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

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1 **Title**

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

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24 **Abstract**

25 This study investigated the production of xylo-oligosaccharides (XOS) from sugarcane straw
26 (SCS) using steam explosion (SE) pretreatment at pilot-scale, as well as co-production of
27 other fermentable sugars and lignin-rich residues for bioethanol and bioenergy, respectively.
28 SE conditions 200 °C; 15 bar; 10 min led to 1) soluble XOS yields of up to 35% (w/w) of
29 initial xylan with ~50% of the recovered XOS corresponding to xylobiose and xylotriose,
30 considered the most valuable sugars for prebiotic applications; 2) fermentable glucose yields
31 from the enzymatic hydrolysis of SE-pretreated SCS of up to ~78%; 3) increase in the energy
32 content of saccharified SCS residues (16%) compared to the untreated material. From an
33 integrated biorefinery perspective, it demonstrated the potential use of SCS for the production
34 of value-added XOS ingredients as well as liquid and solid biofuel products.

35 **1. Introduction**

36 Sugarcane straw (SCS), a residual by-product of the sugarcane industry, is gaining attention
37 as an abundant and low-cost lignocellulosic resource to produce biofuels and high-value
38 products for sugarcane biorefineries (Aguar et al., 2021). This is partly because the practice
39 of burning sugarcane plantations to remove the straw before harvesting is being prohibited
40 due to health and environmental concerns. Moreover, the utilization of SCS yields additional
41 lignocellulosic material without expanding feedstock cultivation areas, hence maximizing the
42 productivity and competitiveness of the existing sugarcane sugar and bioethanol production
43 platforms (Cherubin et al., 2021). For example, in Brazil, which is the leading producer of
44 sugarcane in the world (657 million metric tons in the crop year 2020/2021), at least 20
45 million tonnes of SCS biomass could be harvested for biorefinery applications without
46 impacting soil quality (Carvalho et al., 2017; USDA 2021, 2021).

47 The composition of SCS, typically consisting of dry leaves (~60%) from the stalk and green
48 leaves on the top (~40%), is primarily glucan (~30 to 45% w/w), xylan (~25 to 30% w/w) and
49 lignin (~20 to 30% w/w). Its chemical composition can vary widely from site to site

50 depending on the plant development stage, variety, and collection method (Aguiar et al.,
51 2021). From a biorefinery perspective, the physical and chemical complexities of the
52 lignocellulosic components require deconstruction to be converted into biofuels and
53 biochemicals. In this context, physical (milling and grinding), chemical (alkaline, acid,
54 hydrothermal, and ionic liquids), physicochemical (ammonia fiber expansion – AFEX, and
55 steam explosion – SE), or biological (enzymatic and microorganisms) pretreatment methods
56 are an essential part of the processing steps to overcome the recalcitrance of biomass and in
57 turn, can dictate the efficiency of production as well as the economic and environmental
58 impacts of bio-based products.

59 Several pretreatment strategies for the bioconversion of SCS into value-added bio-products
60 have been explored and are summarized elsewhere (Aguiar et al., 2021). **Among these**
61 **pretreatments, SE is generally considered an environmentally friendly, efficient, chemical-**
62 **free, economic, mild, and fast treatment process suitable for industrial applications and**
63 **various lignocellulosic feedstocks** (Auxenfans et al., 2017). During SE, lignocellulose is
64 saturated with high-pressure (7 to 48 bar) steam at high temperatures (160 to 240 °C) for
65 several minutes (5 to 15 min) and then suddenly de-pressurized, causing the lignocellulosic
66 biomass to undergo an explosive decompression (Yu et al., 2022). Under the high
67 temperature combined with pressure, the hydronium ions formed from water dissociation
68 together with the acetic acid released through the hydrolysis of acetyl groups linked to the
69 xylan backbone act as *in situ* catalysts. As a result, disruption of the glucan-xylan-lignin
70 complex occurs, leading to the depolymerization and removal of the hemicellulose xylan and
71 mixed-linkage glucan, with the limited dissolution of glucan and partial degradation of lignin
72 (Auxenfans et al., 2017; Bhatia et al., 2020b). However, there are also certain disadvantages,
73 such as the incomplete deconstruction of the lignin-carbohydrate complex or production of
74 inhibitors and degradation products depending on the SE severity conditions (Biswas et al.,

75 2015). Please refer to Yu et al., 2022 for a comprehensive review on the impacts of steam
76 explosion on the physical and chemical properties of the biomass feedstock and on the
77 different subsequent conversion processes of biomass including densification for solid pellets
78 (heating value) and enzymatic hydrolysis (cellulose accessibility) for fermentation to liquid
79 biofuels.

80 Recently, there has been a growing interest in producing xylo-oligosaccharides (XOS) from
81 xylan-rich plants, including sugarcane (Prenexus Health, USA) and corncob (Shangdong
82 Longlive Biotechnology, China) (Kumar et al., 2021). With a degree of polymerization (DP)
83 from 2 up to 14 units of xylose, XOS can exhibit multiple properties such as pH (2.5 to 8)
84 and temperature (up to 100°C) stability, low-calorie sweetening potency for sugar and fat
85 alternatives, moisture retention capabilities and prebiotic properties beneficial for health
86 (Álvarez et al., 2020; Amorim et al., 2019). The market price of XOS ranges from 25 \$/kg to
87 50 \$/kg depending on purity level (70 to 95%), and the global market is expected to achieve
88 ~\$130 million by 2025 (Santibáñez et al., 2021; Singh et al., 2018). Hence, XOS sugars have
89 gained commercial interest as animal feed, pharmaceutical, food, and beverage ingredients
90 (Pinales-Márquez et al., 2021).

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

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

103 There is a knowledge gap in pilot-scale SE pretreatment conditions for SCS biomass that
104 seeks maximum hemicellulose solubilization into XOS, which may be advantageous to
105 overcome reports of low XOS conversion yields and/or multi-step downstream processes
106 (Milessi et al., 2021). The primary focus of the study is the production of XOS from SCS
107 using a single step SE pretreatment process, thus avoiding the cost and use of additional
108 catalytic chemicals and enzymes. Important SE parameters to achieve high XOS yields with
109 low DP and low amounts of by-products and sugar degradation products were identified, and
110 a process scheme comprising enzymatic hydrolysis of SE pretreated SCS was proposed for
111 the co-production of fermentable sugars and lignin-rich residues for bioethanol and
112 bioenergy, respectively.

113 **2. Materials and methods**

114 *2.1. Feedstock preparation*

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

120 *2.2. Steam explosion pretreatment*

121 SCS (0.25 kg) were suspended in deionized water at 10:1 water/solid ratio (g/g) and soaked
122 for 2 h at 20 ± 5 °C. The excess liquid of the mixture was drained using a muslin cloth.

123 Aliquots of the recovered liquid were analyzed for total sugar content according to
124 NREL/TP-510-42623 procedure (Sluiter et al., 2008). The strained SCS (~80% MC) was

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

$$136 \text{ SF} = \log_{10} [t * \exp [(T-100) 14.75^{-1}]] \quad (1)$$

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

138 *2.3. Chemical characterization of pretreated solids and hydrolysates*

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

145 *2.4. Analysis of xylo-oligosaccharides, monosaccharides, degradation products, and by-* 146 *products*

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

150 flow rate of 0.3 mL/min and 25 μ L injection volume. The elution program was described
151 previously (Bhatia et al., 2020b). Xylobiose (X2), xylotriose (X3), xylotetraose (X4),
152 xylopentaose (X5), and xylohexaose (X6) purchased from Megazyme were used to construct
153 a calibration curve ranging from 1.25 to 20 μ g/mL.
154 Monosaccharides were quantified by HPAEC-PAD (Thermofisher ICS-5000) using a Dionex
155 CarboPac SA10 guard (4 \times 50 mm) and analytical (4 \times 250 mm) columns at 45 $^{\circ}$ C, a flow rate
156 of 1.5 mL/min with 1 mM KOH as eluent and 25 μ L injection volume. Glucose, xylose,
157 arabinose, galactose, mannose, fructose, sucrose, cellobiose, and fucose were run as
158 calibration standards from 1.25 to 20 μ g/mL. By-products and degradation products were
159 analysed by High-performance liquid chromatography (HPLC) equipped with a refractive
160 index detector using an Aminex HPX-87H column (Bio-Rad) at 55 $^{\circ}$ C, a flow rate of 0.6
161 mL/min with 5 mM H₂SO₄ as eluent according to NREL's standard procedure (Sluiter et al.,
162 2008).

163 *2.5. Enzymatic hydrolysis of pretreated solids*

164 Enzymatic hydrolysis was carried out in triplicate at a solid biomass loading of 1% (w/v)
165 with 0.05 M sodium acetate buffer (pH 5) at 50 $^{\circ}$ C using the commercial cocktail Cellic[®]
166 CTec2 (Novozymes A/S, Denmark) in a total volume of 5.0 mL. The total cellulase activity
167 against filter paper (FPU) measured by the standard IUPAC method was previously reported
168 to be 133 FPU/mL (Kontogianni et al., 2019). The hydrolysis experiment was conducted in
169 15 mL falcon tubes in a rotary shaker set at 150 rpm. Dosage response curve experiments
170 were carried out with enzyme loadings of 5, 10, and 20 mg protein/ g glucan, and samples
171 were withdrawn after 4, 24, 48, and 72 h. The enzymatic hydrolysis was ended by boiling
172 samples at 100 $^{\circ}$ C for 10 min. After centrifugation (10 min, 10,000 \times g), the supernatants
173 were analyzed for glucose and xylose yields by HPAEC-PAD.

174 *2.6. Determination of hemicellulose/holocellulose and lignin/holocellulose using attenuated*
175 *total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy*

176 Pretreated SCS was milled using an MF 10 microfine grinder (IKA[®] Werke GmbH & Co.
177 KG, Germany) to obtain particles that passed through a 0.5 mm sieve. The IR spectra of the
178 samples were collected five times using a Nicolet 6700 FT-IR, Pike Technologies GladiATR
179 diamond spectrometer (Thermo Scientific, Waltham, MA, USA) at 25 °C. The spectral range
180 included was 4000–600 cm⁻¹, and spectra were obtained using 64 scans (128 for the
181 background) and a resolution of 4.0 cm⁻¹. After subtraction of a separate linear background
182 for each peak, the peak area ratio 1732/1160 cm⁻¹ (ranges 1675-1750 and 1142-1182 cm⁻¹,
183 respectively) was used for hemicellulose/holocellulose estimation, while the ratio 1512/1160
184 cm⁻¹ (ranges 1483-1531 and 1142-1182 cm⁻¹, respectively) was used for lignin/holocellulose
185 estimation (Lupoi et al., 2014).

186 *2.7. Thermal analysis*

187 Lignin was isolated from raw SCS using sodium hydroxide (10%, 100 °C, 3 h), recovered by
188 adding concentrated sulfuric acid until pH 2 and dried in an oven at 30 °C until the MC was
189 below 5%. The thermal stability was evaluated in a TGA/DSC analyzer (SDT Q600, TA
190 Instruments). 5 mg of lignin was placed in air-tight aluminum support with a heating rate of
191 10 °C min⁻¹, from 25 to 800 °C under a synthetic air atmosphere.

192 *2.8. Energy content measurement*

193 Energy content measurements of SCS before and after pretreatment and lignin-rich residues
194 remaining after enzymatic hydrolysis were performed in a standard bomb calorimeter (Parr[™]
195 6400 Automatic Isoperibol Calorimeter). All samples were dried in an oven at 30 °C until the
196 MC was below 5%, milled to less than 0.5 mm, and then compressed into pellets using a
197 hydraulic pelletizer before being weighed (~1.5 g of sample was used). Heat content was

198 determined in a sealed steel bomb by burning the samples with an excess of oxygen at a
199 pressure of 430 psi (30 bar).

200 *2.9. Statistical analysis*

201 Statistical analysis was performed using Statistica for 12.0 (StatSoft, Poland). One-way
202 analysis of variance (ANOVA), followed by a post hoc Tukey test at $P < 0.05$, was used to
203 compare the data between SE treatment conditions.

204 **3. Results and Discussion**

205 *3.1. Sugarcane straw composition*

206 **SCS was initially milled and de-ashed to homogenize the SCS particle size and to remove**
207 **extraneous sand and clay residues prior to SE pretreatment. A reduction in ash content was**
208 **observed from ~18% to 5% (w/w).** The chemical composition of the SCS used in this study,
209 consisting of a mix of ground tops and leaves, is shown in Table 1. The untreated SCS
210 material was mainly composed of glucan (~39%), xylan (~24%), lignin (20%), and small
211 amounts of arabinan (~4%) and galactan (~1%) (w/w) on a dry matter (DM) basis,
212 corresponding to **values** previously reported (Brenelli et al., 2020). The initial xylan content,
213 which is of particular interest for the production of XOS, was slightly higher for SCS (~24%)
214 in this study than reported for SCB (~22%) (w/w) (Carvalho et al., 2018; Zhang et al., 2018).
215 The SCS xylan also had ~3% (w/w) of acetyl substituents (Table 1), a critical catalytic source
216 (acetic acid) during autohydrolysis reactions that is required to promote acidic conditions and
217 the depolymerization of the xylan backbone into XOS (Brenelli et al., 2020; Zhang et al.,
218 2018). Hence, like SCB, SCS could be an alternative xylan-rich biomass candidate for
219 producing value-added XOS products in sugarcane biorefineries. The initial SCS glucan
220 (~39%) and lignin (~20%) content was lower (Table 1) when compared to that of SCB glucan
221 (~42 to 44%), and lignin (~23 to 25%) reported previously (Carvalho et al., 2018; Silveira et
222 al., 2018; Zhang et al., 2018). It should be noted that a lower SCS lignin content in this study

223 may be beneficial as it could increase the release of xylan-based XOS from the raw SCS
224 material, and thus help expose glucan to cellulolytic enzymes for improved fermentable
225 glucose yields (Bhatia et al., 2020a). Nonetheless, there is a marked natural variation in the
226 biochemical composition of sugarcane residues (straw and bagasse) due to plant variety and
227 developmental period, including collection site and weather conditions, which must be
228 understood for the biomass to be effectively utilized and processed to produce bioproducts
229 and biomaterials for biorefineries (Aguiar et al., 2021).

230 *3.2. Steam explosion pretreatment to produce XOS*

231 The SCS was submitted to pilot-scale SE pretreatment, and the conditions (SF 3.05 to 4.41)
232 tested were based on previous work on pilot-scale SE pretreatment for XOS production from
233 other biomass feedstocks (Bhatia et al., 2020b; Silveira et al., 2018).

234 The SE conditions 200 °C; 15 bar; 10 min (SF =3.94) resulted in XOS yields of up to ~35%
235 (w/w) of initial DM xylan, corresponding to 8% (w/w) of initial DM biomass, and a low yield
236 of xylose ~8% (w/w) (Table 2a). The percentage recovery of XOS (~35% w/w) was
237 relatively low compared to SE pretreatment of alkali-impregnated sugarcane harvesting
238 residues (sugarcane trash, SCT) (~51% w/w) under similar SE conditions (204 °C, 10 min)
239 (Mihiretu et al., 2019). This higher XOS yield with little or no formation of monomeric
240 xylose from SCT can be attributed to the alkali agents/conditions that preserved the xylan
241 extracts in their oligomeric and polymeric form and that led to significant removal of lignin
242 (up to 70%) due to cleavage of the ester bonds linking lignin with xylan (Mihiretu et al.,
243 2019). Nonetheless, similar to this study, comparable XOS yields (~33%) were attained but
244 with the low formation of xylose (1%) and co-solubilization of lignin (27%) (w/w) for alkali
245 deacetylated SCS subjected to hydrothermal pretreatment (Brenelli et al., 2020). From such
246 alkaline pretreatment studies, it was also inferred that the acetyl side groups cleaved from
247 xylan and released as acetic acid could neutralize and counter-balance the alkalinity of

248 extraction conditions and thus affect the degree of xylan and lignin solubilization. In this
249 context, the comparable XOS yields (~35% w/w) (Table 2a) may also have been achieved
250 during SE by means of buffering of the released acetic acid through the high ash content in
251 SCS (~ 5% w/w) compared to deacetylated SCS (~ 3% w/w) (Brenelli et al., 2020). It should
252 also be noted that a different SE condition (210 °C; 20 bar; 5 min) with a similar SF (3.94) to
253 the optimal condition (200 °C; 15 bar; 10 min) showed a lower XOS yield (~24%) (Table
254 2a), **suggesting that temperature and residence time and not SF *per se*, are the important**
255 **parameters influencing the final properties of the biomass substrate** (Yu et al., 2022).
256 Although SE pretreatment of untreated SCS resulted in relatively high xylose yields (~7%
257 w/w) (Table 2a) compared to deacetylated SCS (1% w/w), nanofiltration membranes or yeast
258 fermentation can be applied to effectively remove and minimize the undesired monomeric
259 xylose sugars to meet the purity requirements of commercial XOS (75 to 95%) (Huang et al.,
260 2019; Wijaya et al., 2020). Regarding the amount and DP of XOS, SE-pretreatment (200 °C;
261 15 bar; 10 min) produced up to ~352.0 g of XOS per kg of initial xylan, with more than 50%
262 comprised of X2 and X3 (~29 and ~25% respectively), ~18% as X4, ~15% as X5, ~9% as
263 X6, and only ~4% as XOS with DP >6 (Table 2b). The effect of the pre-soaking step for SCS
264 under optimal SE conditions for the highest XOS production was also evaluated with similar
265 results to (Bhatia et al., 2020b) (Table 2b) showing that XOS recovery in the hydrolysate did
266 not significantly change with pre-soaking conditions. Moreover, the XOS produced did not
267 undergo significant changes in the distribution of DP 2 to 6 XOS (Table 2b). Certainly,
268 acetyl-assisted autohydrolysis of SCS has the advantage of releasing predominantly XOS
269 with DP 2 to 6 under acidic conditions, whereas alkaline extraction conditions for SCS can
270 lead to xylan solubilization into long-chain XOS with DP ≥ 5 (~85%) (Brenelli et al., 2020).
271 The fact that SCS hemicellulose fragments released during SE pretreatment are partially
272 acetylated is a positive aspect because acetylation contributes to the high solubility of the

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

290 3.3. Chemical composition of pretreatment solids, oligomers, and liquid fraction

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

298 average ~90%, although the SF = 4.12 appeared to expose more fractions of the glucan
299 thereby influencing the dissolution properties of glucan and leading to reduced glucan
300 recovery down to 80%.

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

323 furfural were produced at concentrations of ~0.04 and ~0.14 g/L, equivalent to ~0.43 and
324 ~1.34 g/kg of DM pretreated solids, respectively (see supplementary material). Acetic acid
325 from xylan deacetylation was present at ~1.0 g/L (~9.5 g/kg), while other degradation
326 products and by-products, formic acid (from HMF and furfural degradation) and lactic acid,
327 were found at ~0.45 g/L (~4.4 g/kg), and lactic acid~1.0 g/L (~9.6 g/kg), respectively. The
328 low concentration of degradation and by-products (up to 25 g/kg) compared to XOS
329 concentration, ~8.0 g/L (~80 g/kg) demonstrates that SE is a promising one-step pretreatment
330 strategy to produce XOS from SCS.

331 *3.4. Enzymatic hydrolysis of pretreated solid residues to produce monosaccharides*

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

348 (SF = 3.94) at the same enzyme loading (20 mg protein/g glucan) was ~78% (Figure 1b).
349 This highlights the importance of finding compromise conditions that allow optimal
350 production of both products, although considering the market value of XOS, SE conditions to
351 maximize this fraction would continue to take precedence over maximizing sugars for
352 bioethanol production (Patel and Shah, 2021). The amount of xylose released after enzymatic
353 hydrolysis at 5 and 10 mg protein/ g glucan was negligible and a higher protein loading was
354 needed (20 mg protein/ g glucan) (Figure 1b) compared to glucose released (Figure 1a) as
355 pretreatment severity increased and xylan content sharply decreased (Table 1). **This data**
356 **suggests that the xylan remaining in the pretreated solids at higher SE pretreatment severities**
357 **(SF > 3.94) was less susceptible to the xylanases present in the Cellic® CTec2 enzyme**
358 **cocktail, possibly due to xylanase binding on the residual lignin in the pretreated solids**
359 **during enzymatic hydrolysis** (Jung et al., 2020). Since ~47% of the xylan (Table 1) was
360 retained at the optimal SE pretreatment with the highest XOS yields (Table 2), it can serve as
361 a potential source of fermentable sugars for pentose-utilizing yeast strains (Du et al., 2019).
362 It is noteworthy that the SE pretreatment resulted in negligible removal of lignin (maximum
363 ~6%) under the SF tested (Table 1), even though all pretreated solids had an increase in
364 enzymatic glucan digestibility as the SF increased (Figure 1a). **SCS biomass was likely**
365 **recalcitrant to SE pretreatment due to its high content of guaiacyl lignin sub-unit that is**
366 **highly prone to condensation under acidic conditions** (Yu et al., 2022). **Hence, this could**
367 **partially explain why lignin removal was low (Table 1). Moreover, these observations**
368 **reinforce the notion that extensive delignification during pretreatment is not necessarily an**
369 **essential criterion to improve the digestibility of biomass into fermentable sugars.**
370 Interestingly, previous work showed that alkaline delignification of steam-exploded SCS at
371 high severity conditions (200 °C; 15 min) had a detrimental effect on enzymatic conversion
372 of glucan **as it may have led to the collapse of the network structure, limiting the surface**

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

380 *3.5. Correlations between bulk composition and biomass surface chemical profile during* 381 *xylo-oligosaccharide and monosaccharide production*

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

390 The bulk lignin content did not have a strong correlation ($r = 0.58$) with the extent of glucan
391 conversion (**see supplementary material**). This was expected since the delignification
392 throughout the severity levels remained low, only accounting for at most ~6% at the highest
393 SE pretreatment severity (Table 1). On the other hand, the apparent surface abundance of
394 lignin (ASA-Lig) relative to holocellulose (ASA-Lig/Cell) had a strong positive correlation (r
395 $= 0.80$) with enzymatic digestibility (**see supplementary material**). **The ASA-Lig/Cell**
396 **increased with SE pretreatment severity, especially from SF > 4.0 (Figure 2a). This can be**
397 **attributed to both exposure of lignin surface after preferential removal of hemicellulose**

398 (Table 1) and lignin redistribution after steam pretreatment. Since delignification, as seen in
399 bulk lignin content, did not correlate with improvement in glucan conversion, the increased
400 accessibility of the substrate to hydrolytic enzymes presumably has more to do with reduced
401 lignin surface coverage. However, increased accessible cellulose surface area due to
402 hemicellulose removal is still likely the most important factor in steam-based pretreatment
403 (Djajadi et al., 2017).

404 Concerning the bulk composition of hemicellulose, both arabinoxylan solubilization and
405 reduction in hemicellulose content had notable correlations ($r = \pm 0.80$) with the glucan
406 conversion yield of pretreated solids at different severity levels (see supplementary material).
407 This is expected and has been shown previously for SCS biomass at different severity levels
408 (Batista et al., 2019; Oliveira et al., 2013). In contrast, the apparent surface abundance of
409 hemicellulose (ASA-Hem) relative to holocellulose (ASA-Hem/Cell) had a less strong
410 correlation ($r = 0.66$) with glucan conversion (see supplementary material). Up until SF =
411 3.94, both bulk and surface composition profiles showed a similar trend. A decrease in ASA-
412 Hem/Cell with increasing severity levels (Figure 2a) was in line with increasing
413 hemicellulose removal (Table 1) and XOS production (Table 2). However, from SF > 4.0,
414 where hemicellulose removal increased to more than 75% (Table 1), the hemicellulose
415 relative surface abundance slightly increased with severity (Figure 2a). This change of trend
416 in ASA-Hem/Cell coincided with a reduction of XOS yield (Table 2a), while glucan
417 conversion (Figure 1a) and hemicellulose removal (Table 1) continued to increase.
418 Condensation of lignin with sugar degradation products such as furfural and HMF, i.e.,
419 pseudo-lignin from SF > 4.0, may be one of the reasons behind this observed increase in
420 ASA-Hem/Cell. In any case, assessing biomass surface chemical profile using ATR-FTIR
421 can be limited by the signal-to-noise ratio in each peak. Furthermore, using different biomass
422 sources and using single biomass pretreated at a wide severity range can have different

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

431 When taken together with the chemical composition data obtained in this work and also from
432 previous studies which used SE-pretreated materials with similar or increased lignin contents,
433 it is probable that lignin from SCS is highly resistant to solubilization but is not the primary
434 inhibitor of cellulose hydrolysis (Oliveira et al., 2013). Besides, as previously mentioned,
435 delignification did not correlate with glucan conversion for SE-pretreated SCS (see
436 **supplementary material**). **Instead, hemicellulose removal or modification was more likely to**
437 **account for the increased glucan conversion at a higher severity level, as delignification only**
438 **reached ~6% at maximum severity (Table 1). Recently, a study on wheat straw and poplar**
439 **pointed to the important role of arabinosyl, 4-O-methyl glucuronosyl and acetyl substituents**
440 **of the hemicellulose in recalcitrance** (Deralia et al., 2021). Moreover, other factors such as
441 cellulose crystallinity, inhibitory products, fiber size (which was not accounted for in this
442 work), the ratio between different cell types, and non-productive adsorption of cellulases to
443 lignin can also influence the digestibility of steam-exploded SCS as well as other types of
444 lignocellulose (Barbosa et al., 2020; Yu et al., 2022).

445 *3.6. Overall mass balance of the process and energy content of biomass*

446 The overall mass balance of SCS subjected to SE pretreatment under optimal conditions for
447 XOS production and enzymatic hydrolysis is summarized in Figure 3. Based on 1 kg of DM

448 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 (~84 g) and small amounts of xylose (~18 g), arabinose (~2 g),
451 and glucose (~2 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 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
474 1). The energy levels of untreated SCS (~18 MJ/kg) were higher than SE-pretreated (~15
475 MJ/kg) and lower than saccharified SCS (~21 MJ/kg) (Table 3). Lignin enrichment in
476 pretreated and saccharified SCS was expected to benefit energy levels compared to the
477 untreated sample (Li et al., 2013). However, the ash content was found to be 2-fold higher in
478 steam-exploded SCS compared to the untreated material (~10% and ~5%, respectively), and
479 according to another report under similar SE conditions (Oliveira et al., 2013). The **higher** ash
480 content (~10%) may have produced an inert effect on the combustion by causing a reduction
481 in the share of combustible carbon matter and the calorific value of the biomass. Hence, the
482 relation between the **amount of ash and** the detrimental effect on the calorific value of the
483 biomass for combined heat and power production requires further investigation. Nonetheless,
484 the higher energy content of the saccharified SCS by about 16% and potentially improved
485 solid pellet quality could reduce the total energy input and costs associated with the XOS
486 production process. The residual lignin from the enzymatically pretreated solid residue could
487 also be hydrolyzed through alkaline or acid treatment and used to produce lignin-based
488 materials and value-added molecules, providing several options for maximizing the value
489 streams in biorefineries (Wang et al., 2019). **Lastly, simulation studies on the techno-**
490 **economic and environmental assessment of SCS-based biorefineries to produce XOS,**
491 **fermentable sugars, and bioenergy under different handling processes such as milling and**
492 **grinding, SE pretreatments, enzymatic saccharification, and fermentation conditions or**
493 **pelletization of the saccharified solids are crucial for the successful commercialization of the**
494 **integrated biorefinery.**

495 **4. Conclusions**

496 Under the SE conditions tested, XOS yields up to 35% w/w of initial xylan were obtained,
497 and ~50% of the recovered XOS were low-DP XOS (X2 and X3), known for their higher

498 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 **Livia 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 &
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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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676

677 **Figure and Table captions**

678 **Figure 1.** Glucan (A) and xylan (B) conversion (%) to glucose and xylose, respectively, after
679 72 h of enzymatic hydrolysis of SE-pretreated solids obtained at different severity factors and
680 enzyme loadings.

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

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

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

689 **Table 2.** XOS yield (w/w % of initial SCS), xylose/XOS recovered (w/w % of initial DM
690 xylan) at different severity factors ($\log R_0$) (A), and the soaking effect prior SE-pretreatment
691 conditions 200 °C; 15 bar; 10 min on the total XOS g/kg (initial xylan) and XOS profile (B).

692 **Table 3.** Lignin and energy content in untreated, SE-pretreated and saccharified SCS.

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701 **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 ± 0.7 ^f	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 ± 0.6 ^d	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 ± 0.1 ^a	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 ± 1.2 ^e	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 ± 0.5 ^a	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 ± 1.1 ^c	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 ± 3.6 ^a	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 ± 0.5 ^a	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 ± 0.9 ^b	91.5 ± 3.5 ^{ab}	93.8 ± 2.1 ^c	5.9 ± 1.3 ^e	96.3 ± 2.4 ^b

702 ^aLignin is total acid-soluble and acid-insoluble lignin (Klason).703 ^bIncludes galactan, extractives, ash and other solids.704 ^cPulp recovered (%) = gram of DM residual straw recovered after pretreatment/ 100 g DM untreated straw.705 ^dGlucan recovery (%) = (Glucan content in pretreated straw × straw recovered) / Total glucan in untreated straw.706 ^eXylan removal (%) = 100 – Xylan recovery (%) in pretreated straw.707 ^fLignin removal (%) = 100 – Lignin recovery (%) in pretreated straw.708 ^gDeacetylation (%) = 100 – Acetyl groups (%) in pretreated straw.

709 DM, dry matter; nd, not detected. Data are means ± standard error (n≥2) from technical and experimental replicates. Different letters in the same

710 column indicate significant statistical differences based on ANOVA (p ≤ 0.05).

711 **Table 2.**

A				
Condition	Severity	XOS yield	Xylose yield	XOS yield
	factor	(w/w %, initial xylan)	(w/w %, initial xylan)	(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	35.19 ± 3.24 ^e	7.76 ± 0.19 ^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	Relative Percentage (%)					
	(Initial xylan)	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

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

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

715 statistical differences based on ANOVA (p ≤ 0.05).

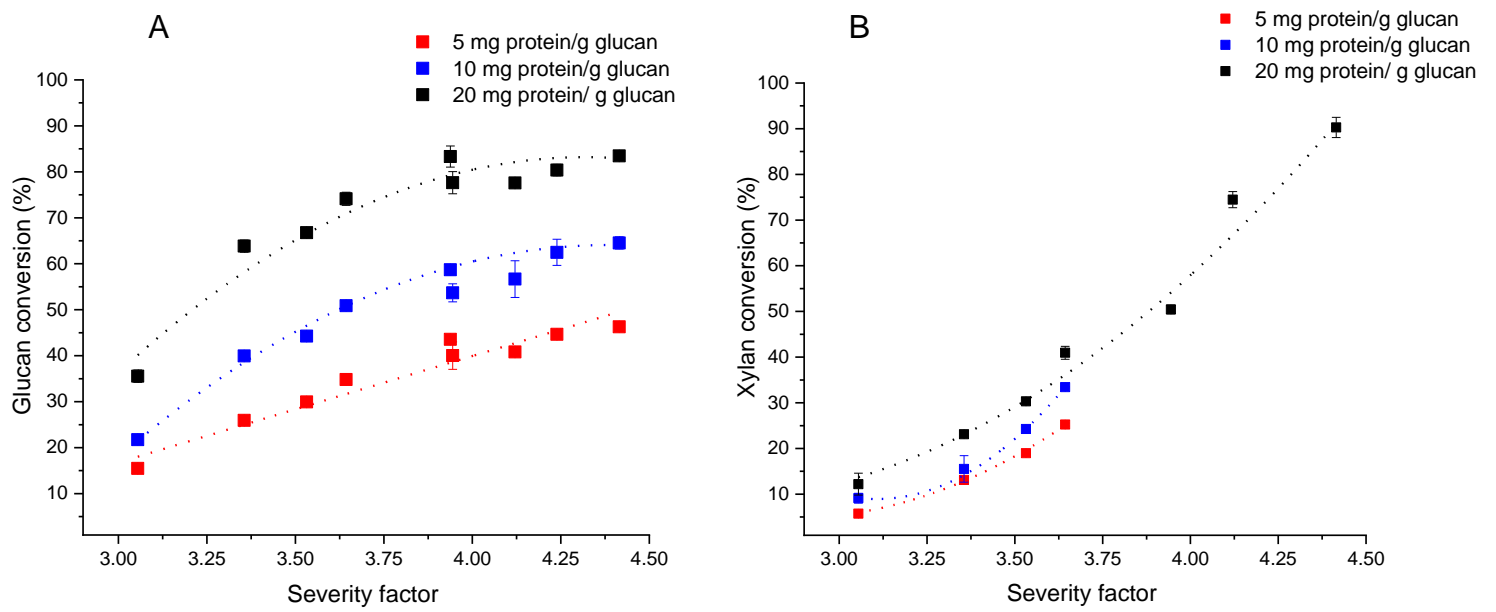
Table 3.

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.

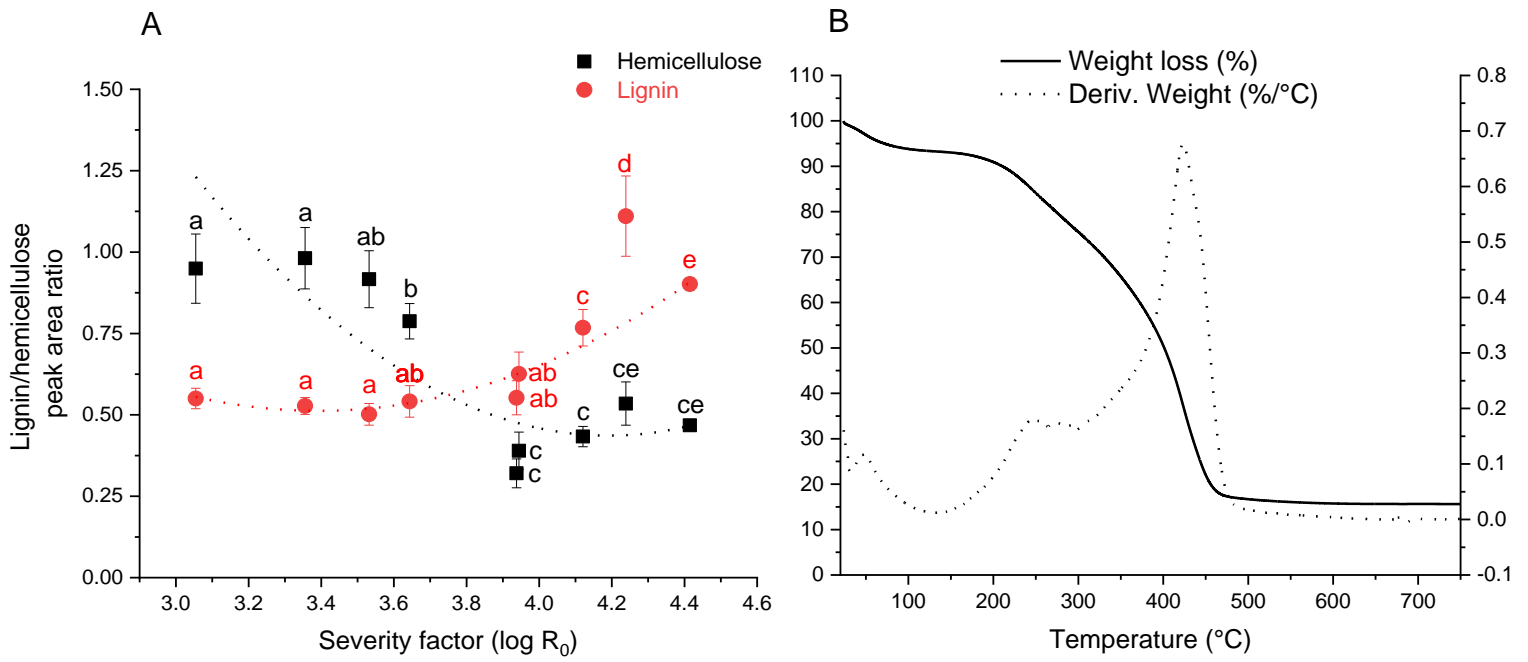
Data are means ± standard error (n≥2) from technical and experimental replicates. Different letters in the same column indicate significant statistical differences based on ANOVA (p ≤ 0.05).

Figure 1.



Data points represent the average and standard deviation from three experimental replicates.

Figure 2.



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.

