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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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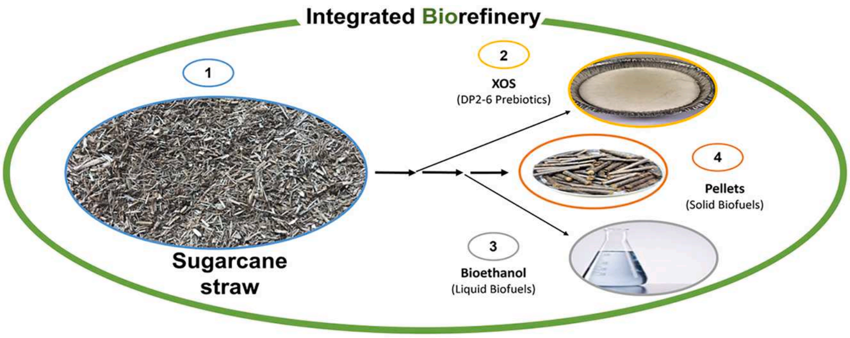
HIGHLIGHTS

- SE pretreatment is effective to produce low DP (2 to 6) XOS from SCS.
- Up to 35 % (w/w) of initial xylan in SCS can be recovered as high market value XOS.
- ~50 % of the recovered XOS were xylobiose and -triose, known as important prebiotics.
- Up to 78 % enzymatic hydrolysis of glucan to glucose from SE pretreated SCS.
- The energy content of SE and enzymatically saccharified SCS increased by up to 16 %.

GRAPHICAL ABSTRACT



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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 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 35 % (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.

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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).

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 from site to site depending on the plant development stage, variety, and collection method (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 steam (7 to 48 bar) 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 hemicelluloses 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). Please refer to Yu et al., 2022 for a comprehensive review on the impacts of steam explosion on the physical and chemical properties of the biomass feedstock and on the different subsequent conversion processes of biomass including densification for solid pellets (heating value) and enzymatic hydrolysis (cellulose accessibility) for fermentation to liquid biofuels.

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 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 range (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 a single step SE pretreatment process, thus avoiding the cost and use of additional catalytic chemicals and enzymes. Important SE parameters to achieve high XOS yields with low DP and low amounts of by-products and sugar degradation products were identified, and a process scheme comprising 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 °C 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}]] (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.

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

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

2.7. *Thermal analysis*

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

2.8. *Energy content measurement*

Energy content measurements of SCS before and after pretreatment and lignin-rich residues remaining after enzymatic hydrolysis were performed in a standard bomb calorimeter (Parr™ 6400 Automatic Isoperibol Calorimeter). All samples were dried in an oven at 30 °C until the MC was below 5 %, milled to less than 0.5 mm, and then compressed into pellets using a hydraulic pelletizer before being weighed (~1.5 g of sample was used). Heat content was determined in a sealed steel bomb by burning the samples with an excess of oxygen at a pressure of 430 psi (30 bar).

2.9. *Statistical analysis*

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

3. Results and discussion

3.1. *Sugarcane straw composition*

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

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

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}

DM, dry matter; nd, not detected. Data are means ± standard error ($n \geq 2$) from technical and experimental replicates. Different letters in the same column indicate significant statistical differences based on ANOVA ($p \leq 0.05$).

[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.

cellulolytic enzymes for improved fermentable glucose yields (Bhatia et al., 2020a). Nonetheless, there is a marked natural variation in the biochemical composition of sugarcane residues (straw and bagasse) due to plant variety and developmental period, including collection site and weather conditions, which must be understood for the biomass to be effectively utilized and processed to produce bioproducts and biomaterials for biorefineries (Aguiar et al., 2021).

3.2. *Steam explosion pretreatment to produce XOS*

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

The SE conditions 200 °C; 15 bar; 10 min (SF = 3.94) resulted in XOS yields of up to ~35 % (w/w) of initial DM xylan, corresponding to 8 % (w/w) of initial DM biomass, and a low yield of xylose ~8 % (w/w) (Table 2a). The percentage recovery of XOS (~35 % w/w) was relatively low compared to SE pretreatment of alkali-impregnated sugarcane harvesting residues (sugarcane trash, SCT) (~51 % w/w) under similar SE conditions (204 °C, 10 min) (Mihiretu et al., 2019). This higher XOS yield with little or no formation of monomeric xylose from SCT can be attributed to the alkali agents/conditions that preserved the xylan extracts in their oligomeric and polymeric form and that led to significant removal of lignin (up to 70 %) due to cleavage of the ester bonds linking lignin with xylan (Mihiretu et al., 2019). Nonetheless, similar to this study, comparable XOS yields (~33 %) were attained but with the low formation of xylose (1 %) and co-solubilization of lignin (27 %) (w/w) for alkali deacetylated SCS subjected to hydrothermal pretreatment (Brenelli et al., 2020). From such alkaline pretreatment studies, it was also inferred that the acetyl side groups cleaved from xylan and released as acetic acid could neutralize and counter-balance the alkalinity of extraction conditions and thus affect the degree of xylan and lignin solubilization. In this context, the comparable XOS yields (~35 % w/w) (Table 2a) may also have been achieved during SE by means of buffering of the released acetic acid through the high ash content in SCS (~5 % w/w) compared to deacetylated SCS (~3 % w/w) (Brenelli et al., 2020). It should also be noted that a different SE condition (210 °C; 20 bar; 5 min) with a similar SF (3.94) to the optimal condition (200 °C; 15 bar; 10 min) showed a lower XOS yield (~24 %) (Table 2a), suggesting that temperature and residence time and not SF *per se*, are the important parameters influencing the final properties of the biomass substrate (Yu et al., 2022). Although SE pretreatment of SCS resulted in relatively high xylose yields (~8 % w/w) (Table 2a) compared to deacetylated SCS (1 % w/w), nanofiltration membranes or yeast fermentation can be applied to effectively remove and minimize the undesired monomeric xylose sugars to meet the purity requirements of commercial XOS (75 to 95 %) (Huang et al., 2019; Wijaya et al., 2020). Regarding the amount and DP of XOS, SE-pretreatment (200 °C; 15 bar; 10 min) produced up to ~ 352.0 g of XOS per kg of initial xylan, with >50 % comprised of X2 and X3 (~29 and ~ 25 % respectively), ~18 % as X4, ~15 % as X5, ~9 % as X6, and only ~4 % as XOS with DP > 6 (Table 2b). The effect of the pre-soaking step for SCS under optimal SE conditions for the highest XOS production was also evaluated with similar results to (Bhatia et al., 2020b) (Table 2b) showing that XOS recovery in the hydrolysate did not significantly change with pre-soaking conditions. Moreover, the XOS produced did not undergo significant changes in the distribution of DP 2 to 6 XOS (Table 2b). Certainly, acetyl-assisted autohydrolysis of SCS has the advantage of releasing predominantly XOS with DP 2 to 6 under acidic conditions, whereas alkaline extraction conditions for SCS can lead to xylan solubilization into long-chain XOS with DP ≥ 5 (~85 %) (Brenelli et al., 2020). The fact that SCS hemicellulose fragments released during SE pretreatment are partially acetylated is a positive feature 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

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

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	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 ± 1.0^{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.
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 ($p \leq 0.05$).

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 to 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 to 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 ~0.2 to 3 % (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 compared to the untreated SCS. Thus, glucan recovered in the SE-pretreated solids was on average ~90 %, although the SF = 4.12 appeared to expose more fractions of the glucan thereby influencing the dissolution properties of glucan and leading to reduced glucan recovery down to 80 %.

An increase in glucan and lignin content and reduction in xylan has been observed in other studies related to SE-pretreatment of SCS for cellulosic ethanol production (Oliveira et al., 2013; Zhang et al., 2018). In general, the degree of solubilization of SCS increased moderately at all temperatures as the reaction time increased, but the main biomass components exhibited different extents of solubilization (Table 1). Xylan solubilization and removal increased as the SE pretreatment severity increased and reached 94 % at SF = 4.41. Because acetyl groups are linked to the xylan backbone, deacetylation followed the same pattern, although a significant degree of deacetylation (>60 %) was observed at all temperatures after 5 min. Lignin solubilization and, therefore, delignification was minimal under all the tested SE conditions. The maximum delignification (~6 %) was achieved at the highest severity factor (SF = 4.41). It is widely known that the acidic conditions of SE-pretreatment typically induce lignin depolymerization followed by condensation to minimize its surface area and deposition onto the fibers combined with ash, extractives, and other components (Heikkinen et al., 2014). This may explain why the lignin content in pretreated solids mostly increased as the SE pretreatment temperature increased. The oligosaccharides analysed in the liquid fraction were mainly composed of xylose (8.1 %), arabinose (0.5 %), galactose (0.3 %), acetyl (0.7 %) and glucose (1.4 %) (w/w). The composition of undesired products and degradation products in the XOS-rich hydrolysates was also assessed to inform the design of subsequent processing methods for XOS recovery and purification (see supplementary material). As expected, all the aforementioned products increased with 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) (Fig. 1). Generally, glucan hydrolysis after 72 h increased as the severity level increased for all enzyme loadings tested (Fig. 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 % (Fig. 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) (Fig. 1b) compared to glucose released (Fig. 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 on 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 (Fig. 1a). SCS biomass was likely recalcitrant to SE pretreatment due to its 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 (Table 1). 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 (Fig. 2a). This can be attributed to both exposure of lignin surface after preferential removal of hemicellulose (Table 1) and lignin redistribution after steam

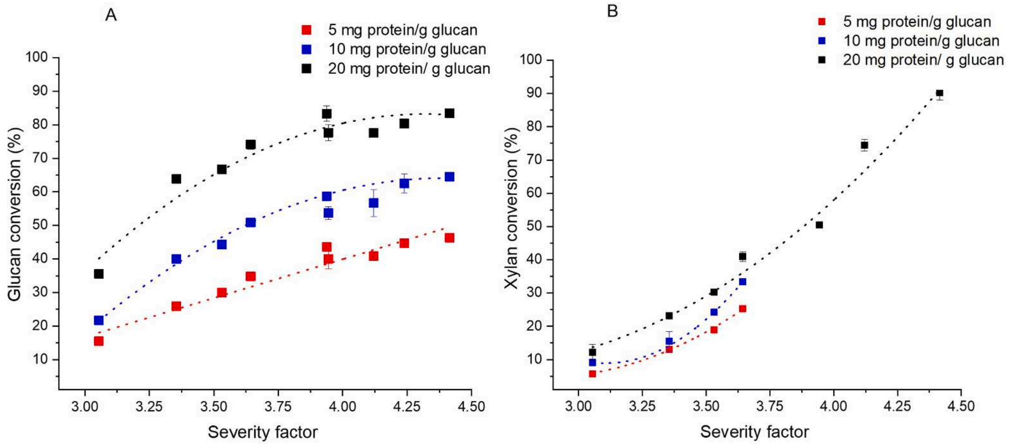

Fig. 1. Glucan (A) and xylan (B) conversion (%) to glucose and xylose, respectively, after 72 h of enzymatic hydrolysis of SE-pretreated solids obtained at different severity factors and enzyme loadings.

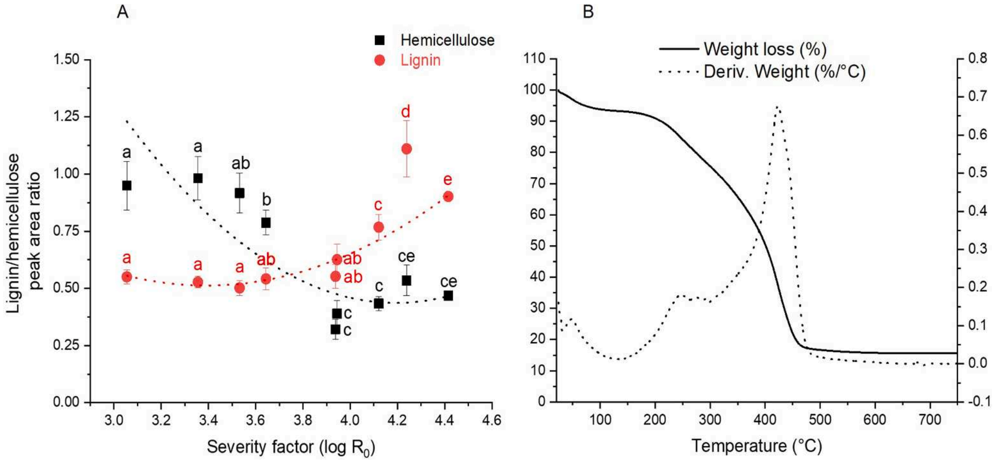

Fig. 2. ATR-FTIR peak area ratio of wavenumbers representing hemicellulose (1732 cm^{-1}) and lignin (1512 cm^{-1}) each relative to that of holocellulose (1160 cm^{-1}) for SE-pretreated SCS under different severity factors (A) and Thermogravimetric curve recorded for alkaline lignin extracted from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

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 presumably has more to do with reduced lignin surface coverage. However, increased accessible cellulose surface area due to hemicellulose removal is still likely the most important factor in steam-based pretreatment (Djajadi et al., 2017).

Concerning the bulk composition of hemicellulose, both xylan solubilization and reduction in hemicellulose content had notable correlations (r = ± 0.80) with the 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 (Fig. 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 >75 % (Table1), the hemicellulose relative surface abundance slightly increased with severity (Fig. 2a). This change of trend in ASA-Hem/Cell coincided with a reduction of XOS yield (Table 2a), while glucan conversion (Fig. 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 (Fig. 2b). Thus, the mobilization of lignin, which can be inferred from the FTIR data to have occurred at temperatures higher than 200 °C (Fig. 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, hemicellulose removal or modification was more likely to account for the increased glucan conversion at a higher severity level, as delignification only reached ~6 % at maximum severity (Table 1). Recently, a study on wheat straw and poplar pointed to the important role of arabinosyl, 4-O-methyl glucuronosyl and acetyl substituents of the hemicellulose in recalcitrance (Deralia et al., 2021). 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 Fig. 3. Based on 1 kg of DM SCS feedstock, ~83 % was obtained as a solid fraction comprised of ~339 g glucan, ~111 g xylan, ~5 g acetyl, and ~197 g lignin and the remaining ~17 % was dissolved into the hydrolysate as soluble XOS (~84 g) and small amounts of xylose (~18 g), arabinose (~2 g), and glucose (~2 g). As previously stated, XOS could be used either as ingredients in functional foods after purification or for fermentation to bioproducts using microorganisms capable of metabolizing oligomers (Amorim et al., 2019). The former is of particular interest as short-chained XOS, i.e., X2 and X3, are known for their potential prebiotic activity and higher sweetness than sucrose (Moura et al., 2007; Park et al., 2017). After enzymatic hydrolysis, the glucan-rich solids using the highest enzyme loading (20 mg protein/g glucan) and low biomass loading (1 % w/v) for 72 h produced ~263 g of glucose and ~56 g of xylose. Both sugars can be further fermented to obtain bio-based fuels and chemicals (Santos et al., 2019). Processive *endo*-glucanases could also be used to produce cello-oligomers, glucose polymers with potential applications in the food and bioenergy industry, but this would either require auxiliary enzymes or cellulose decrystallization (Barbosa et al., 2020).

Energy content is an important property for determining the attractiveness of a potential biofuel. Biomass with a higher energy level and density is more energy efficient for conversion into biofuel and mitigates against transportation costs and expenses associated with storage, handling, and distribution (Albashabsheh and Heier Stamm, 2021). Lignin has a higher energy content (~27 MJ/kg) than glucan and

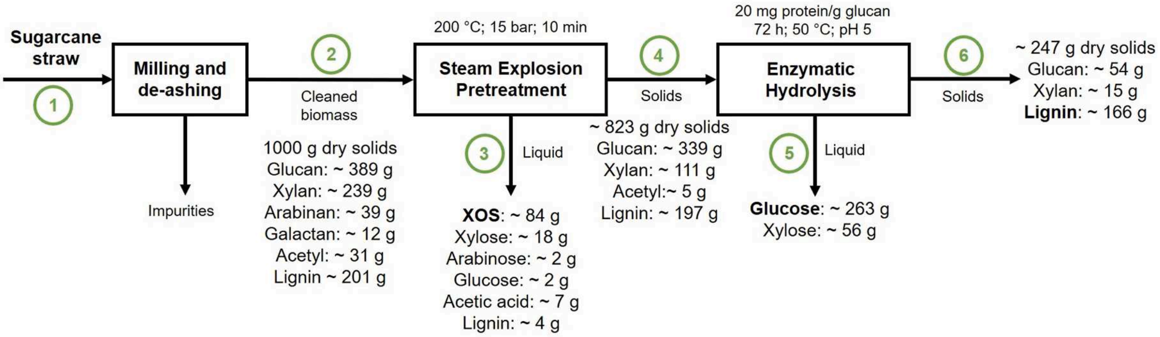

Fig. 3. Overall mass balance of SCS under SE-pretreatment.

xylan (∼18 MJ/kg), making the lignin-rich residue after hydrolysis of pretreated material a good candidate for combustion and the provision of heat for the aforementioned biorefinery process. The lignin content in the SE-pretreated solids increased from ∼20 to ∼24 % (w/w) after SE-pretreatment under optimal conditions for XOS production (Table 1) and reached ∼67 % after saccharification (Table 3) due to the efficient enzymatic conversion of polysaccharides into monosaccharides (Fig. 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 35 % 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 released as fermentable glucose and the remaining lignin-rich (67 % w/w) saccharified solids exhibited a 16 % higher energy content than untreated SCS. A potential value chain is presented for sugarcane biorefineries using SCS via value-added XOS production and co-production of renewable liquid and solid biofuels.

CRediT authorship contribution statement

Lívia B. Brenelli: Validation, Investigation, Writing – original draft, Writing – review & editing, Visualization, Visualization, Project administration. **Rakesh Bhatia:** Validation, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Demi T. Djajadi:** Validation, Writing – review & editing, Visualization. **Lisbeth G. Thygesen:** Validation, Writing – review & editing. **Sarita C. Rabelo:** Validation, Writing – review & editing. **David J. Leak:** Conceptualization, Validation, Writing – review & editing, Funding acquisition. **Telma T. Franco:** Conceptualization, Validation, Writing – review & editing, Funding acquisition. **Joe A. Gallagher:** Validation, Writing – review & editing, Supervision, Funding acquisition.

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

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, 72 h)	67.1 ± 1.8^{b}	20.59 ± 0.20^{c}

*Extractives free basis.
Data are means ± standard error ($n \geq 2$) from technical and experimental replicates. Different letters in the same column indicate significant statistical differences based on ANOVA ($p \leq 0.05$)

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127093.

References

Aguiar, A., Milessi, T.S., Mulinari, D.R., Lopes, M.S., da Costa, S.M., Candido, R.G., 2021. Sugarcane straw as a potential second generation feedstock for biorefinery and white biotechnology applications. Biomass Bioenerg. 144, 105896.
Albashabsheh, N.T., Heier Stamm, J.L., 2021. Optimization of lignocellulosic biomass-to-biofuel supply chains with densification: Literature review. Biomass Bioenerg. 144, 105888.

Álvarez, C., González, A., Alonso, J.L., Sáez, F., Negro, M.J., Gullón, B., 2020. Xylooligosaccharides from steam-exploded barley straw: Structural features and assessment of bifidogenic properties. Food Bioprod. Process. 124, 131–142.

Amorim, C., Silvério, S.C., Prather, K.L.J., Rodrigues, L.R., 2019. From lignocellulosic residues to market: Production and commercial potential of xylooligosaccharides. Biotechnol. Adv. 37 (7), 107397.

Arai, T., Biely, P., Uhliariková, I., Sato, N., Makishima, S., Mizuno, M., Nozaki, K., Kaneko, S., Amano, Y., 2019. Structural characterization of hemicellulose released from corn cob in continuous flow type hydrothermal reactor. J. Biosci. Bioeng. 127 (2), 222–230.

Auxenfans, T., Crônier, D., Chabbert, B., Paës, G., 2017. Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment. Biotechnol. Biofuels. 10 (1), 1–16.

Barbosa, F.C., Martins, M., Brenelli, L.B., Ferrari, F.A., Forte, M.B.S., Rabelo, S.C., Franco, T.T., Goldbeck, R., 2020. Screening of potential endoglucanases, hydrolysis conditions and different sugarcane straws pretreatments for cello-oligosaccharides production. Bioresour. Technol. 123918, 2020.

Batista, G., Souza, R.B.A., Pratto, B., dos Santos-Rocha, M.S.R., Cruz, A.J.G., 2019. Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. Bioresour. Technol. 275, 321–327.

Bhatia, R., Lad, J.B., Bosch, M., Bryant, D.N., Leak, D., Hallett, J.P., Franco, T.T., Gallagher, J.A., 2021. Production of oligosaccharides and biofuels from Miscanthus using combinatorial steam explosion and ionic liquid pretreatment. Bioresour. Technol. 323, 124625.

Bhatia, R., Winters, A., Bryant, D.N., Bosch, M., Clifton-Brown, J., Leak, D., Gallagher, J., 2020b. Pilot-scale production of xylo-oligosaccharides and fermentable sugars from Miscanthus using steam explosion pretreatment. Bioresour. Technol. 296, 122285.

Biswas, R., Uellendahl, H., Ahring, B.K., 2015. Wet Explosion: a universal and efficient pretreatment process for lignocellulosic biorefineries. Bioenergy Res. 8 (3), 1101–1116.

Brenelli, L.B., Figueiredo, F.L., Damasio, A., Franco, T.T., Rabelo, S.C., 2020. An integrated approach to obtain xylo-oligosaccharides from sugarcane straw: From lab to pilot scale. Bioresour. Technol. 313, 123637.

Carvalho, A.F.A., Marcondes, W.F., de Oliva Neto, P., Pastore, G.M., Saddler, J.N., Arantes, V., 2018. The potential of tailoring the conditions of steam explosion to produce xylo-oligosaccharides from sugarcane bagasse. Bioresour. Technol. 250, 221–229.

Carvalho, J.L.N., Nogueirol, R.C., Menandro, L.M.S., Bordonal, R.d.O., Borges, C.D., Cantarella, H., Franco, H.C.J., 2017. Agronomic and environmental implications of sugarcane straw removal: a major review. GCB Bioenergy 9 (7), 1181–1195.

Cherubin, M.R., Bordonal, R.O., Castioni, G.A., Guimarães, E.M., Lisboa, I.P., Moraes, L. A.A., Menandro, L.M.S., Tenelli, S., Cerri, C.E.P., Karlen, D.L., Carvalho, J.L.N., 2021. Soil health response to sugarcane straw removal in Brazil. Ind Crops Prod. 163, 113315.

de Freitas, C., Terrone, C.C., Masarin, F., Carmona, E.C., Brienzo, M., 2021. In vitro study of the effect of xylooligosaccharides obtained from banana pseudostem xylan by enzymatic hydrolysis on probiotic bacteria. Biocatal. Agric. Biotechnol. 33, 101973.

Deralia, P.K., Jensen, A., Felby, C., Thygesen, L.G., 2021. Chemistry of lignin and hemicellulose structures interacts with hydrothermal pretreatment severity and affects cellulose conversion. Biotechnol Prog. 37 (5), e3189.

Djajadi, D.T., Hansen, A.R., Jensen, A., Thygesen, L.G., Pinelo, M., Meyer, A.S., Jørgensen, H., 2017. Surface properties correlate to the digestibility of hydrothermally pretreated lignocellulosic Poaceae biomass feedstocks. Biotechnol. Biofuels. 10 (1), 1–15.

Du, C., Li, Y., Zhao, X., Pei, X., Yuan, W., Bai, F., Jiang, Y., 2019. The production of ethanol from lignocellulosic biomass by Kluyveromyces marxianus CICC 1727–5 and Spathaspora passalidarum ATCC MYA-4345. Appl. Microbiol. Biotechnol. 103 (6), 2845–2855.

Espírito Santo, M.C.D., Cardoso, E.B., Guimaraes, F.E.G., deAzevedo, E.R., Cunha, G.P.D., Novotny, E.H., Pellegrini, V.D.O.A., Chandel, A.K., Silveira, M.H.L., Polikarpov, I., 2019. Multifaceted characterization of sugarcane bagasse under different steam explosion severity conditions leading to distinct enzymatic hydrolysis yields. Ind Crops Prod. 139, 111542.

Ferrão, L.L., Ferreira, M.V.S., Cavalcanti, R.N., Carvalho, A.F.A., Pimentel, T.C., Silva, H. L.A., Silva, R., Esmerino, E.A., Neto, R.P.C., Tavares, M.I.B., Freitas, M.Q., Menezes, J.C.V., Cabral, L.M., Moraes, J., Silva, M.C., Mathias, S.P., Raices, R.S.L., Pastore, G.M., Cruz, A.G., 2018. The xylooligosaccharide addition and sodium reduction in requeijão cremoso processed cheese. Int. Food Res. J. 107, 137–147.

Heikkinen, H., Elder, T., Maaheimo, H., Rovio, S., Rahikainen, J., Kruus, K., Tamminen, T., 2014. Impact of steam explosion on the wheat straw lignin structure studied by solution-state nuclear magnetic resonance and density functional methods. J. Agric. Food Chem. 62 (43), 10437–10444.

Ho, A.L., Kosik, O., Lovegrove, A., Charalampopoulos, D., Rastall, R.A., 2018. In vitro fermentability of xylo-oligosaccharide and xylo-polysaccharide fractions with different molecular weights by human faecal bacteria. Carbohydr. Polym. 179, 50–58.

Huang, C., Wang, X., Liang, C., Jiang, X., Yang, G., Xu, J., Yong, Q., 2019. A sustainable process for procuring biologically active fractions of high-purity xylooligosaccharides and water-soluble lignin from Moso bamboo prehydrolyzate. Biotechnol. Biofuels 12 (1), 1–13.

Jung, W., Sharma-Shivappa, R., Park, S., Kolar, P., 2020. Effect of cellulolytic enzyme binding on lignin isolated from alkali and acid pretreated switchgrass on enzymatic hydrolysis. 3. Biotech 10 (1), 1–10.

Kontogianni, N., Barampouti, E.M., Mai, S., Malamis, D., Loizidou, M., 2019. Effect of alkaline pretreatments on the enzymatic hydrolysis of wheat straw. Environ. Sci. Pollut. Res. Int. 26 (35), 35648–35656.

Kumar, V., Bahuguna, A., Ramalingam, S., Kim, M., 2021. Developing a sustainable bioprocess for the cleaner production of xylooligosaccharides: An approach towards lignocellulosic waste management. J. Clean. Prod. 316, 128332.

Li, C., Tanjore, D., He, W., Wong, J., Gardner, J.L., Sale, K.L., Simmons, B.A., Singh, S., 2013. Scale-up and evaluation of high solid ionic liquid pretreatment and enzymatic hydrolysis of switchgrass. Biotechnol. Biofuels 6 (1), 1–13.

Lupoi, J.S., Singh, S., Simmons, B.A., Henry, R.J., 2014. Assessment of Lignocellulosic Biomass Using Analytical Spectroscopy: An Evolution to High-Throughput Techniques. Bioenergy Res. 7 (1), 1–23.

Míguez, B., Gullón, P., Cotos-Yáñez, T., Massot-Cladera, M., Pérez-Cano, F.J., Vila, C., Alonso, J.L., 2021. Manufacture and Prebiotic Potential of Xylooligosaccharides Derived From Eucalyptus nitens Wood. Front. Chem. Eng. 3, 670440.

Mihiretu, G.T., Chimphango, A.F., Görgens, J.F., 2019. Steam explosion pre-treatment of alkali-impregnated lignocelluloses for hemicelluloses extraction and improved digestibility. Bioresour. Technol. 294, 122121.

Milessi, T.S., Corradini, F.A.S., Marçal, J.V.M., Baldez, T.O., Kopp, W., Giordano, R.C., Giordano, R.L.C., 2021. Xylooligosaccharides production chain in sugarcane biorefineries: From the selection of pretreatment conditions to the evaluation of nutritional properties. Ind Crops Prod. 172, 114056.

Moura, P., Barata, R., Carvalheiro, F., Gírio, F., Loureiro-Dias, M.C., Esteves, M.P., 2007. In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by Bifidobacterium and Lactobacillus strains. LWT-Food Sci. Tech. 40 (6), 963–972.

Oliveira, F.M.V., Pinheiro, I.O., Souto-Maior, A.M., Martin, C., Gonçalves, A.R., Rocha, G. J.M., 2013. Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products. Bioresour. Technol. 130, 168–173.

Overend, R.P., Chornet, E., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences 321 (1561), 523–536.

Park, H.W., Kim, M.J., Seo, S., Yoo, S., Hong, J.H., 2017. Relative sweetness and sweetness quality of Xylobiose. Food Sci. Biotechnol. 26 (3), 689–696.

Patel, A., Shah, A.R., 2021. Integrated lignocellulosic biorefinery: Gateway for production of second generation ethanol and value added products. J. Bioresour. Bioprod. 6 (2), 108–128.

Pihlajaniemi, V., Sipponen, M.H., Liimatainen, H., Sirviö, J.A., Nyyssölä, A., Laakso, S., 2016. Weighing the factors behind enzymatic hydrolyzability of pretreated lignocellulose. Green Chem. 18 (5), 1295–1305.

Pinales-Márquez, C.D., Rodríguez-Jasso, R.M., Araújo, R.G., Loredo-Treviño, A., Nabarlatz, D., Gullón, B., Ruiz, H.A., 2021. Circular bioeconomy and integrated biorefinery in the production of xylooligosaccharides from lignocellulosic biomass: A review. Ind Crops Prod. 162, 113274.

Santibáñez, L., Henríquez, C., Corro-Tejeda, R., Bernal, S., Armijo, B., Salazar, O., 2021. Xylooligosaccharides from lignocellulosic biomass: A comprehensive review. Carbohydr. Polym. 251, 117118.

Santos, F., de Matos, M., Rabelo, S.C., Eichler, P., 2019. Sugarcane biorefinery, technology and perspectives, Sugarcane Biorefinery. Academic Press, Technology and Perspectives.

Silveira, M.H.L., Chandel, A.K., Vanelli, B.A., Sacilotto, K.S., Cardoso, E.B., 2018. Production of hemicellulosic sugars from sugarcane bagasse via steam explosion employing industrially feasible conditions: Pilot scale study. Bioresour. Technol. Rep. 3, 138–146.

Singh, R.D., Banerjee, J., Sasmal, S., Muir, J., Arora, A., 2018. High xylan recovery using two stage alkali pre-treatment process from high lignin biomass and its valorisation to xylooligosaccharides of low degree of polymerisation. Bioresour. Technol. 256, 110–117.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Technical Report. NREL/TP-510-42623.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618, US National Renewable Energy Laboratory. Laboratory Analytical Procedure (LAP), Golden, Colorado.

USDA, 2021. Brazil: Sugar Annual. https://www. https://www.fas.usda.gov/data/brazil-sugar-annual-7. (accessed 03.02.22).

Wang, H., Pu, Y., Ragauskas, A., Yang, B., 2019. From lignin to valuable products–strategies, challenges, and prospects. Bioresour. Technol. 271, 449–461.

Wijaya, H., Sasaki, K., Kahar, P., Rahmani, N., Hermiati, E., Yopi, Y., Ogino, C., Prasetya, B., Kondo, A., 2020. High enzymatic recovery and purification of xylooligosaccharides from empty fruit bunch via nanofiltration. Processes 5, 619.

Yu, Y., Wu, J., Ren, X., Lau, A., Rezaei, H., Takada, M., Bi, X., Sokhansanj, S., 2022. Steam explosion of lignocellulosic biomass for multiple advanced bioenergy processes: A review. Renew. Sust. Energ. Rev. 154, 111871.

Zhang, W., You, Y., Lei, F., Li, P., Jiang, J., 2018. Acetyl-assisted autohydrolysis of sugarcane bagasse for the production of xylo-oligosaccharides without additional chemicals. Bioresour. Technol. 265, 387–393.