed in other
304 studies related to SE-pretreatment of SCS for cellulosic ethanol production (Oliveira et al.,
305 2013; Zhang et al., 2018). In general, the degree of solubilization of SCS increased
306 moderately at all temperatures as the reaction time increased, but the main biomass
307 components exhibited different extents of solubilization (Table 1). Xylan solubilization and
308 removal increased as the SE pretreatment severity increased and reached 94% at SF = 4.41.
309 Because acetyl groups are linked to the xylan backbone, deacetylation followed the same
310 pattern, although a significant degree of deacetylation (> 60%) was observed at all
311 temperatures after 5 min. Lignin solubilization and, therefore, delignification was minimal
312 under all the tested SE conditions. The maximum delignification (~6%) was achieved at the
313 highest severity factor (SF = 4.41). It is widely known that the acidic conditions of SE-
314 pretreatment typically induce lignin depolymerization followed by condensation to minimize
315 its surface area and deposition onto the fibers combined with ash, extractives, and other
316 components (Heikkinen et al., 2014). This may explain why the lignin content in pretreated
317 solids mostly increased as the SE pretreatment temperature increased. The oligosaccharides
318 analysed in the liquid fraction were mainly composed of xylose (8.1%), arabinose (0.5%),
319 galactose (0.3%), acetyl (0.7%) and glucose (1.4%) (w/w). The composition of undesired
320 products and degradation products in the XOS-rich hydrolysates was also assessed to inform
321 the design of subsequent processing methods for XOS recovery and purification (see
322 supplementary material). As expected, all the aforementioned products increased with
323 increased severity factors. Under the SE conditions which yielded maximum XOS

production, degradation compounds from hexose and pentose sugars, such as HMF and furfural were produced at concentrations of ~0.04 and ~0.14 g/L, equivalent to ~0.43 and ~1.34 g/kg of DM pretreated solids, respectively (see supplementary material). Acetic acid from xylan deacetylation was present at ~1.0 g/L (~9.5 g/kg), while other degradation products and by-products, formic acid (from HMF and furfural degradation) and lactic acid, were found at ~0.45 g/L (~4.4 g/kg), and lactic acid~1.0 g/L (~9.6 g/kg), respectively. The low concentration of degradation and by-products (up to 25 g/kg) compared to XOS concentration, ~8.0 g/L (~80 g/kg) demonstrates that SE is a promising one-step pretreatment strategy to produce XOS from SCS.

3.4. Enzymatic hydrolysis of pretreated solid residues to produce monosaccharides

The enzymatic hydrolysis of the SE-pretreated solids rich in glucan that could be processed into fermentable sugars to produce liquid biofuels was also investigated. The digestibility of SE-pretreated SCS obtained at different severity levels, in terms of glucose and xylose released after hydrolysis, was assessed over a 72 h period using various enzyme loadings (5 to 20 mg protein/g glucan) (Figure 1). Generally, glucan hydrolysis after 72 h increased as the severity level increased for all enzyme loadings tested (Figure 1a) (see supplementary material). This may suggest that the high SF effectively increased the cellulose surface area by removal of hemicellulose, thereby enhancing the accessibility of glucan in the pretreated SCS to hydrolytic enzymes (Pihlajaniemi et al., 2016). Indeed, a linear correlation was observed between hemicellulose removal, SE pretreatment severity, and enzymatic glucan to glucose conversion (see supplementary material). Similar findings were observed previously using SE-pretreatment under comparable conditions on different grasses (Bhatia et al., 2020b; Zhang et al., 2018). The highest glucan conversion (~84%) was obtained by enzymatic hydrolysis using the highest enzyme loading (20 mg protein/g glucan) on pretreated solid obtained at the highest severity factor tested (SF = 4.41). In comparison, the glucan

conversion yield of pretreated solids generated under conditions for optimal XOS production (SF = 3.94) at the same enzyme loading (20 mg protein/g glucan) was ~78% (Figure 1b). This highlights the importance of finding compromise conditions that allow optimal production of both products, although considering the market value of XOS, SE conditions to maximize this fraction would continue to take precedence over maximizing sugars for bioethanol production (Patel and Shah, 2021). The amount of xylose released after enzymatic hydrolysis at 5 and 10 mg protein/ g glucan was negligible and a higher protein loading was needed (20 mg protein/ g glucan) (Figure 1b) compared to glucose released (Figure 1a) as pretreatment severity increased and xylan content sharply decreased (Table 1). This data suggests that the xylan remaining in the pretreated solids at higher SE pretreatment severities (SF > 3.94) was less susceptible to the xylanases present in the Cellic® CTec2 enzyme cocktail, possibly due to xylanase binding with the residual lignin in the pretreated solids during enzymatic hydrolysis (Jung et al., 2020). Since ~47% of the xylan (Table 1) was retained at the optimal SE pretreatment with the highest XOS yields (Table 2), it can serve as a potential source of fermentable sugars for pentose-utilizing yeast strains (Du et al., 2019). It is noteworthy that the SE pretreatment resulted in negligible removal of lignin (maximum ~6%) under the SF tested (Table 1), even though all pretreated solids had an increase in enzymatic glucan digestibility as the SF increased (Figure 1a). SCS biomass was likely recalcitrant to SE pretreatment due to the high content of guaiacyl lignin sub-unit that is highly prone to condensation under acidic conditions (Yu et al., 2022). Hence, this could partially explain why lignin removal was low. Moreover, these observations reinforce the notion that extensive delignification during pretreatment is not necessarily an essential criterion to improve the digestibility of biomass into fermentable sugars. Interestingly, previous work showed that alkaline delignification of steam-exploded SCS at high severity conditions (200 °C; 15 min) had a detrimental effect on enzymatic conversion of glucan as it

may have led to the collapse of the network structure, limiting the surface availability to enzymes and hydrolysis (Oliveira et al., 2013). Besides, it has already been shown that auto-hydrolysis effectively and indirectly increases the surface area of cellulose via the dissolution of hemicellulose, and the presence of lignin associated with small pores is not deleterious for enzymatic hydrolysis of the ensuing pretreated biomass (Espírito Santo et al., 2019). Lastly, hydrolysis optimization through reducing reaction time and increasing the total solids loading as well as the fermentation of both hexose and pentose sugars in the enzymatic hydrolysates would need to be undertaken to ensure maximal process economics.

3.5. Correlations between bulk composition and biomass surface chemical profile during xylo-oligosaccharide and monosaccharide production

ATR-FTIR spectroscopy has previously been applied to evaluate changes in the surface chemical profile of hydrothermally pretreated grasses. Compared to bulk composition analysis, it has been instrumental in correlating enzymatic digestibility with biomass origin and pretreatment severity (Djajadi et al., 2017). In this work, both bulk composition and the surface chemical profile of SE-pretreated SCS at different severity levels obtained using FTIR were correlated with the extent of glucan conversion and XOS production yields. Another interesting point was finding the correlation between XOS production and glucan conversion yields with regard to the changes in the structural components.

The bulk lignin content did not have a strong correlation ($r = 0.58$) with the extent of glucan conversion (see supplementary material). This was expected since the delignification throughout the severity levels remained low, only accounting for at most ~6% at the highest SE pretreatment severity (Table 1). On the other hand, the apparent surface abundance of lignin (ASA-Lig) relative to holocellulose (ASA-Lig/Cell) had a strong positive correlation ($r = 0.80$) with enzymatic digestibility (see supplementary material). The ASA-Lig/Cell increased with SE pretreatment severity, especially from SF > 4.0 (Figure 2a). This can be

attributed to both exposure of lignin surface after preferential removal of hemicellulose (Table 1) and lignin redistribution after steam pretreatment. Since delignification, as seen in bulk lignin content, did not correlate with improvement in glucan conversion, the increased accessibility of the substrate to hydrolytic enzymes can be accounted more on the lignin surface abundance. However, considering the whole process and other components, increased cellulose surface area mostly by hemicellulose removal is likely the most important factor in steam-based pretreatment (Djajadi et al., 2017).

Concerning the bulk composition of hemicellulose, both arabinoxylan solubilization and reduction in hemicellulose content had notable correlations ($r = \pm 0.80$) with a glucan conversion yield of pretreated solids at different severity levels (see supplementary material). This is expected and has been shown previously for SCS biomass at different severity levels (Batista et al., 2019; Oliveira et al., 2013). In contrast, the apparent surface abundance of hemicellulose (ASA-Hem) relative to holocellulose (ASA-Hem/Cell) had a less strong correlation ($r = 0.66$) with glucan conversion (see supplementary material). Up until SF = 3.94, both bulk and surface composition profiles showed a similar trend. A decrease in ASA-Hem/Cell with increasing severity levels (Figure 2a) was in line with increasing hemicellulose removal (Table 1) and XOS production (Table 2). However, from SF > 4.0, where hemicellulose removal increased to more than 75% (Table1), the hemicellulose relative surface abundance slightly increased with severity (Figure 2a). This change of trend in ASA-Hem/Cell coincided with a reduction of XOS yield (Table 2a), while glucan conversion (Figure 1a) and hemicellulose removal (Table 1) continued to increase. Condensation of lignin with sugar degradation products such as furfural and HMF, i.e., pseudo-lignin from SF > 4.0, may be one of the reasons behind this observed increase in ASA-Hem/Cell. In any case, assessing biomass surface chemical profile using ATR-FTIR can be limited by the signal-to-noise ratio in each peak. Furthermore, using different biomass

sources and using single biomass pretreated at a wide severity range can have different sensitivity ranges (Djajadi et al., 2017). Therefore, it needs to be recognized that the peak area ratio using ATR-FTIR is either qualitative or semi-quantitative at best. Interestingly, thermogravimetric analysis (TG) and its derivative profiles (DTG) obtained from lignin isolated from raw SCS showed that the interval from 200 to 250 °C corresponded to 7% of the total mass loss (%) while the maximum degradation rate (Tmax) occurred at 420 °C (Figure 2b). Thus, the mobilization of lignin, which can be inferred from the FTIR data to have occurred at temperatures higher than 200 °C (Figure 2b), may also improve the hydrolysis yield.

When taken together with the chemical composition data obtained in this work and also from previous studies which used SE-pretreated materials with similar or increased lignin contents, it is probable that lignin from SCS is highly resistant to solubilization but is not the primary inhibitor of cellulose hydrolysis (Oliveira et al., 2013). Besides, as previously mentioned, delignification did not correlate with glucan conversion for SE-pretreated SCS (see supplementary material). Instead, the hemicellulose removal was more likely to account for the increased glucan conversion at a higher severity level, mainly because delignification reached a maximum of ~6% (Table 1). Moreover, other factors such as cellulose crystallinity, inhibitory products, fiber size (which was not accounted for in this work), the ratio between different cell types, and non-productive adsorption of cellulases to lignin can also influence the digestibility of steam-exploded SCS as well as other types of lignocellulose (Barbosa et al., 2020; Yu et al., 2022).

3.6. Overall mass balance of the process and energy content of biomass

The overall mass balance of SCS subjected to SE pretreatment under optimal conditions for XOS production and enzymatic hydrolysis is summarized in Figure 3. Based on 1 kg of DM SCS feedstock, ~83% was obtained as a solid fraction comprised of ~339 g glucan, ~111 g

449 xylan, ~5 g acetyl, and ~197 g lignin and the remaining ~17% was dissolved into the
450 hydrolysate as soluble XOS (~72 g) and small amounts of xylose (~14 g), arabinose (~1.7 g),
451 and glucose (~1.9 g). As previously stated, XOS could be used either as ingredients in
452 functional foods after purification or for fermentation to bioproducts using microorganisms
453 capable of metabolizing oligomers (Amorim et al., 2019). The former is of particular interest
454 as short-chained XOS, i.e., X2 and X3, are known for their potential prebiotic activity and
455 higher sweetness than sucrose (Moura et al., 2007; Park et al., 2017). After enzymatic
456 hydrolysis, the glucan-rich solids using the highest enzyme loading (20 mg protein/g glucan)
457 and low biomass loading (1% w/v) for 72 h produced ~263 g of glucose and ~56 g of xylose.
458 Both sugars can be further fermented to obtain bio-based fuels and chemicals (Santos et al.,
459 2019). Processive *endo*-glucanases could also be used to produce cello-oligomers, glucose
460 polymers with potential applications in the food and bioenergy industry, but this would either
461 require auxiliary enzymes or cellulose decrystallization (Barbosa et al., 2020). Hydrolysis
462 optimization through reducing reaction time and increasing the total solids loading is crucial
463 to improving the overall process economics.
464 Energy content is an important property for determining the attractiveness of a potential
465 biofuel. Biomass with a higher energy level and density is more energy efficient for
466 conversion into a biofuel and mitigates against transportation costs and expenses associated
467 with storage, handling, and distribution (Albashabsheh and Heier Stamm, 2021). Lignin has a
468 higher energy content (~27 MJ/kg) than glucan and xylan (~18 MJ/kg), making the lignin-
469 rich residue after hydrolysis of pretreated material a good candidate for combustion and the
470 provision of heat for the aforementioned biorefinery process. The lignin content in the SE-
471 pretreated solids increased from ~20 to ~24% (w/w) after SE-pretreatment under optimal
472 conditions for XOS production (Table 1) and reached ~67% after saccharification (Table 3)
473 due to the efficient enzymatic conversion of polysaccharides into monosaccharides (Figure

1). The energy levels of untreated SCS (~18 MJ/kg) were higher than SE-pretreated (~15 MJ/kg) and lower than saccharified SCS (~21 MJ/kg) (Table 3). Lignin enrichment in pretreated and saccharified SCS was expected to benefit energy levels compared to the untreated sample (Li et al., 2013). However, the ash content was found to be 2-fold higher in steam-exploded SCS compared to the untreated material (~10% and ~5%, respectively), and according to another report under similar SE conditions (Oliveira et al., 2013). The higher ash content (~10%) may have produced an inert effect on the combustion by causing a reduction in the share of combustible carbon matter and the calorific value of the biomass. Hence, the relation between the amount of ash and the detrimental effect on the calorific value of the biomass for combined heat and power production requires further investigation. Nonetheless, the higher energy content of the saccharified SCS by about 16% and potentially improved solid pellet quality could reduce the total energy input and costs associated with the XOS production process. The residual lignin from the enzymatically pretreated solid residue could also be hydrolyzed through alkaline or acid treatment and used to produce lignin-based materials and value-added molecules, providing several options for maximizing the value streams in biorefineries (Wang et al., 2019). Lastly, simulation studies on the techno-economic and environmental assessment of SCS-based biorefineries to produce XOS, fermentable sugars, and bioenergy under different handling processes such as milling and grinding, SE pretreatments, enzymatic saccharification, and fermentation conditions or pelletization of the saccharified solids are crucial for the successful commercialization of the integrated biorefinery.

4. Conclusions

Under the SE conditions tested, XOS yields up to 30% w/w of initial xylan were obtained, and ~50% of the recovered XOS were low-DP XOS (X2 and X3), known for their higher prebiotic potential. Up to 78% of the glucan in the SE-pretreated SCS was enzymatically

499 released as fermentable glucose and the remaining lignin-rich (67% w/w) saccharified solids
500 exhibited a 16% higher energy content than untreated SCS. A potential value chain is
501 presented for sugarcane biorefineries using SCS via value-added XOS production and co-
502 production of renewable liquid and solid biofuels.
503 E-supplementary data for this work can be found in the e-version of this paper online

504 **CRediT authorship contribution statement**

505 **Lívia B. Brenelli:** Validation, Investigation, Writing - original draft, Writing - review &
506 editing, Visualization, Project administration. **Rakesh Bhatia:** Validation, Investigation,
507 Writing - original draft, Writing - review & editing, Visualization, Project administration.
508 **Demi T. Djajadi:** Validation, Writing - review & editing, Visualization. **Lisbeth G.**
509 **Thygesen:** Validation, Writing - review & editing. **Sarita C. Rabelo:** Validation, Writing -
510 review & editing. **David J. Leak:** Conceptualization, Validation, Writing - review & editing,
511 Funding acquisition. **Telma T. Franco:** Conceptualization, Validation, Writing - review &
512 editing, Funding acquisition. **Joe A. Gallagher:** Validation, Writing - review & editing,
513 Supervision, Funding acquisition.

514 **Declaration of competing interest**

515 The authors declare that they have no known competing financial interests or personal
516 relationships that could have influenced the work reported in this paper.

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674 **Figure and Table captions**

675 **Figure 1.** Glucan (A) and xylan (B) conversion (%) to glucose and xylose, respectively, after
676 72 h of enzymatic hydrolysis of SE-pretreated solids obtained at different severity factors and
677 enzyme loadings.

678 **Figure 2.** ATR-FTIR peak area ratio of wavenumbers representing hemicellulose (1732
679 cm^{-1}) and lignin (1512 cm^{-1}) each relative to that of holocellulose (1160 cm^{-1}) for SE-
680 pretreated SCS under different severity factors (A) and Thermogravimetric curve recorded
681 for alkaline lignin extracted from untreated SCS. Dotted lines represent the derivative curve
682 (B).

683 **Figure 3.** Overall mass balance of SCS under SE pretreatment.

684 **Table 1.** Effect of SE pretreatment on the composition of SCS-pretreated solids and mass
685 balance of biomass components.

686 **Table 2.** XOS yield (w/w % of initial SCS), xylose/XOS recovered (w/w % of initial DM
687 xylan) at different severity factors (log R_0) (a), and the soaking effect prior SE-pretreatment
688 conditions 200 °C; 15 bar; 10 min on the total XOS g/kg (initial xylan) and XOS profile (b).

689 **Table 3.** Lignin content and energy density in untreated, pretreated and saccharified SCS.

690 **Table 1.**

Condition	Biomass composition (w/w % of DM solids)						Pulp recovered[c] (w/w % DM solids)	Glucan recovery[d] (%)	Xylan removal[e] (%)	Lignin removal[f] (%)	Deacetylation[g] (%)
	Glucan	Xylan	Arabinan	Acetyl	Lignin[a]	Others[b]					
Untreated	38.9 ± 0.1^{a}	23.9 ± 0.0^{a}	3.9 ± 0.0^{e}	3.1 ± 0.1^{e}	20.1 ± 0.1^{a}	10.1 ± 0.2^{b}	-	-	-	-	-
180 °C (9 bar) 5 min	38.1 ± 0.8^{a}	24.1 ± 0.2^{a}	2.4 ± 0.1^{d}	2.4 ± 0.1^{d}	20.2 ± 0.8^{a}	10.2 ± 0.1^{ab}	98.9	96.7 ± 1.3^{b}	0.1 ± 0.1^{d}	0.5 ± 0.0^{a}	24.3 ± 0.0^{c}
180 °C (9 bar) 10 min	39.0 ± 0.2^{ab}	24.4 ± 0.3^{a}	2.0 ± 0.1^{c}	2.3 ± 0.2^{d}	22.4 ± 1.4^{ab}	8.2 ± 0.1^{d}	89.0	89.2 ± 2.1^{a}	9.2 ± 0.1^{e}	0.9 ± 0.1^{ab}	34.3 ± 0.1^{d}
180 °C (9 bar) 15 min	42.0 ± 0.4^{abc}	19.2 ± 0.1^{g}	1.5 ± 0.2^{b}	1.5 ± 0.1^{c}	24.3 ± 1.9^{abc}	4.6 ± 0.3^{c}	81.7	88.1 ± 3.1^{ac}	29.7 ± 2.4^{a}	1.3 ± 0.3^{abc}	61.3 ± 0.2^{g}
200 °C (15 bar) 5 min	38.1 ± 0.7^{a}	17.9 ± 0.2^{f}	nd	1.8 ± 0.1^{c}	21.6 ± 1.7^{ab}	12.5 ± 0.4^{a}	93.5	91.6 ± 1.8^{ab}	29.9 ± 1.2^{a}	0.6 ±0.1^{a}	44.8 ± 0.1^{e}
200 °C (15 bar) 10 min	41.2 ± 1.2^{ab}	13.5 ± 0.1^{e}	nd	0.6 ± 0.2^{b}	23.9 ± 2.3^{abc}	11.2 ± 0.1^{ab}	82.3	87.2 ± 2.3^{ac}	53.3 ± 2.4^{f}	2.0 ± 0.1^{abc}	83.2 ± 2.1^{a}
200 °C (15 bar) 15 min	43.0 ± 2.3^{bc}	7.5 ± 0.3^{d}	nd	0.6 ± 0.1^{b}	22.2 ± 1.4^{ab}	12.5 ± 0.1^{a}	74.0	81.7 ± 1.0^{c}	76.9 ± 3.3^{g}	2.4 ± 0.4^{bcd}	85.2 ± 1.3^{a}
210 °C (20 bar) 5 min	46.2 ± 2.1^{c}	4.0 ± 0.5^{c}	0.4 ± 0.1^{a}	0.2 ± 0.0^{a}	25.3 ± 0.4^{bc}	12.3 ± 0.4^{a}	77.2	91.7 ± 2.3^{ab}	87.2 ± 3.2^{b}	2.9 ± 0.5^{cd}	52.0 ± 1.2^{f}
210 °C (20 bar) 10 min	42.3 ± 1.5^{abc}	2.1 ± 0.1^{b}	0.2 ± 0.2^{a}	0.3 ± 0.0^{a}	23.4 ± 0.2^{ab}	19.8 ± 0.5^{e}	82.4	92.7 ± 3.2^{ab}	89.6 ± 2.8^{bc}	4.1 ± 1.1^{d}	92.8 ± 2.2^{b}

210 °C (20 bar) 15 min	52.3 ± 2.6[d]	2.2 ± 0.3[b]	nd	0.2 ± 0.0[a]	27.8 ± 2.4[c]	5.8 ± 1.2[c]	68.1	91.5 ± 3.5[ab]	93.8 ± 2.1[c]	5.9 ± 1.3[e]	96.3 ± 2.4[b]

[a]Lignin is total acid-soluble and acid-insoluble lignin (Klason).
[b]Includes galactan, extractives, ash and other solids.
[c]Pulp recovered (%) = gram of DM residual straw recovered after pretreatment/ 100 g DM untreated straw.
[d]Glucan recovery (%) = (Glucan content in pretreated straw × straw recovered) / Total glucan in untreated straw.
[e]Xylan removal (%) = 100 – Xylan recovery (%) in pretreated straw.
[f]Lignin removal (%) = 100 – Lignin recovery (%) in pretreated straw.
[g]Deacetylation (%) = 100 – Acetyl groups (%) in pretreated straw.
DM, dry matter; nd, not detected.

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700 <Footnote: Data are means ± standard error (n≥2) from technical and experimental replicates. Different letters in the same column indicate

701 significant statistical differences based on ANOVA ($p \leq 0.05$).>

Table 2.

a

Condition	Severity factor	XOS yield (w/w %, initial xylan)	Xylose yield (w/w %, initial xylan)	XOS yield (w/w %, initial biomass)
180 °C, 9 bar, 5 min	3.05	4.45 ± 0.23[a]	0.20 ± 0.02[a]	1.07 ± 0.05[a]
180 °C, 9 bar, 10 min	3.36	11.95 ± 1.10[c]	0.47 ± 0.06[a]	2.86 ± 0.26[d]
180 °C, 9 bar, 15 min	3.53	17.98 ± 0.59[bc]	1.51 ± 0.29[a]	4.48 ± 0.21[b]
200 °C, 15 bar, 5 min	3.64	26.37 ± 1.60[de]	1.46 ± 0.10[a]	6.35 ± 0.39[c]
200 °C, 15 bar, 10 min	3.94	31.25 ± 2.45[e]	6.85 ± 0.99[b]	7.98 ± 0.25[e]
200 °C, 15 bar, 15 min	4.12	19.00 ± 1.92[b]	12.09 ± 0.47[c]	4.95 ± 0.45[bc]
210 °C, 20 bar, 5 min	3.94	23.92 ± 3.40[bd]	7.34 ± 1.01[b]	5.91 ± 0.81[bc]
210 °C, 20 bar, 10 min	4.24	4.69 ± 0.15[a]	11.35 ± 0.40[c]	1.76 ± 0.14 [ad]
210 °C, 20 bar, 15 min	4.41	1.25 ± 0.14[a]	3.77 ± 0.23[d]	0.75 ± 0.04[a]

b

Condition	Total XOS g/kg (Initial xylan)	Relative Percentage (%)					
		X2	X3	X4	X5	X6	XOS dp >6
200 °C, 15 bar, 10 min (No pre-soaking)	351.93 ± 32.38[a]	29.23 ± 0.69	25.28 ± 0.32	17.99 ± 0.12	15.08 ± 0.13	8.67 ± 0.09	3.76 ± 1.17
200 °C, 15 bar, 10 min (pre-soaking, 2 h, 25 °C)	337.07 ± 17.37[a]	26.95 ± 0.88	24.36 ± 0.92	17.69 ± 0.61	15.33 ± 0.17	9.16 ± 0.05	6.51 ± 2.29
200 °C, 15 bar, 10 min (pre-soaking, 2 h, 70 °C)	337.98 ± 5.58[a]	30.68 ± 1.18	25.79 ± 0.71	18.46 ± 0.40	14.61 ± 0.28	8.23 ± 0.17	2.23 ± 1.84

X2, xylobiose; X3, xylotriose, X4, xylotetraose; X5, xylopentaose; X6, xylohexaose; dp, degree of polymerization.

<Footnote: Data are means ± standard error (n≥2) from technical and experimental replicates. Different letters in the same column indicate significant statistical differences based on ANOVA ($p \leq 0.05$).>

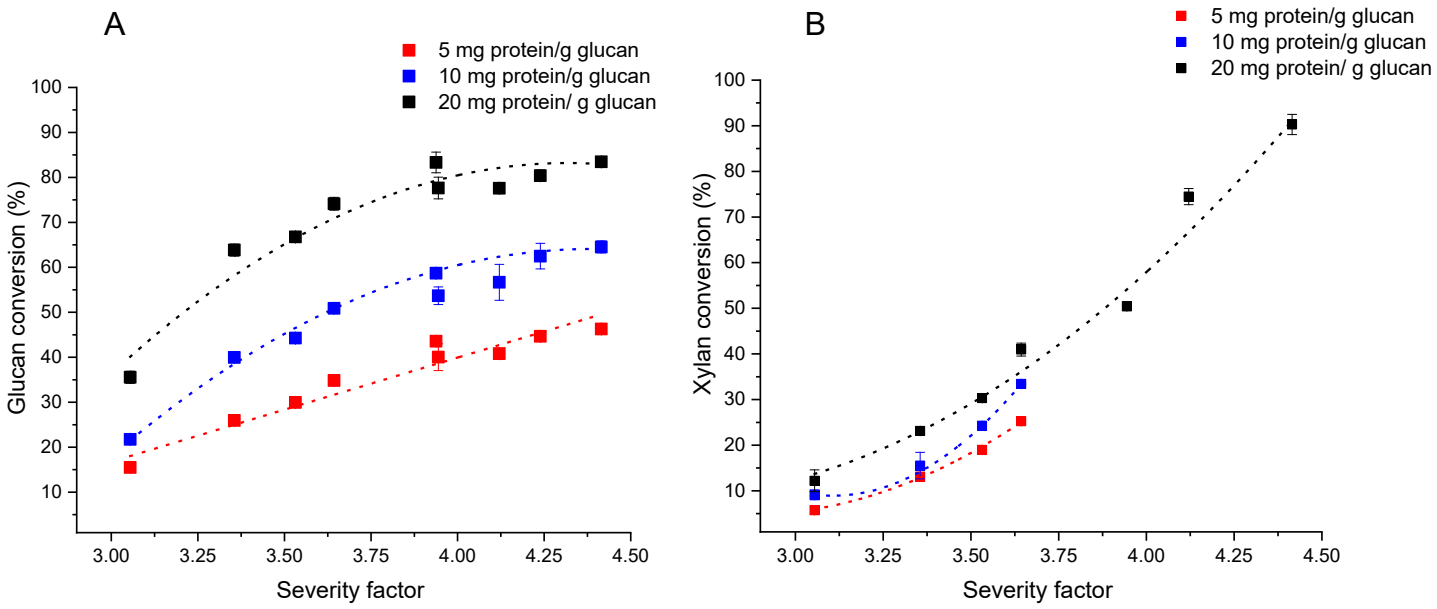
Table 3.

SCS biomass	Lignin* (% w/w)	Energy Content (MJ/kg)
Untreated	20.1± 0.1[a]	17.71 ± 0.11[a]
Pretreated (200 °C, 15 bar, 10 min)	23.9 ± 2.3[a]	14.69 ± 0.13[b]
Saccharified (20 mg protein/g glucan, 72h)	67.1 ± 1.8[b]	20.59 ± 0.20[c]

*Extractives free basis.

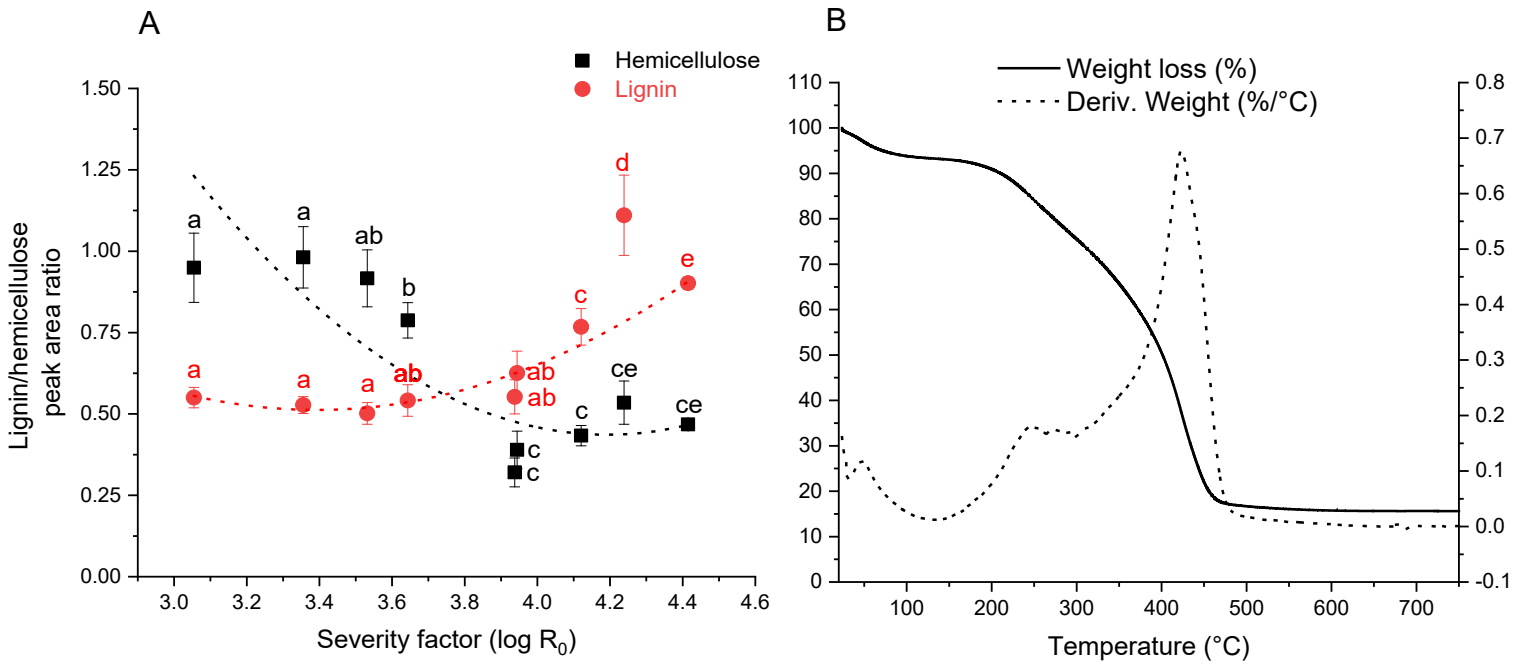
<Footnote: Data are means ± standard error (n≥2). Different letters in the same column indicate significant statistical differences based on ANOVA ($p \leq 0.05$).>

Figure 1.



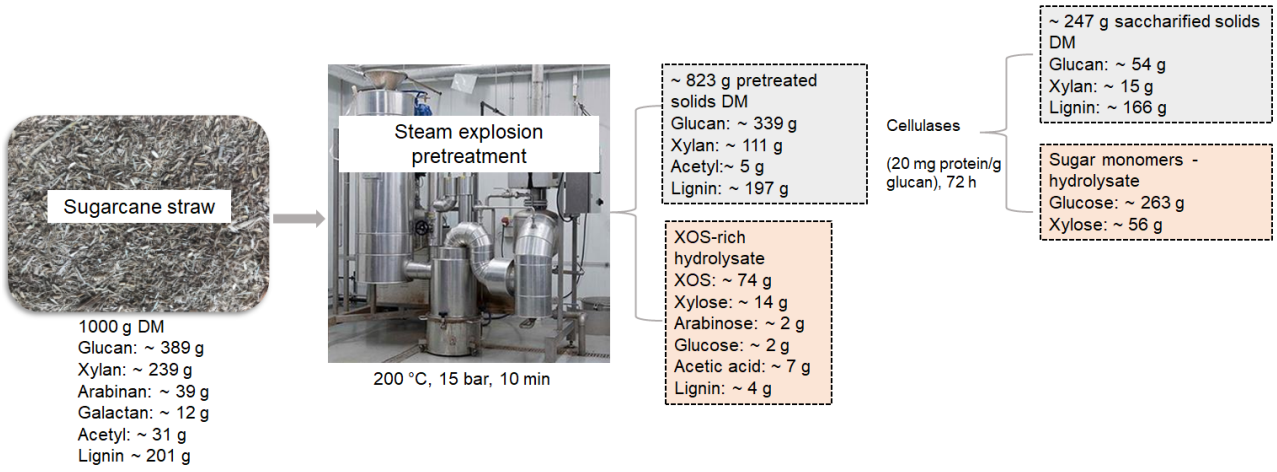
<Footnote: Data points represent the average and standard deviation from three experimental replicates.>

Figure 2.



<Footnote: Data points represent the average and standard deviation from five technical replicates. Different letters indicate significant statistical differences based on ANOVA ($p \leq 0.05$).>

Figure 3.


Sugarcane straw
1000 g DM
Glucan: ~ 389 g
Xylan: ~ 239 g
Arabinan: ~ 39 g
Galactan: ~ 12 g
Acetyl: ~ 31 g
Lignin ~ 201 g
Steam explosion pretreatment
200 °C, 15 bar, 10 min
~ 823 g pretreated solids DM
Glucan: ~ 339 g
Xylan: ~ 111 g
Acetyl:~ 5 g
Lignin: ~ 197 g
XOS-rich hydrolysate
XOS: ~ 74 g
Xylose: ~ 14 g
Arabinose: ~ 2 g
Glucose: ~ 2 g
Acetic acid: ~ 7 g
Lignin: ~ 4 g
Cellulases
(20 mg protein/g glucan), 72 h
~ 247 g saccharified solids DM
Glucan: ~ 54 g
Xylan: ~ 15 g
Lignin: ~ 166 g
Sugar monomers - hydrolysate
Glucose: ~ 263 g
Xylose: ~ 56 g