

RESEARCH

Open Access



# Coproduction of xylo-oligosaccharides and glucose from sugarcane bagasse in subcritical CO<sub>2</sub>-assisted seawater system

Leping Zhang<sup>1</sup>, Xiankun Zhang<sup>1</sup>, Fuhou Lei<sup>2</sup>, Jianxin Jiang<sup>1\*</sup> and Li Ji<sup>1\*</sup>

## Abstract

Abundant seawater resources can replace the shortage of freshwater resources. The co-production of xylo-oligosaccharides and glucose from sugarcane bagasse by subcritical CO<sub>2</sub>-assisted seawater pretreatment was studied in this paper. We investigated the effects of pretreatment conditions of temperature, CO<sub>2</sub> pressure and reaction time on the yield of xylo-oligosaccharides in subcritical CO<sub>2</sub>-assisted seawater systems. The maximum xylo-oligosaccharide yield of 68.23% was obtained at 165 °C/2 MPa/5 min. After further enzymatic hydrolysis of the solid residue, the highest glucose yield of 94.45% was obtained. In this system, there is a synergistic effect of mixed ions in seawater and CO<sub>2</sub> to depolymerize xylan into xylo-oligosaccharides with a lower degree of polymerization. At the same time, the addition of CO<sub>2</sub> increased the pore size and porosity of sugarcane bagasse, improved the efficiency of enzymatic hydrolysis and increased the yield of glucose. Therefore, this study provides a more environmentally friendly and sustainable process for the co-production of xylo-oligosaccharides and glucose from sugarcane bagasse, and improves the utilization of seawater resources.

**Keywords:** Sugarcane bagasse, Xylo-oligosaccharides, Subcritical CO<sub>2</sub>-assisted seawater pretreatment, Glucose, Enzymatic hydrolysis

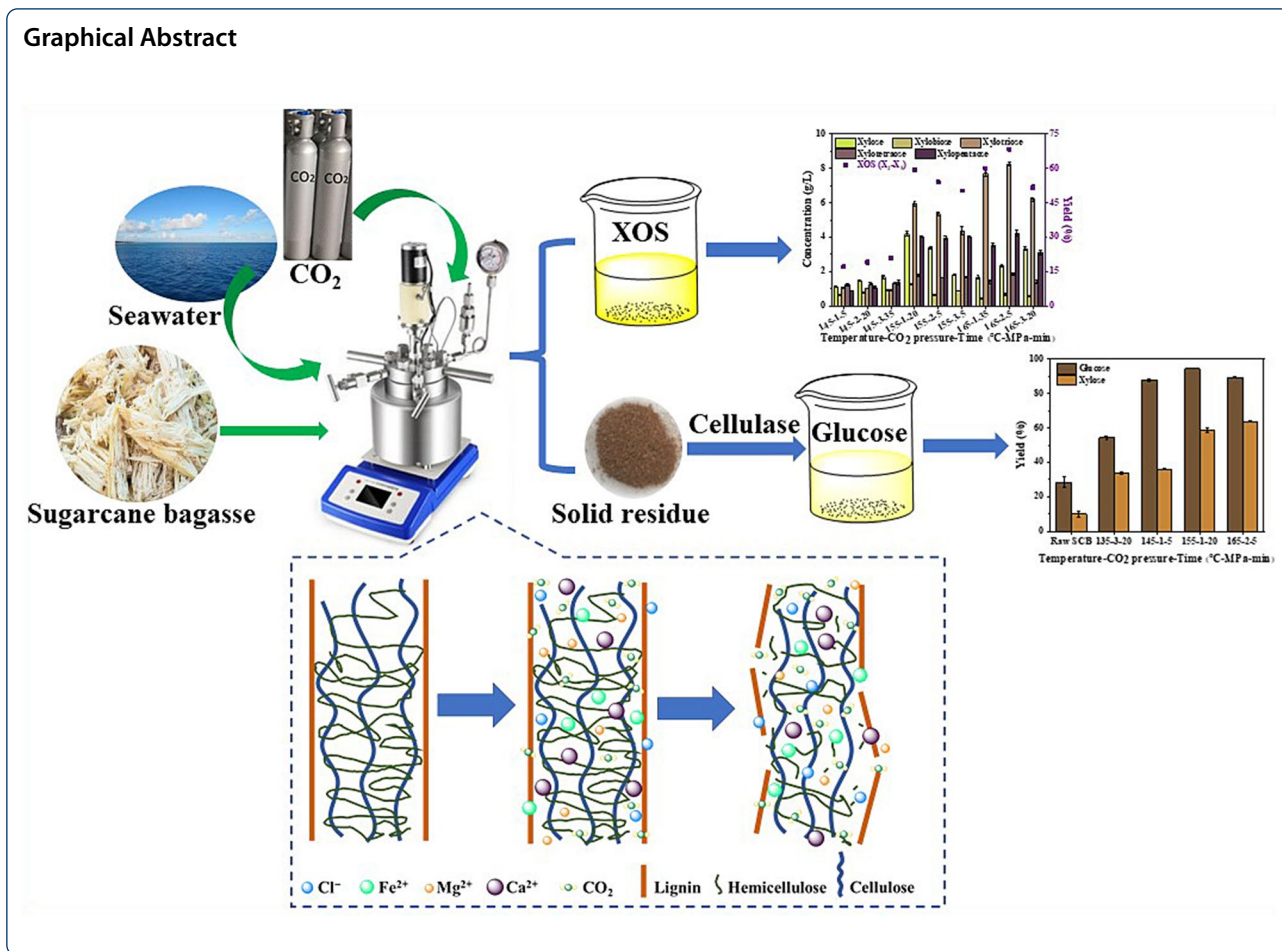
\*Correspondence: [jiangjx2004@hotmail.com](mailto:jiangjx2004@hotmail.com); [bjfu090524116@163.com](mailto:bjfu090524116@163.com)

<sup>1</sup> Department of Chemistry and Chemical Engineering, MOE Engineering Research Center of Forestry Biomass Materials and Bioenergy, Beijing Forestry University, Beijing 100083, China

Full list of author information is available at the end of the article



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.



## Introduction

The rapid depletion of fossil fuels and severe environmental pollution prompt people to seek sustainable resources to replace non-renewable fossil resources (Gong et al. 2021; Ni et al. 2021). Lignocellulosic biomass is an ideal resource for biofuel and high value-added chemicals production due to its wide distribution, abundant reserves, low cost and renewability (Wu et al. 2021b). As one of the lignocellulosic biomass materials, sugarcane bagasse (SCB) is abundant and consists of three main components: cellulose, hemicellulose and lignin (Ajala et al. 2021). The cell wall structure of SCB is formed by the cross-linking of a lignin–hemicellulose–cellulose (Ire et al. 2016), which makes it challenging to produce biofuels or chemicals directly. Therefore, hunting for a proper pretreatment method is necessary to improve the utilization of hemicellulose, enhance the enzyme digestibility of cellulose and maintain the structural integrity of lignin, achieving the utilization of the whole composition of lignocellulose finally (Liu et al. 2019; Zhang et al. 2020a).

Xylo-oligosaccharide (XOS) is a soluble carbohydrate produced by partial hydrolysis of hemicellulose at the high temperature. It consists of xylose chains of lengths 2–10, with or without arabinose or glucuronic acid side groups (Jaichakan et al. 2021). The biological activity of XOS will depend on their degree of polymerization. According to its different biological activities, XOS is primarily used in the functional food, pharmaceutical and chemical industries (de Freitas et al. 2019).

At present, a large number of pretreatment methods for XOS production have been developed, such as acid or alkaline hydrolysis (Liao et al. 2021a; Yang et al. 2021; Zhang et al. 2021a), autohydrolysis (Zhang et al. 2020b), biological pretreatment (Valladares-Diestra et al. 2021), inorganic salt pretreatment (You et al. 2020; Zhang et al. 2019) and the combination of some of them (Liao et al. 2021b; Nuntawat et al. 2018). These methods have their advantages and disadvantages, but they all have one thing in common: they all use many freshwater resources as the main solvent. However, if XOS is produced on a

large scale, it may cause excessive use of freshwater resources. Seawater resources are abundant, accounting for about 97% of the world's water resources. Our previous research has shown that seawater containing a variety of inorganic salt ions can catalyze the production of XOS from SCB (Zhang et al. 2020b). The concentrations of iron, magnesium, calcium and NaCl in the seawater are  $1.25 \pm 0.01$  mg/mL,  $414.40 \pm 0.20$  mg/mL,  $199.05 \pm 0.05$  mg/mL and  $27.13 \pm 0.00$  mg/mL, respectively (Zhang et al. 2020b). The metal ions in these inorganic salts provide an indirect weak acidic (Lewis acid) environment. In this subacidic environment, chloride ions can cut off the hydrogen bond between glycan molecules, to promote the degradation of highly polymerized glycans into smaller polysaccharides or even monosaccharides (Jiang et al. 2018; Zhang et al. 2020b).

During these available pretreatment processes, a large amount of hemicellulose was degraded into XOS and retained in the liquid components, while most cellulose and lignin were still kept in the solid parts (Zhang et al. 2021b, 2020a, 2020b; Zhu et al. 2021). Our previous study demonstrated that the seawater hydrothermal pretreatment of SCB is a highly efficient and environmentally friendly method for XOS production (Zhang et al. 2020b). It combined the advantages of automatic hydrolysis and ion pretreatment, which was green, efficient, lower cost, non-toxic and without additional chemical reagents. Seawater hydrothermal pretreatment can not only obtain a high yield of XOS, but also a high yield of glucose in the enzymatic hydrolysis stage of solid residue. However, in addition to receiving high-yield XOS, the existing pretreatment conditions are still severe (higher temperature and severity parameter). CO<sub>2</sub> pretreatment is a non-toxic, environmentally friendly, and efficient method for the pretreatment of lignocellulosic biomass (Ge et al. 2020). In the pretreatment process, CO<sub>2</sub> can form a weak acid (H<sub>2</sub>CO<sub>3</sub>) catalyst with water to degrade hemicellulose and further destroy the lignin-carbohydrate complex (LCC) compound structure. After the reaction, rapid release and separation of CO<sub>2</sub> can be achieved through decompression (Gurgel et al. 2014; Liu et al. 2019; Park and Lee 2020). The separation methods of XOS include solvent extraction, membrane separation and chromatography, in which chromatography is the most efficient one to obtain high-purity XOS (Xiao et al. 2018). Moreover, the metal ions (iron, magnesium and calcium) contained in seawater are beneficial to human body (Zhang et al. 2018).

Therefore, an effective lignocellulosic pretreatment was proposed using subcritical CO<sub>2</sub>-assisted seawater pretreatment (SCSP) to improve the production of XOS and the enzymolysis efficiency in this study. We

systematically studied the effects of pretreatment temperature, CO<sub>2</sub> pressure and reaction time on the production of XOS, and analyzed the impact of CO<sub>2</sub> on the enzymatic hydrolysis of solid residues. The composition of various pretreated substrates, supernatant, as well as subsequent enzymatic hydrolysates and residues was investigated. The synergistic mechanism of subcritical CO<sub>2</sub> and inorganic ions in seawater was also studied. The co-production of functional oligosaccharides and fermentable sugars provides a promising method for the comprehensive utilization of SCB.

## Materials and methods

### Materials

SCB was obtained from Guitang Co., Ltd. (Guangxi, China). It was washed with tap water, then dried and ground to 40-mesh size, and finally stored in a sealed place at room temperature for further use. The chemical composition of SCB consisted of  $37.61 \pm 0.23\%$  glucan,  $21.87 \pm 0.17\%$  xylan,  $20.60 \pm 0.20\%$  acid-insoluble lignin (AIL) and  $2.30 \pm 0.22\%$  ash. The seawater was taken from Qingdao (Shandong, China), sterilized at 121 °C and passed through a 0.22- $\mu$ m filter, and stored at 4 °C for subsequent use.

All the quantitative standard products such as glucose, xylose (X<sub>1</sub>), xylobiose (X<sub>2</sub>), xylotriose (X<sub>3</sub>), xyloetraose (X<sub>4</sub>), xylopentaose (X<sub>5</sub>), furfural (FF), 5-hydroxymethylfurfural (HMF) and acetic acid (AA) were purchased from Sigma, USA. Cellulase purchased from Novozymes (Copenhagen, Denmark), and its filter paper activity was 145.2 U/mL, endo-1, 4- $\beta$ -D-glucanase activity and  $\beta$ -glucosidase activity were 3256.0 U/mL and 2774.2 IU/mL, respectively.

### Subcritical CO<sub>2</sub>-assisted seawater pretreatment of sugarcane bagasse

The SCSP of SCB was carried out in a 100 mL multifunctional stainless-steel reaction kettle (TGYF-C), which was automatically heated, stirred and timed. Based on the experiments we have studied, the pretreatment temperature was set as 135, 145, 155 and 165 °C, the reaction

**Table 1** L<sub>9</sub> (3<sup>3</sup>) factor and level of orthogonal experiment

Levels	A Pretreatment temperature (°C)	B CO <sub>2</sub> pressure (MPa)	C Reaction time (min)
1	145	1	5
2	155	2	20
3	165	3	35

time was set as 5, 20, 35 and 50 min, and the CO<sub>2</sub> pressure was 0, 1, 2 and 3 MPa (Zhang et al. 2020b). In addition, the yield of XOS as the objective, the L<sub>9</sub> (3<sup>3</sup>) orthogonal experiment was designed with the pretreatment temperature, CO<sub>2</sub> pressure and reaction time as factors (Table 1). The conditions for pretreatment were determined using the severity parameter (SP), defined according to Eq. (1):

$$\text{Glucose(\%)} = \frac{\text{Glucose after the enzymatic hydrolysis(g)} \times 0.9}{\text{Glucan in pretreated solid residues(g)}} \times 100\%. \quad (3)$$

$$\text{SP} = \log \left\{ \int_0^t P^n e^{\left(\frac{T-100}{14.75}\right)} dt \right\}, \quad (1)$$

where  $t$  is the reaction time (min),  $T$  is the pretreatment temperature (°C), 14.75 is an empirical parameter related to temperature and activation energy,  $P$  is the CO<sub>2</sub> pressure (MPa), and  $n$  is an arbitrary constant (0.849 for acidic medium).

Raw SCB (5 g) and seawater (50 mL) were successively added into the reactor tank, and then the kettle cover was covered. CO<sub>2</sub> was inserted into the gas inlet of the reactor, and the method was as follows. First, a small amount of gas was continuously injected for 2–3 min to eliminate the air in the reactor to ensure that the reactor was filled with CO<sub>2</sub>. Then, CO<sub>2</sub> gas was continuously injected to achieve the required pressure and the gas outlet was closed. Next, the switch of the reaction kettle was turned on. When the reaction was over, the kettle body was cooled to room temperature quickly with tap water, the kettle cover opened and CO<sub>2</sub> released rapidly. Finally, the resulting mixture was vacuumed and filtered to separate the solid and liquid components. The solid components were washed to neutral with excess deionized water and stored at 4 °C for subsequent cellulase hydrolysis and component analysis. The supernatant was stored at –20 °C for further analysis of XOS (X<sub>2</sub>–X<sub>5</sub>), monosaccharides (glucose and xylose) and by-products (FF and HMF). The XOS yield ( $Y$ ) was calculated as follows from Eq. (2):

$$Y(\%) = \frac{\text{XOS in the supernatant(g)}}{\text{Xylan in raw SCB(g)}} \times 100\%. \quad (2)$$

#### Enzymatic hydrolysis of the solid residues

Enzymatic hydrolysis experiments were carried out on the untreated SCB and the pretreated solid residue in a 50-mL centrifuge tube, which was placed in a vibrating

incubator at 48 °C and reacted at 150 rpm for 72 h. Substrate concentration was 5%, cellulase dosage was 30 FPU/g cellulose, and acetic acid–sodium acetate buffer (pH 4.8) was used as enzymatic hydrolysis system. Samples were taken at 4 and 72 h, and centrifuged to obtain supernatant for sugar analysis. The enzymatic hydrolysis residues were dried and stored for future analysis. The glucose yield (%) of pretreated solid residues after 72 h enzymatic hydrolysis is calculated as Eq. (3):

#### High-performance liquid chromatography analysis

According to the method of the National Renewable Energy Laboratory (NREL) (Sluiter et al. 2008), the chemical composition (glucan, xylan, and AIL) of SCB and solid residues under different pretreatment conditions was determined. The sugars of raw SCB and liquid after enzymatic hydrolysis were determined by high-performance liquid chromatography (HPLC) (Waters e2695, USA) system with an Aminex HPX-87P column (300 × 7.8 mm; Bio-Rad Laboratories, USA) at 85 °C with a refractive index detector (RID) at 35 °C, and ultra-pure water at 0.6 mL/min was used as the mobile phase. In addition, the concentrations of by-products (AA, FF, HMF) after pretreatment were measured by HPLC with an HPX-87H column (300 × 7.8 mm; Bio-Rad Laboratories, USA) at 65 °C, using 5 mM H<sub>2</sub>SO<sub>4</sub> as the eluent. Supernatant after SCSP was determined XOS content by a Sugar-Pak 1 column at 85 °C with ultra-pure water as the mobile phase at a flow rate of 0.5 mL/min. All liquids are filtered with the 0.22-μm filter before detection.

#### Characterization methods of raw materials and solid residues

Physical adsorption analysis was performed using an automatic specific surface characteristics analyzer (Autosorb-IQ, Canta, USA) to evaluate the specific surface area and pore size distribution characteristics of samples. Test type was mesoporous, adsorption gas was nitrogen (N<sub>2</sub>), temperature was 120 °C, and degassing time was 3 h. The specific surface area and pore size distribution of all samples were calculated by Brunauer–Emmett–Teller (BET) equation, and the average pore size and pore volume were obtained (Iruretagoyena et al. 2020).

Surface morphology was analyzed by scanning electron microscopy (SEM) (SU8010, Japan). The samples were treated with gold spray in a vacuum and attached to the electron microscope sample table. The acceleration voltage of the electron beam was 5 kV.



The crystallinity of SCB and pretreated substrate were examined by X-ray diffraction (XRD) analysis (Rigaku Ultima IV diffractometer, Japan). The test parameters were as follows: the scanning range was in a  $2\theta$  range between  $10^\circ$  and  $80^\circ$ , scanning speed was  $5^\circ/\text{min}$ , the scanning voltage and current was 40 kV and 40 mA. The crystallinity index (CrI) was calculated using Eq. (4):

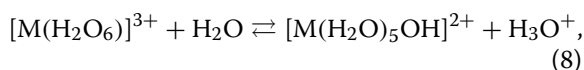
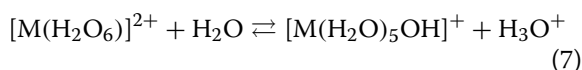
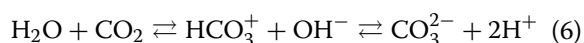
$$\text{CrI}(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100\%, \quad (4)$$

where  $I_{002}$  is the scattered intensity at a peak of around  $22.5^\circ$  and  $I_{am}$  is the scattered intensity of the amorphous portion around  $18.1^\circ$  (Segal et al. 1959).

## Results and discussion

### Reaction mechanisms of subcritical $\text{CO}_2$ -assisted seawater pretreatment on hemicellulose degradation

According to previous studies, the mechanism of  $\text{CO}_2$ -assisted seawater pretreatment was speculated as follows: during the hydrothermal pretreatment at high temperature, the water was ionized into hydrogen ions, which can act as catalysts in acidic hydrolysis reactions, leading to degradation and dissolution of hemicelluloses (Wu et al. 2021a). In addition, complex ions in seawater first penetrated the SCB, and metal ions such as  $\text{Mg}^{2+}$  and then acted as Lewis acids combined with hydronium ion to break the glycosidic bonds of xylan, with  $\text{Cl}^-$  further converting xylo-oligomers into short-chain xylo-oligomers, simultaneously  $\text{H}_2\text{CO}_3$  and acetic acid produced during the process assisted in this conversion (Zhang et al. 2020b).  $[\text{M}(\text{H}_2\text{O})_n]^{z+}$  is the generic formula of metal ions ligand complexes, where M is the metal ion, z is the oxidation state of the cation, and n is the solvation number usually ranging from 4 to 9. The metal cations can play the role of Lewis acids and facilitate the breakage of glycosidic linkages (C–O–C) (Loow et al. 2015; Kamireddy et al. 2013; Zhang et al. 2019). The whole process involved chemical reactions are as follows Eqs. (5)–(8):



where M is the metal ion.

**Table 2** The arrangement and results of orthogonal experiment

Number	A ( $^\circ\text{C}$ )	B (MPa)	C (min)	$Y^a$ (%)	SP
1	145	1	5	17.16	2.02
2	145	2	20	19.14	2.88
3	145	3	35	20.81	3.27
4	155	1	20	59.32	2.92
5	155	2	35	53.95	3.42
6	155	3	5	50.26	2.72
7	165	1	35	60.04	3.46
8	165	2	5	68.23	2.87
9	165	3	20	51.67	3.62
$K_1$	19.04	45.51	45.22		
$K_2$	54.51	47.11	43.38		
$K_3$	59.98	40.91	44.93		
R	45.45	2.65	3.23		

SP: severity parameter

<sup>a</sup> Y is the XOS ( $X_2 - X_1$ ) yield

### Orthogonal experimental results of XOS yield

The optimum pretreatment temperature ( $155^\circ\text{C}$ ),  $\text{CO}_2$  pressure (2 MPa) and reaction time (5 min) were determined by a single factor experiment (Additional file 1: Figs. S1–S3). Under this pretreatment condition, the yield of XOS reached 58.13%. In order to optimize the pretreatment conditions and obtain a higher XOS yield, the orthogonal experiment was carried out with XOS yield as the index.

As can be seen from the data in Table 2, the primary and secondary factors affecting the yield of XOS were  $A > C > B$ . That was, pretreatment temperature had the most significant influence on XOS yield, followed by reaction time and  $\text{CO}_2$  pressure. Meanwhile, the optimal pretreatment process was  $A_3B_2C_1$  ( $165^\circ\text{C}$ -2 MPa-5 min) according to K value, under which the yield of XOS was 68.23%.

Table 2 shows the SP of reaction conditions for SCSP. Different from our previous exploration of seawater hydrothermal pretreatment (Zhang et al. 2020b), the addition of  $\text{CO}_2$  in this study was a crucial factor for XOS production. It can be found that  $\text{CO}_2$  pressure effectively reduces the SP under the condition of obtaining a similar XOS yield. For example, in the SCSP process, when the yields of XOS were 59.32% ( $155^\circ\text{C}$ -1 MPa-20 min) and 68.23% ( $165^\circ\text{C}$ -2 MPa-5 min), respectively. The corresponding SP values were 2.92 and 2.87, respectively. However, in the seawater hydrothermal pretreatment process, the XOS yields were 60.58% ( $165^\circ\text{C}$ -70 min) and 67.12% ( $175^\circ\text{C}$ -30 min), the corresponding SP values were 3.76 and 3.68, respectively (Additional file 1:

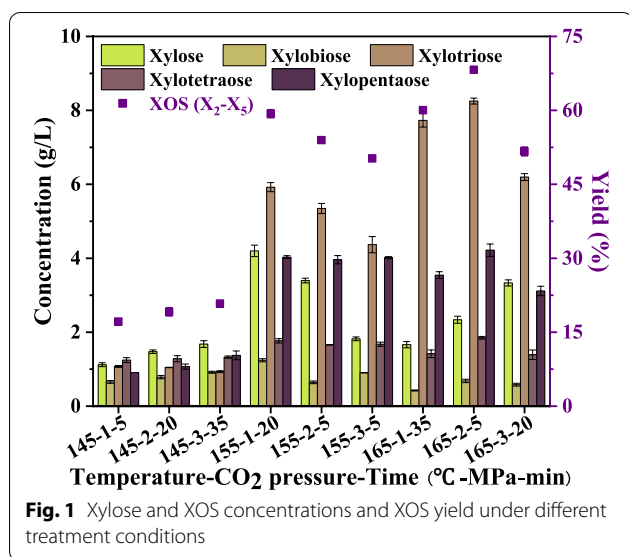


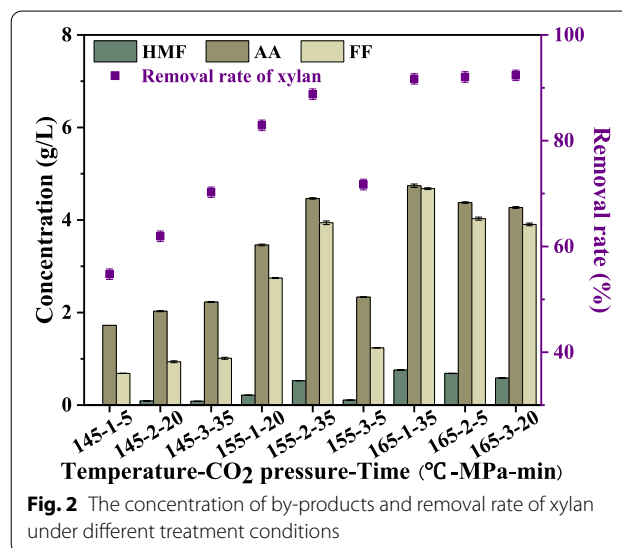
Table S1). In other words, under the same pretreatment temperature and reaction time, CO<sub>2</sub> will promote the degradation of more hemicellulose to XOS, which significantly improves the yield of XOS.

#### Effects of different pretreatment conditions on XOS and by-products

Subcritical CO<sub>2</sub>-assisted seawater pretreatment, breaks down the linkages among hemicellulose, cellulose, and lignin of the SCB and releases or degrades the hemicellulose firstly (Liu et al. 2019). Pretreatment temperature, CO<sub>2</sub> pressure and reaction time have different effects on the degradation of hemicellulose into XOS with different degrees of polymerization. The degree of polymerization of XOS varies under different pretreatment conditions. Still, the concentration of X<sub>3</sub> was higher (Fig. 1), which inferred that the X<sub>3</sub> was more stable and easier to form in these conditions, consistent with the study of Liu et al. (2019). When the pretreatment temperature was 145 °C, the concentration of XOS and xylose in the supernatant was generally low. With the increase of CO<sub>2</sub> pressure and reaction time, the concentration of XOS and xylose increased, but the increasing trend was steady. However, when the pretreatment temperature was increased to 155 °C and 165 °C, the concentration of XOS increased significantly. When XOS (X<sub>2</sub>–X<sub>5</sub>) yield was 59.32% (155 °C-1 MPa-20 min), this was consistent with the XOS yield under the same pretreatment conditions in the single factor experiment (Additional file 1: Fig. S2). Under the optimal condition (165 °C-2 MPa-5 min), the yield of XOS (X<sub>2</sub>–X<sub>5</sub>) was 68.23%, the concentrations of X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub> and X<sub>5</sub> were 0.68 g/L, 8.25 g/L, 1.85 g/L and 4.22 g/L, respectively.

In addition, the influences of different solvents (seawater and freshwater) on the production of XOS in the pretreatment system were compared (Additional file 1: Fig. S5). It could be found that the yield of XOS in the seawater system was significantly higher than that in the freshwater system. The difference in XOS (X<sub>2</sub>–X<sub>5</sub>) concentration was slight (0.48 g/L) at a lower temperature (145 °C) but significant (5.84 and 8.28 g/L) at higher temperatures (155 and 165 °C). In summary, it was proved once again that temperature was the most critical factor affecting the yield of XOS. In other words, the pretreatment must reach a specific temperature to obtain XOS with higher concentration and yield. The XOS yield in the seawater system increased by 37.88% compared with the freshwater system (165 °C-2 MPa-5 min). The results showed that the synergistic effect of seawater and CO<sub>2</sub> was more substantial than that of freshwater and CO<sub>2</sub>. This phenomenon means that the ions in seawater and CO<sub>2</sub> have an excellent synergistic effect.

SP values and by-products (HMF, AA, and FF) concentrations indirectly reflected the intensity of pretreatment conditions (Table 2 and Fig. 2). The concentration of by-products and the removal rate of xylan were closely related to SP values. At the same temperature, the removal rate of xylan increased with the increase of SP value. For example, when the SP value increased from 2.02 to 3.27, the removal rate of xylan rose from 50.76% to 70.26%, and the yield of XOS increased from 17.16 to 20.81% at 145 °C. HMF and FF are degradation products of glucose and xylose. Under all pretreatment conditions, the concentration of HMF was lower than that of FF, indicating that xylose was more easily degraded (Fig. 1 and Fig. 2). In addition, the removal rate of xylan increased gradually with the deepening of pretreatment conditions,



**Table 3** The three components yield of sugarcane bagasse after SCSP of orthogonal experiment

A (°C)	B (MPa)	C (min)	Glucan <sup>a</sup> (%)	Xylan <sup>b</sup> (%)	AIL <sup>c</sup> (%)
145	1	5	51.10 ± 0.29	13.56 ± 0.06	19.70 ± 3.97
	2	20	52.65 ± 0.10	11.68 ± 0.02	25.00 ± 0.67
	3	35	53.32 ± 0.59	9.60 ± 0.54	25.80 ± 0.80
155	1	20	58.17 ± 0.75	5.85 ± 0.02	26.64 ± 1.36
	2	35	56.96 ± 0.58	3.91 ± 0.03	31.80 ± 2.73
	3	5	53.07 ± 2.76	9.55 ± 0.40	24.97 ± 1.23
165	1	35	57.32 ± 0.68	2.95 ± 0.03	28.64 ± 4.16
	2	5	56.29 ± 0.20	2.85 ± 0.03	32.40 ± 0.87
	3	20	56.91 ± 0.45	2.75 ± 0.02	30.97 ± 2.64

All values are mean ± standard deviation of two replicate determinations.

<sup>a</sup> Glucan yield = (glucan in solid residues (%) × solid residue (g)/glucan in raw substrate (g)) × 100%

<sup>b</sup> Xylan yield = (xylan in solid residues (%) × solid residue (g)/xylan in raw substrate (g)) × 100%

<sup>c</sup> AIL yield = (AIL in solid residues (%) × solid residue (g)/AIL in raw substrate (g)) × 100%

and the maximum xylan removal rate reached 92.35% (165 °C-3 MPa-20 min).

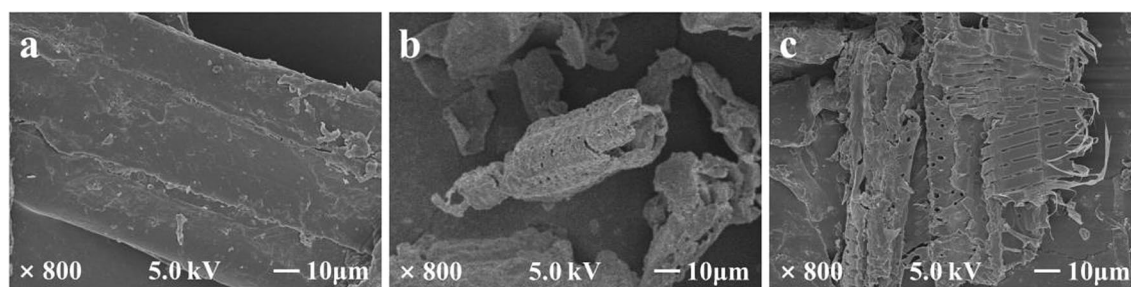
To some extent, the composition of solid residues after SCSP also explained the degradation sequence of lignin, cellulose and hemicellulose (Table 3). With the increase of pretreatment temperature, the xylan content in the solid residue decreased gradually. For example, an increase in pretreatment temperature from 135 °C to 165 °C resulted in a reduction in xylan content from 19.75% to 3.38%. But for the other two conditions (CO<sub>2</sub> pressure and reaction time), the reduction in xylan content was to a lesser extent (Additional file 1: Table S2). When CO<sub>2</sub> pressure increased from 1 to 3 MPa, xylan content decreased only 3.71%; the reaction time was extended from 5 to 50 min, and the xylan content decreased by 3.63%. This result indicated that compared with the pretreatment temperature, the degradation effect of CO<sub>2</sub> pressure and reaction time on xylan was weaker, consistent with the above conclusion (Table 2).

In conclusion, the results of single factor and orthogonal experiments showed that the X<sub>3</sub> content accounted for the largest proportion of XOS under most SCSP processes. However, the X<sub>4</sub> content accounted for the largest proportion of XOS in the seawater hydrothermal pretreatment process. The results showed an internal relationship between CO<sub>2</sub> and mixed ions in seawater, which can better promote the transformation of XOS from a high degree of polymerization to a low degree of polymerization. Previous study has shown that XOS with average DP of 3–4 has better prebiotic effect than XOS with DP of 5–6 (Moura et al. 2007), which further proves the advantage of SCSP.

### Characterization of raw materials and pretreated solid residues

The morphological structure of raw SCB and pretreated solid residues (seawater hydrothermal pretreatment and SCSP) were displayed by SEM (Fig. 3). Raw SCB showed a smooth, complete, solid, continuous, flat surface and highly fibrillary state. However, due to the degradation of hemicellulose and lignin and the decomposition between lignin and carbohydrates, the pretreated SCB structure showed separation and disorder, and the surface became rough. After pretreatment, many large and small holes appeared on the SCB surface, especially in the solid residue by SCSP. This phenomenon can be explained as follows: CO<sub>2</sub> increased the pressure of the reaction system; CO<sub>2</sub> gas and water reacted to generate H<sub>2</sub>CO<sub>3</sub> and penetrated into the internal structure of SCB. After the reaction finished, rapid decompression made CO<sub>2</sub> gas released from SCB and expanded the pores on the fiber surface, and made the pretreated SCB have a higher pore area and porosity (Ge et al. 2020). SCSP removed a large amount of hemicellulose and part of lignin, destroyed the internal structure or carbohydrate composite structure of SCB, loosened the surface structure, and leaked more cellulose for the accessibility of enzymes (Ge et al. 2020; Huang et al. 2018; Lin et al. 2020).

The BET surface area, micropore surface area, total pore volume, and average pore size of the SCB and



**Fig. 3** SEM of SCB before and after pretreatment. **a** Raw SCB, **b** 175 °C-30 min, **c** 155 °C-1 MPa-20 min

**Table 4** BET surface area, total pore volume and average pore size of SCB and solids pretreated by different processes

Samples	BET surface area (m <sup>2</sup> /g)	Total pore volume (cm <sup>3</sup> /g)	Average pore size (nm)
Raw SCB	2.47	0.01	4.74
175 °C-30 min	5.72	0.02	19.30
155 °C-1 MPa-20 min	7.88	0.03	32.49

solids pretreated by different pretreatment processes are shown in Table 4. The BET surface area of SCB after seawater hydrothermal pretreatment (175 °C-30 min) increased from 2.47 to 5.72 m<sup>2</sup>/g, and the average pore size increased from 4.74 to 19.30 nm. After SCSP (155 °C-1 MPa-20 min), the BET surface area increased by 218.57% (from 2.47 m<sup>2</sup>/g to 7.88 m<sup>2</sup>/g), the total pore volume increased by 200% (from 0.01 cm<sup>3</sup>/g to 0.03 cm<sup>3</sup>/g), and the average pore diameter increased by 585.44% (from 4.74 nm to 32.49 nm). The experimental results were consistent with the above SEM phenomena. It was proved that CO<sub>2</sub> gas with a certain pressure increased the porosity of SCB and was more conducive to the penetration of inorganic salts, thus promoting the reaction of XOS production. At the same time, the contact area between cellulase and substrate was increased in subsequent enzymatic hydrolysis.

XRD analysis was performed for the untreated and pretreated SCB to study the crystallinity index (CrI) from the diffraction patterns. The changes of the crystallization region after pretreatment can be seen in

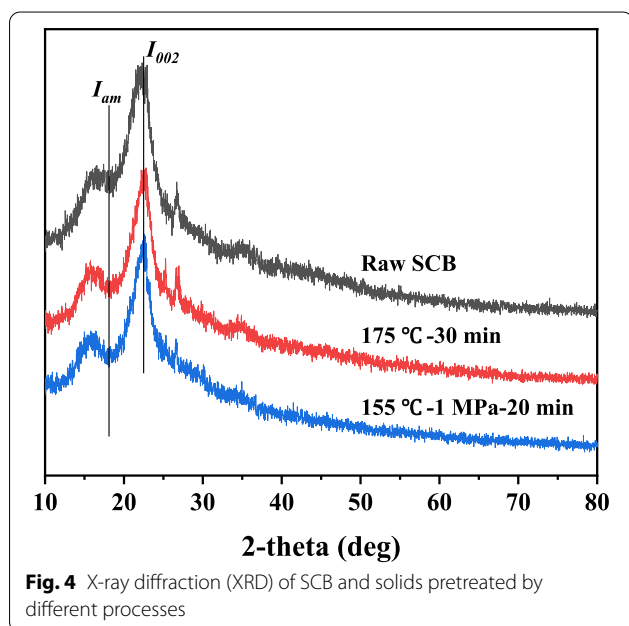
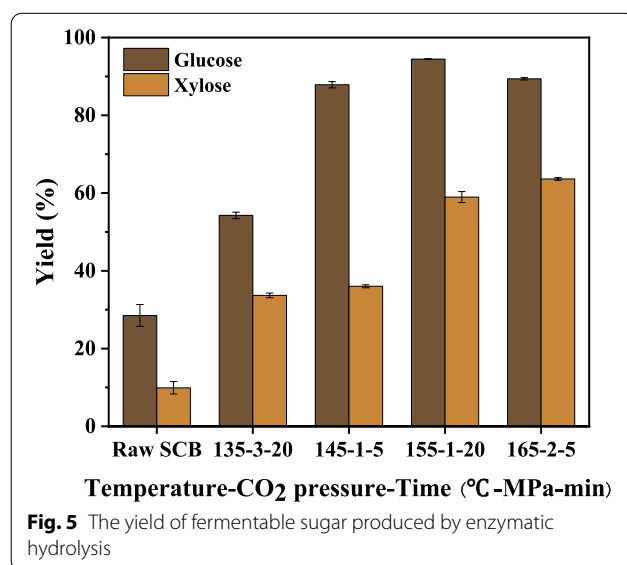


Fig. 4. In the pretreatment process, two factors determine the crystallinity of pretreated SCB: decrystallizing by swelling and dissolving the crystalline fraction of cellulose, and increasing CrI by reducing amorphous cellulose, lignin and hemicellulose (Chourasia et al. 2021). CrI calculated for untreated SCB was found to be 36%, while seawater hydrothermal pretreated SCB was 52%, and SCSP pretreated SCB was 61%. The addition of CO<sub>2</sub> in the reaction process made more hemicellulose removed. Therefore, the results showed an increase in CrI after pretreatment implying removal of amorphous fractions of SCB. These results thus showed the consistency with SCB composition analysis, SEM and BET results before and after treatment.

**Effects of different pretreatment conditions on enzymatic hydrolysis**

It has been proved that SCSP can reduce the reaction conditions and produce high-yield XOS. At the same time, in order to make better use of the solid residue after pretreatment, the cellulase hydrolysis experiment of the solid residue under partial pretreatment conditions was carried out. The presence of CO<sub>2</sub> increased the pores in the solid residue, to increase the specific surface area of contact between cellulase and cellulose. Moreover, the pretreated solid residue was rich in cellulose, and more glucose products can be obtained during enzymatic hydrolysis and thus, the utilization rate of solid residue was increased.

The results of enzymatic hydrolysis of solid residues are shown in Fig. 5. SCSP process improved the yield of enzymatic hydrolysis of glucose. For example, the glucose yield of raw SCB was 28.52% without any treatment, while the highest glucose yield of pretreated residue is 94.45%





(155 °C-1 MPa-20 min). In addition, it had been proved from the above discussion that the temperature was the main factor affecting the yield of XOS, so the temperature was selected as a variable to pretreat the residue for enzymatic hydrolysis. It can be seen that the glucose yield was different at different temperatures, but there was an obvious boundary (145 °C). Before this boundary, the temperature had a significant effect on the glucose yield, and the influence decreased after this boundary. For example, when the pretreatment temperature was 135 °C, although the CO<sub>2</sub> pressure was high (3 MPa) and the reaction time was long (20 min), the yield of glucose was still low (54.26%); when the pretreatment temperature increased to 145 °C, the CO<sub>2</sub> pressure was 1 MPa and the reaction time was 5 min, the yield of glucose increased significantly (87.86%); the increase of glucose yield was not evident when the temperature continued to grow. The appropriate pretreatment temperature was the key factor to improve the yield of glucose. In other words, only after the pretreatment reaches a specific temperature can a large amount of hemicellulose and some lignin be removed and more cellulose is exposed, to promote the enzymatic hydrolysis reaction (Liu et al. 2019). Similarly, the changing trend of xylose yield was similar to that of glucose. However, the xylose yield of enzymatic hydrolysis of solid residue was generally low. Most of the hemicellulose was degraded after pretreatment, and few can be retained in the solid residue for enzymatic hydrolysis. In summary, SCSP can substantially increase the cellulolytic digestibility of SCB and increase the yield of glucose (Zhang et al. 2017).

### Competitive advantage analysis of subcritical CO<sub>2</sub>-assisted seawater pretreatment

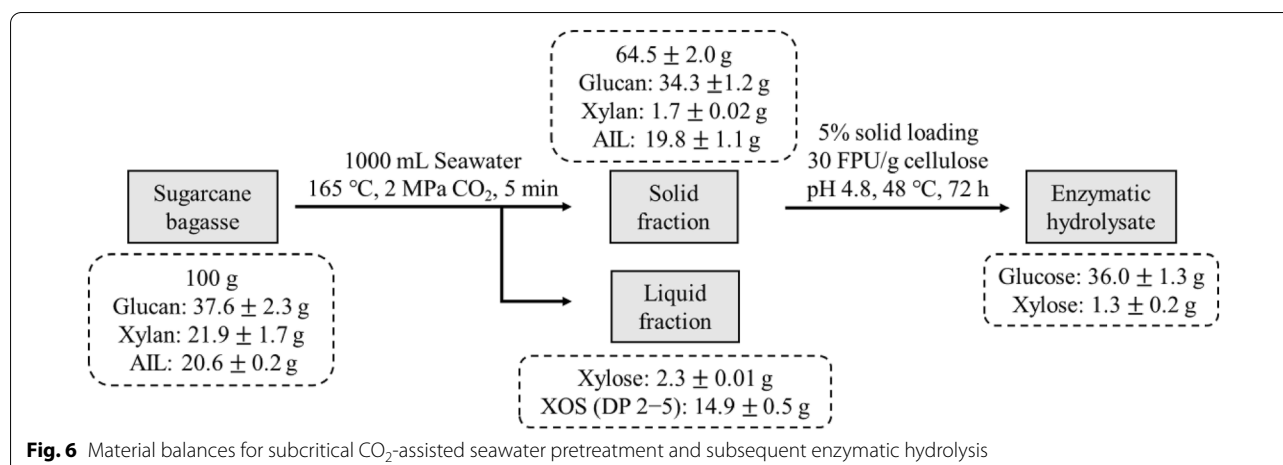
Table 5 shows the comparison of XOS and fermentable sugars produced by some pretreatment methods. It can be seen that the yield of XOS and fermentable sugar produced is different due to different raw materials and pretreatment methods. SCSP can produce XOS with high yield at lower temperature and shorter reaction time compared with other methods. Similarly, the type of enzyme and enzymatic hydrolysis conditions also affect the yield of fermentable sugars (relative to the content of glucan/xylose in the raw material). Compared with the other results, through the ingenious coupling of seawater and CO<sub>2</sub>, this study achieved a higher XOS yield under lower pretreatment conditions (165 °C-2 MPa-5 min), promoted the subsequent enzymatic hydrolysis reaction and obtained 94.45% glucose.

### Material balance at optimal pretreatment conditions

In this study, XOS and fermentable sugars were co-produced efficiently by SCSP and enzymatic hydrolysis. Figure 6 summarizes the material balance based on 100 g dry SCB under the optimum conditions. From 100 g of dry raw SCB (comprising 37.6 g glucan, 21.9 g xylan, and 20.6 g AIL), 64.5 g of SCB containing 34.3 g glucan, 1.7 g xylan and 19.8 g AIL were recovered by SCSP, which indicated most of hemicellulose were degraded. At the same time, 2.3 g xylose and 14.9 g XOS (DP 2–5) were released from the pretreatment liquid. After enzymatic hydrolysis, about 36.0 g glucose and 1.3 g xylose were recovered. The

**Table 5** XOS yield and enzymatic hydrolysis performance under different pretreatment strategies

Raw material	Pretreatment conditions	XOS yield	Enzyme	Enzymatic hydrolysis conditions	Yield	Ref
Poplar sawdust	6.5% acetic acid, 170 °C, 27 min	(DP 2–6) 36.0%	C2730, Celluclast® 1.5 L	50 °C, pH 4.8, 20 FPU/g cellulose, 108 h	Glucose: 51.0%	Huang et al. (2018)
Poplar	1% H <sub>2</sub> SO <sub>4</sub> delignification; 2% lactic acid, 170 °C, 30 min	(DP 2–6) 42.7%	Cellulase (Cellic CTec2)	50 °C, pH 5.0, 10 mg cellulase protein/g dry matter, 72 h	Glucose: 88.9%; xylose: 66.2%	Yang et al. (2019)
SCB: white birch=(65:35)	Autohydrolysis; 200 °C, 10 min	(DP 2–5) 52.99%	Cellulase (Cellic CTec2)	50 °C, pH 4.8, 18 FPU/g cellulose, 72 h	Glucose: 84.74%; xylose: 72.54%	Zhang et al. (2018)
SCB	0.1 M MgCl <sub>2</sub> , 180 °C, 10 min	(DP 2–5) 53.79%	Cellulase (Cellic CTec2)	50 °C, pH 4.8, 18 FPU/g cellulose, 72 h	Glucose: 71.62%; xylose: 66.75%	Zhang et al. (2019)
Corn straw	5 MPa CO <sub>2</sub> , 170 °C, 40 min	(DP 2–5) 40%	Commercial cellulase	50 °C, pH 4.8, 20 FPU/g cellulose, 72 h	Glucose: 90.2%; xylose: 81.7%	(Liu et al. 2019)
SCB	Seawater hydrothermal pretreatment; 165 °C, 2 MPa CO <sub>2</sub> , 5 min	(DP 2–5) 68.23%	Commercial cellulase	48 °C, pH 4.8, 30 FPU/g cellulose, 72 h	Glucose: 94.45%	This study



yield of glucose was 94.45%, accounting for 86.17% of the glucose content in the raw SCB. The results showed that a small amount of sugar was lost during pretreatment and washing, most of the sugar was retained.

## Conclusions

In this study, SCSP was explored for transforming SCB into XOS and fermentable sugar. Seawater, the only reaction solvent, saves freshwater resources. A small amount of subcritical CO<sub>2</sub> reduces the pretreatment temperature and reaction time, thus reducing the cost. The process is green and pollution-free without adding any chemical reagent, and the highest yield of XOS is 68.23% (165 °C-2 MPa-5 min), and the highest yield of glucose after enzymatic hydrolysis is 94.45%. The combined production of XOS and glucose realizes the high-value utilization of SCB. Future work can further study the full utilization of lignocellulosic biomass and expand the application range of each component.

## Abbreviations

AA: Acetic acid; AIL: Acid-insoluble lignin; FF: Furfural; HMF: 5-Hydroxymethylfurfural; LCC: Lignin-carbohydrate complex; SCB: Sugarcane bagasse; SCSP: Subcritical CO<sub>2</sub>-assisted seawater pretreatment; X<sub>1</sub>: Xylose; X<sub>2</sub>: Xylobiose; X<sub>3</sub>: Xylotriose; X<sub>4</sub>: Xylotetraose; X<sub>5</sub>: Xylopentaose; XOS: Xylo-oligosaccharide.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40643-022-00525-3>.

**Additional file 1: Fig. S1.** Effect of pretreatment temperature on the yield (a) and concentration (b) of xylose and XOS. **Fig. S2.** Effect of CO<sub>2</sub> pressure on the yield (a) and concentration (b) of xylose and XOS. **Fig. S3.** Effect of reaction time on the yield (a) and concentration (b) of xylose and XOS. **Fig. S4.** Effect of different conditions on the concentration of by-products. **Fig. S5.** Effect of different solvents (seawater and freshwater) on the yield and concentration of XOS. **Table S1.** The severity parameter (SP) about seawater hydrothermal pretreatment. **Table S2.** The three components yield of sugarcane bagasse after subcritical CO<sub>2</sub>-assisted seawater pretreatment of single factor experiments.

## Acknowledgements

Not applicable.

## Authors' contributions

LZ and XZ designed the experiment and carried out all of the experiments. LZ calculated all data and drafted the manuscript. FL provided supervision and carried out a part of data analysis. JJ and LJ supervised the experiments, reviewed and revised the manuscript. All authors read and approved the final manuscript.

## Funding

This work was financially supported by the National Key R&D Program of China (2019YFD1002404) and the Guangxi Key Laboratory of Chemistry Engineering of Forest Products (GXFK2002/2005).

## Availability of data and materials

Not applicable.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All the authors agreed to submit the manuscript in this journal.

### Competing interests

The authors declare that there is no competing interest.

### Author details

<sup>1</sup>Department of Chemistry and Chemical Engineering, MOE Engineering Research Center of Forestry Biomass Materials and Bioenergy, Beijing Forestry University, Beijing 100083, China. <sup>2</sup>Key Laboratory of Chemistry and Engineering of Forest Products, State Ethnic Affairs Commission, Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Nanning 530006, China.

Received: 15 December 2021 Accepted: 15 March 2022

Published online: 28 March 2022

## References

Ajala EO, Ighalo JO, Ajala MA, Adeniyi AG, Ayanshola AM (2021) Sugarcane bagasse: a biomass sufficiently applied for improving global energy, environment and economic sustainability. *Bioresour Bioprocess* 8:87

- Chourasia VR, Pandey A, Pant KK, Henry RJ (2021) Improving enzymatic digestibility of sugarcane bagasse from different varieties of sugarcane using deep eutectic solvent pretreatment. *Bioresour Technol* 337:125480
- de Freitas C, Carmona E, Brienza M (2019) Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. *Bioact Carbohydr Diet Fibre* 18:100184
- Ge S, Wu Y, Peng W, Xia C, Mei C, Cai L, Shi SQ, Sonne C, Lam SS, Tsang YF (2020) High-pressure CO<sub>2</sub> hydrothermal pretreatment of peanut shells for enzymatic hydrolysis conversion into glucose. *Chem Eng J* 385:123949
- Gong Z, Yang G, Song J, Zheng P, Liu J, Zhu W, Huang L, Chen L, Luo X, Shuai L (2021) Understanding the promoting effect of non-catalytic protein on enzymatic hydrolysis efficiency of lignocelluloses. *Bioresour Bioprocess* 8:9
- Gurgel LVA, Pimenta MTB, da Silva Curvelo AA (2014) Enhancing liquid hot water (LHW) pretreatment of sugarcane bagasse by high pressure carbon dioxide (HP-CO<sub>2</sub>). *Ind Crops Prod* 57:141–149
- Huang K, Luo J, Cao R, Su Y, Xu Y (2018) Enhanced xylooligosaccharides yields and enzymatic hydrolyzability of cellulose using acetic acid catalysis of poplar sawdust. *J Wood Chem Technol* 38:371–384
- Ire FS, Ezebuio V, Ogugbue CJ (2016) Production of bioethanol by bacterial co-culture from agro-waste-impacted soil through simultaneous saccharification and co-fermentation of steam-exploded bagasse. *Bioresour Bioprocess* 3:26
- Irrettagoyena D, Bikane K, Sunny N, Lu H, Kazarian SG, Chadwick D, Pini R, Shah N (2020) Enhanced selective adsorption desulfurization on CO<sub>2</sub> and steam treated activated carbons: Equilibria and kinetics. *Chem Eng J* 379:122356
- Jaichakan P, Nakphaichit M, Rungchang S, Weerawatanakorn M, Phongthai S, Klangpetch W (2021) Two-stage processing for xylooligosaccharide recovery from rice by-products and evaluation of products: promotion of lactic acid-producing bacterial growth and food application in a high-pressure process. *Food Res Int* 147:110529
- Jiang Z, Budarin VL, Fan J, Remón J, Li T, Hu C, Clark JH, (2018) Sodium chloride-assisted depolymerization of xylo-oligomers to xylose. *ACS Sustain Chem Eng* 6:4098–4104
- Kamireddy SR, Li J, Tucker M, Degenstein J, Ji Y (2013) Effects and mechanism of metal chloride salts on treatment and enzymatic digestibility of corn stover. *Ind Eng Chem Res* 52:1775–1782
- Liao H, Li X, Lian Z, Xu Y, Zhang J (2021a) Two-step acetic acid/sodium acetate and xylanase hydrolysis for xylooligosaccharides production from corn-cob. *Bioresour Technol* 342:125979
- Liao H, Xu Y, Zhang J (2021b) Efficient production of xylooligosaccharides and fermentable sugars from corncob by propionic acid and enzymatic hydrolysis. *Bioresour Technol* 342:125680
- Lin W, Xing S, Jin Y, Lu X, Huang C, Yong Q (2020) Insight into understanding the performance of deep eutectic solvent pretreatment on improving enzymatic digestibility of bamboo residues. *Bioresour Technol* 306:123163
- Liu X, Wei W, Wu S (2019) Subcritical CO<sub>2</sub>-assisted autohydrolysis for the co-production of oligosaccharides and fermentable sugar from corn straw. *Cellulose* 26:7889–7903
- Loow YL, Wu TY, Tan KA, Lim YS, Slow LF, Jahim JM, Mohammad AW, Teoh WH (2015) Recent advances in the application of inorganic salt treatment for transforming lignocellulosic biomass into reducing sugars. *J Agric Food Chem* 63:8349–8363
- Moura P, Barata R, Carvalho F, Gírio F, Loureiro-Dias MC, Esteves MP (2007) In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium* and *Lactobacillus* strains. *LWT* 40:963–972
- Ni J, Di J, Ma C, He Y (2021) Valorisation of corncob into furfuryl alcohol and furoic acid via chemoenzymatic cascade catalysis. *Bioresour Bioprocess* 8:113
- Nuntawat K, Bhagavathi SS, Sasithorn S, Narissara L, Sartjin P, Chaiyavat C (2018) Optimization of alkaline pretreatment and enzymatic hydrolysis for the extraction of xylooligosaccharide from rice husk. *AMB Expr* 8:115
- Park C, Lee J (2020) Recent achievements in CO<sub>2</sub>-assisted and CO<sub>2</sub>-catalyzed biomass conversion reactions. *Green Chem* 22(9):2628–2642
- Segal L, Creely JJ, Martin AE Jr, Conrad CM (1959) Crystallinity of native cellulose using the X-ray diffractometer. *Text Res J* 29:786–794
- Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Determination of structural carbohydrates and lignin in biomass. *Lab Anal Proced* 1617:1–16
- Valladares-Diestra KK, de Souza Porto Vandenberghe L, Soccol CR, (2021) A biorefinery approach for enzymatic complex production for the synthesis of xylooligosaccharides from sugarcane bagasse. *Bioresour Technol* 333:125174
- Wu R, Liu W, Li L, Jiang C, Hou Q (2021a) Combination of hydrothermal and chemi-mechanical pretreatments to enhance enzymatic hydrolysis of poplar branches and insights on cellulase adsorption. *Bioresour Technol* 342:126024
- Wu Y, Guo H, Rahman MS, Chen X, Zhang J, Liu Y, Qin W (2021b) Biological pretreatment of corn stover for enhancing enzymatic hydrolysis using *Bacillus* sp. P3. *Bioresour Bioprocess* 8:92
- Xiao X, Wen J, Wang Y, Bian J, Li M, Peng F, Sun R (2018) NMR and ESI-MS spectrometry characterization of autohydrolysis xylo-oligosaccharides separated by gel permeation chromatography. *Carbohydr Polym* 195:303–310
- Yang Q, Ying W, Wen P, Zhu J, Xu Y, Zhang J (2021) Delignification of poplar for xylo-oligosaccharides production using lactic acid catalysis. *Bioresour Technol* 342:125943
- You Y, Zhang X, Li P, Lei F, Jiang J (2020) Co-production of xylooligosaccharides and activated carbons from *Camellia oleifera* shell treated by the catalysis and activation of zinc chloride. *Bioresour Technol* 306:123131
- Zhang H, Xu Y, Yu S (2017) Co-production of functional xylooligosaccharides and fermentable sugars from corncob with effective acetic acid prehydrolysis. *Bioresour Technol* 234:343–349
- 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
- Zhang W, Zhang X, Lei F, Jiang J (2020a) Co-production bioethanol and xylooligosaccharides from sugarcane bagasse via autohydrolysis pretreatment. *Renew Energy* 162:2297–2305
- Zhang W, Lei F, Li P, Zhang X, Jiang J (2019) Co-catalysis of magnesium chloride and ferrous chloride for xylo-oligosaccharides and glucose production from sugarcane bagasse. *Bioresour Technol* 291:121839
- Zhang X, Zhang W, Lei F, Yang S, Jiang J (2020b) Coproduction of xylooligosaccharides and fermentable sugars from sugarcane bagasse by seawater hydrothermal pretreatment. *Bioresour Technol* 309:123385
- Zhang F, Lan W, Li Z, Zhang A, Tang B, Wang H, Wang X, Ren J, Liu C (2021a) Co-production of functional xylo-oligosaccharides and fermentable sugars from corn stover through fast and facile ball mill-assisted alkaline peroxide pretreatment. *Bioresour Technol* 337:125327
- Zhang L, Zhang W, Zhang F, Jiang J (2021b) Xylo-oligosaccharides and lignin production from *Camellia oleifera* shell by malic acid hydrolysis at mild conditions. *Bioresour Technol* 341:125897
- Zhu J, Zhang H, Jiao N, Xiao Y, Shi D, Xu Y (2021) A green process for producing xylooligosaccharides from poplar: Endoxylanase assisted autohydrolysis, activated carbon separation, and spent liquor for rice growth. *Ind Crops Prod* 174:114187

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)