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Xylo-oligosaccharide oriented biorefining of corncob through citric acid/sodium citrate buffer hydrolysis and enzymatic upgrading

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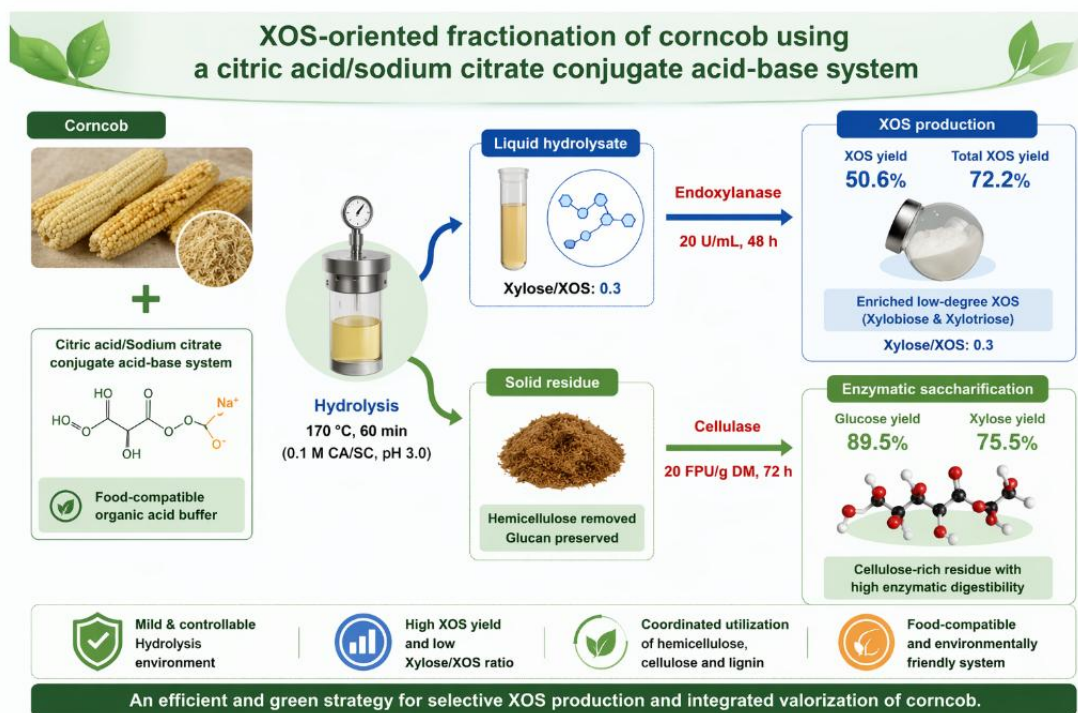
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Abstract

Selective production of xylo-oligosaccharides (XOS) from corncob is hindered by secondary degradation during acid hydrolysis. Here, a citric acid/sodium citrate (CA/SC) conjugate acid-base system was developed for XOS-oriented fractionation of corncob. Compared with the citric acid hydrolysis, the optimal CA/SC pretreatment increased the XOS yield from 44.3% to 50.5%, indicating a more favorable hydrolysis environment for XOS accumulation. Subsequent endoxylanase hydrolysis further upgraded residual high-degree soluble oligomers and increased the XOS yield to 60.2%, with clear enrichment of xylobiose and xylotriose. Meanwhile, the pretreatment selectively removed hemicellulose while largely preserving glucan, producing cellulose-rich solid residues with enhanced enzymatic digestibility. Cellulase hydrolysis gave glucose and xylose yields of 89.5% and 75.5%, respectively. Mass balance based on 1000 g of raw corncob gave 185.4 g XOS, 329.2 g glucose, and a lignin-rich final residue, demonstrating the potential of the CA/SC system for integrated corncob valorization.

Keywords: xylan, citric acid, endoxylanase, monosaccharides, lignin residue

Graphical Abstract



1 Introduction

Xylo-oligosaccharides (XOS) are widely recognized as high-value functional oligosaccharides because of their prebiotic and health-promoting effects [1, 2]. They are mainly produced through the hydrolysis of xylan, the major hemicellulose component in lignocellulosic biomass. Corncob is an attractive feedstock for XOS production because of its high xylan content [3, 4]. However, the key challenge in XOS production is not simply promoting xylan depolymerization, but controlling the reaction so that xylan is converted preferentially into XOS rather than being excessively degraded into xylose, furfural, and other by-products. Such secondary degradation not only lowers XOS yield and selectivity, but also increases the complexity and cost of downstream purification.

At present, both enzymatic and acid hydrolysis are used for XOS production [5-7]. Enzymatic routes are relatively selective, but they usually require prior xylan extraction and purification, which increases process complexity and cost [8, 9]. Acid hydrolysis is simpler and faster, but mineral acids often cause equipment corrosion and severe secondary degradation of XOS, resulting in low selectivity and high by-product formation [6, 10, 11]. Organic acids provide a milder alternative and generally produce fewer inhibitors than inorganic acids, but controlling over-hydrolysis during acid treatment remains a major challenge for selective XOS production.

One feasible way to improve selectivity is to use organic acid buffer systems [12, 13]. Previous studies have shown that buffered hydrolysis can moderate proton release, suppress excessive degradation of XOS, and reduce furfural and 5-hydroxymethylfurfural formation, thereby improving XOS production [14-16]. Among the available organic acids, citric acid is particularly attractive because it is

inexpensive, biodegradable, and widely used in food and feed applications [17, 18]. Despite these advantages, the use of a citric acid/sodium citrate buffer system for direct XOS production from corncob has been rarely reported.

In this study, a citric acid/sodium citrate buffer system was applied to hydrolyze corncob for XOS production, followed by endoxylanase treatment to further regulate the oligomer distribution. The effects of hydrolysis time, temperature, buffer concentration, and pH on XOS formation and by-product generation were systematically investigated, with particular emphasis on reducing secondary degradation and improving XOS yield and selectivity. Changes in the chemical composition and structure of the solid residue were also characterized, and its enzymatic digestibility was evaluated. This work aims to provide a simple and potentially cost-effective route for selective XOS production from corncob, while reducing downstream separation and purification demand and enabling more complete utilization of corncob carbohydrates.

2 Material and Method

2.1 Materials

Corn cob was obtained from Dongtai, Jiangsu Province, China. The raw material was milled and sieved, and the fraction passing through a 40-mesh sieve, corresponding to a particle size of no more than 0.425 mm, was collected for subsequent experiments. The initial chemical composition of the corncob was determined in duplicate according to the standard analytical procedures of the National Renewable Energy Laboratory (NREL) [19]. The corncob mainly consisted of glucan (33.0%), xylan (30.8%), acid-insoluble lignin (13.3%), and acid-soluble lignin (3.9%).

Cellulase (Cellic® CTec2) was provided by Novozymes A/S (Denmark). Its filter

paper unit (FPU) activity and protein content were quantified as 123 FPU/mL and 170.8 mg/mL, respectively. Endoxylanase (X2753, Pentopan Mono BG) had an enzyme activity of 100 U/mL, as provided by the supplier, Novozymes A/S, Denmark. Commercial standards for XOS (xylobiose to xylohexaose, X2-X6) were purchased from Megazyme (Ireland). All other chemicals and reagents were of analytical grade and acquired from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2 Citric acid/sodium citrate buffer hydrolysis

Hydrolysis of raw corncob was carried out in citric acid (CA) or citric acid/sodium citrate (CA/SC) buffer system using 30 mL stainless steel batch reactors. In a typical experiment, 1.0 g of corncob was suspended in 10 mL of CA solution or CA/SC buffer. The reactor was then sealed and immersed in a preheated silicone oil bath at 160 to 180 °C for 30 to 80 min. After hydrolysis, the reactors were immediately cooled in an ice-water bath to quench the reaction. The resulting slurries were quantitatively collected and vacuum-filtered to separate the solid residues from the liquid hydrolysates. The solid residues were thoroughly washed with deionized water, dried, and weighed for subsequent compositional analysis and enzymatic saccharification. The liquid hydrolysates were stored at –20 °C prior to the analysis of XOS, monosaccharides, and degradation products, including furfural, HMF, and formic acid. All hydrolysis experiments were performed in duplicate, and the mean values are reported.

2.3 Endoxylanase hydrolysis

To modify the XOS profile, the CA/SC liquid hydrolysate obtained from CA/SC hydrolysis of corncob was subjected to further hydrolysis using a commercial endoxylanase. The reactions were carried out in 10 mL centrifuge tubes with a working volume of 3 mL, and the hydrolysis conditions were maintained at 50 °C in a

shaking incubator (150 rpm) for periods of 12, 24, and 48 h. The effect of endoxylanase dosage on XOS production from corncob was investigated at levels of 5, 10, and 20 U per mL of hydrolysate. After the hydrolysis, the enzymes were inactivated by heating at 100 °C for 5 min. The samples were then centrifuged, and the supernatants were analyzed for XOS and xylose concentrations. Each condition was tested in triplicate (n=3).

2.4 Enzymatic saccharification of pretreated corncob solid

To modify the XOS profile, the liquid hydrolysate obtained from CA/SC hydrolysis of corncob was further treated with a commercial endoxylanase. Enzymatic hydrolysis was performed in 10 mL centrifuge tubes with a working volume of 3 mL at 50 °C in a shaking incubator at 150 rpm for 12, 24, or 48 h. The effect of enzyme dosage on XOS production was evaluated at 5, 10, and 20 U/mL hydrolysate. After hydrolysis, the enzyme was inactivated by heating at 100 °C for 5 min. The reaction mixtures were then centrifuged, and the supernatants were collected for the analysis of XOS and xylose. All experiments were conducted in triplicate, and the results are presented as mean values.

2.5 Analytical methods

The chemical compositions of the raw and pretreated corncob solids were determined following the NREL standard laboratory analytical procedures [19]. Monosaccharides (glucose, xylose) and degradation by-products (furfural, HMF, formic acid) in the liquid samples were quantified using high-performance liquid chromatography (HPLC, Agilent 1260) equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column. The column was operated at 35 °C with 5 mM H₂SO₄ as the mobile phase at a flow rate of 0.6 mL/min [20].

The concentrations of XOS with degrees of polymerization of 2 to 6 were

determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD, Dionex ICS-3000). XOS were separated on a CarboPac PA200 analytical column using a gradient elution program with eluent A consisting of 0.1 M NaOH and eluent B consisting of 0.5 M sodium acetate in 0.1 M NaOH. The flow rate was 0.3 mL/min [21].

The yields of xylose, and glucose, as well as the component removal rates, were calculated according to the equations described by [22]. The XOS (DP 2-6) yield was calculated based on the xylan content in raw corncob, as shown in equation (1).

$$\text{XOS yield (\%)} = \frac{\text{Mass of XOS (DP 2-6) in hydrolysate}}{\text{Initial mass of xylan in corncob}} \times 100 \quad (1)$$

The crystallinity index (CI) of corncob samples was measured by X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer at 5°/min in the range of 5°–50°. Calculation of CI was according to the following equation [23].

$$\text{CI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

I_{002} ($2\theta = 22^\circ - 22.5^\circ$) and I_{am} ($2\theta = 18.5^\circ$) represent the maximum value of the crystalline region and the minimum value of the amorphous region, respectively.

The surface atomic composition and chemical environment of corncob samples were determined by X-ray photoelectron spectroscopy (XPS) using a Kratos Analytical AXIS UltraDLD spectrometer equipped with monochromatic Al K α radiation ($h\nu = 1486.6$ eV). The binding energies were calibrated with reference to the C 1s peak at 284.8 eV [24].

3 Results and discussion

3.1 Citric acid hydrolysis for XOS production from corncob

3.1.1 Effect of citric acid concentration on XOS production

The concentration of CA had a pronounced effect on the hydrolysis behavior of corncob xylan at 170 °C for 30 min. In the absence of added CA, 22.1% XOS and

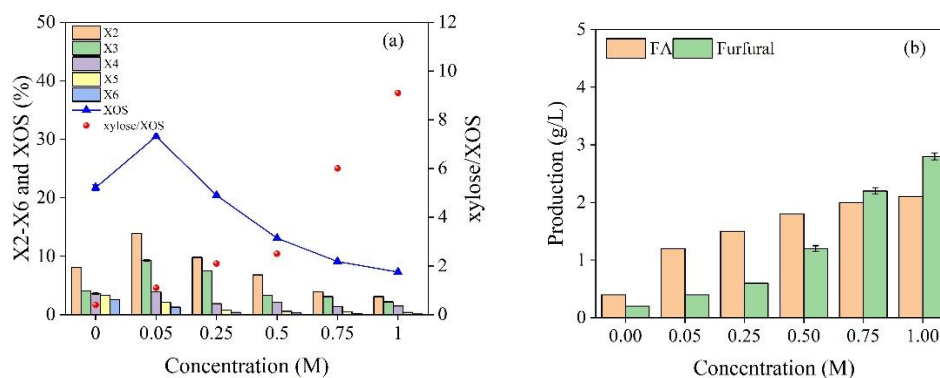


Fig. 1 Effects of citric acid (0.0-1.0 M) concentrations on XOS and by-products from corncob at 160 °C and pH 3.4 for 60 min. (A) XOS yield; (B) by-products yield

8.1% xylose were obtained, indicating that corncob underwent appreciable autohydrolysis under these conditions (Fig. 1a). When 0.05 M CA was introduced, the XOS yield increased to 30.4%, which was the highest value among all tested conditions. At the same time, the yields of xylobiose and xylotriose increased to 13.7% and 9.5% (Fig. 1a), respectively, suggesting that a low citric acid concentration promoted xylan depolymerization and favored the formation of low-degree XOS.

With further increase in CA concentration, the XOS yield decreased from 20.4% at 0.25 M to 7.5% at 1.0 M, whereas the xylose yield increased from 37.0% to 56.9% (Fig. 1a). Meanwhile, the xylose/XOS ratio increased continuously from 2.1 to 8.6, indicating that the further hydrolysis of XOS became progressively more severe at higher acid concentrations. These results indicated that low CA loading was beneficial for XOS production, whereas higher concentrations accelerated the conversion of XOS into xylose [25, 26]. Therefore, although CA promotes the hydrolysis of xylan, excessively acidic conditions are not conducive to the selective accumulation of XOS.

The composition of by-products in the hydrolysate further reflected the effect of CA concentration on the hydrolysis pathway. As the CA concentration increased from 0 to 1.0 M, the concentrations of FA and furfural increased from 0.3 to 2.0 g/L and 0.2 to 2.8 g/L, respectively (Fig. 1b). Among them, furfural showed the most

pronounced increase, especially at CA concentrations above 0.25 M. This result was consistent with the continuous increase in xylose yield and the decline in XOS yield, indicating that higher acid concentrations promoted not only xylan depolymerization but also the further dehydration of xylose to furfural [18, 27].

3.1.2 Effect of pretreatment temperature and time on XOS production

Pretreatment temperature and time markedly affected the hydrolysis behavior of corncob in 0.05 M CA. The highest XOS yield of 44.3% was obtained at 160 °C for 30 min, together with a xylose yield of 16.2% and a xylose/XOS ratio of 0.42 (Table 1). Under the same temperature, however, further extending the reaction time to 40 and 50 min decreased the XOS yield to 36.0% and 31.7%, respectively, while the xylose yield increased to 22.1% and 26.4% (Table 1). This result indicated that secondary hydrolysis of XOS became more pronounced at longer reaction times [28].

A similar trend was observed with increasing temperature. At 20 min, increasing the temperature from 160 to 170 and 180 °C decreased the XOS yield from 34.7% to 30.4% and 22.2%, respectively, whereas the xylose yield increased from 12.1% to 25.8% and 34.8% (Table 1). At 30 min, the XOS yield further decreased from 44.3% at 160 °C to 30.4% at 170 °C and 16.9% at 180 °C, while the xylose yield increased from 16.2% to 28.9% and 47.6% (Table 1). Meanwhile, the yields of X4 to X6 declined markedly with increasing temperature and time, showing that higher-degree oligomers were less stable under more severe conditions (Table 1).

The changes in by-product composition further reflected the effect of reaction time on the hydrolysis pathway at 160 °C. As the reaction time increased from 20 to 50 min, the concentrations of formic acid, acetic acid, HMF, and furfural increased from 0.6 to 0.9 g/L, 0.9 to 1.5 g/L, 0 to 0.2 g/L, and 0.1 to 0.3 g/L, respectively (Table 1). The relatively low levels of HMF throughout the tested period suggested that

213 **Table 1** Effects of temperature and time on the production of XOS (X2-X6) and by-products (xylose, FA, HMF and furfural) obtained from corncob (10%, w/v) by
214 0.05 M CA hydrolysis

Temperature- time (°C-min)	Yields (%)							Xylose/XOS	FA(g/L)	HMF(g/L)	Furfural(g/L)
	X2	X3	X4	X5	X6	XOS	Xylose				
160-20	12.7±0.1	8.2±0.0	7.8±0.1	4.5±0.1	3.9±0.1	37.0±2.7	12.0±0.0	0.4±0.0	0.6±0.0	0.1±0.0	0.1±0.0
160-30	14.7±0.1	13.7±0.2	7.5±0.2	5.1±0.2	4.2±0.1	45.2±1.8	16.6±0.1	0.4±0.0	0.8±0.0	0.1±0.0	0.2±0.0
160-40	14.9±0.2	9.2±0.1	5.7±0.0	3.5±0.0	2.1±0.0	35.5±0.5	22.4±0.2	0.7±0.0	1.0±0.0	0.2±0.0	0.3±0.0
160-50	13.2±0.2	8.1±0.0	3.9±0.0	3.2±0.1	2.4±0.0	30.8±1.6	26.0±0.4	1.0±0.0	0.9±0.0	0.2±0.0	0.3±0.0
170-20	12.2±0.5	9.5±0.1	3.9±0.0	2.3±0.0	1.7±0.1	29.6±1.1	26.4±0.8	1.0±0.0	1.0±0.0	0.4±0.0	0.4±0.0
170-30	13.9±0.0	9.3±0.1	3.9±0.0	2.1±0.0	1.3±0.0	30.5±0.0	29.9±1.7	1.1±0.0	1.1±0.0	0.4±0.0	0.6±0.0
180-20	11.7±0.1	5.6±0.1	2.6±0.0	1.2±0.0	0.4±0.0	21.4±1.2	36.5±2.8	1.9±0.1	1.3±0.0	0.5±0.0	0.6±0.0
180-30	9.7±0.2	4.2±0.1	1.4±0.0	0.8±0.0	0.4±0.1	16.5±0.4	48.4±1.2	3.3±0.0	1.6±0.0	0.5±0.0	0.7±0.0

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hexose degradation was limited under these conditions. In contrast, the gradual increase in furfural was consistent with the increase in xylose yield and the decline in XOS yield at longer reaction times, indicating progressively enhanced secondary degradation of xylan-derived products. The increase in acetic acid was mainly associated with deacetylation of hemicellulose during hydrolysis [29, 30]. These results suggested that extending the reaction time beyond 30 min intensified secondary reactions, which was unfavorable for the selective accumulation of XOS.

3.2 Citric acid/sodium citrate hydrolysis of corncob for XOS production

3.2.1 Effect of citric acid/sodium citrate concentration on XOS production

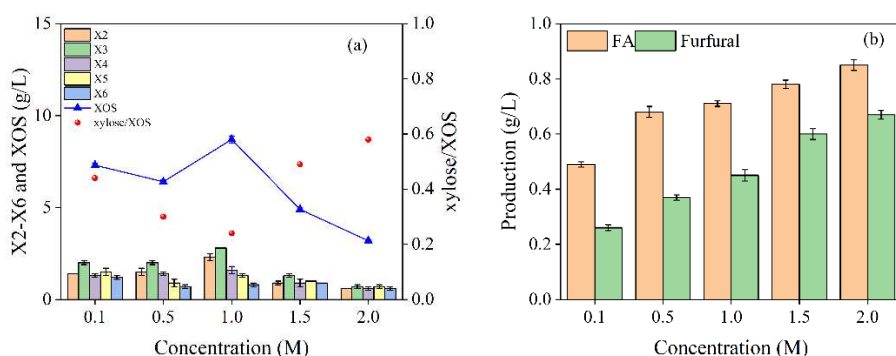


Fig. 2 Effects of CA/SC (0.1-2.0 M) concentrations on XOS and by-products from corncob at 160 °C and pH 3.4 for 60 min. (A) XOS yield; (B) by-products yield

In comparison with CA hydrolysis alone, the CA/SC buffer system showed a clear advantage in promoting XOS accumulation and maintaining a lower extent of secondary hydrolysis at 160 °C for 60 min. The concentration of the CA/SC had a marked influence on XOS production from corncob. Here, the buffer concentration refers to the combined concentration of citric acid and sodium citrate, with a fixed molar ratio of 1:1. As the total CA/SC concentration increased from 0.1 to 1.0 M, the XOS yield increased from 22.4% to 29.9%, while the xylose yield increased only slightly from 6.3% to 8.2% (Fig. 2a). Meanwhile, the xylose/XOS ratio remained low in the range of 0.27 to 0.31, indicating that XOS accumulation was favored over

excessive secondary hydrolysis under these conditions. Among the tested concentrations, 1.0 M gave the highest XOS yield together with the lowest xylose/XOS ratio, suggesting that this condition provided the most suitable buffer environment for selective xylan conversion into XOS.

The distribution of individual oligomers also changed with buffer concentration. At 0.1 and 0.5 M, the product mixture contained relatively high proportions of X4 to X6, indicating that xylan hydrolysis was still incomplete at lower buffer concentrations. When the CA/SC concentration increased to 1.0 M, the yields of X2, X3, and X4 increased to 7.9%, 9.5%, and 5.5%, respectively (Fig. 2a), showing that a moderate increase in buffer concentration promoted the conversion of higher-degree oligomers into target low-degree XOS. However, further increasing the CA/SC concentration to 1.5 and 2.0 M led to a sharp decrease in total XOS yield to 16.7% and 11.1%, respectively, while the xylose/XOS ratio increased to 0.53 and 0.91 (Fig. 2a). This result indicated that excessive buffer concentration accelerated the further degradation of XOS and was therefore unfavorable for their selective accumulation.

A critical finding of this study was the effective suppression of degradation products by the buffering action of the CA/SC system. The concentrations of formic acid (FA) and furfural increased with CA/SC concentration, yet remained at comparatively low levels. For instance, at the optimal XOS yield condition (1.0 M CA/SC), FA and furfural concentrations were only 0.70 and 0.61 g/L, respectively (Fig. 2b). In contrast, when citric acid was used alone at a concentration of 1.0 M, the concentrations of FA and furfural reached 2.0 and 2.8 g/L, respectively (Fig. 1b). This result here demonstrated that the CA/SC hydrolysis generated fewer by-products compared to citric acid hydrolysis, thereby leading to a higher XOS product purity.

3.2.2 Effect of pH on XOS production in the CA/SC buffer system

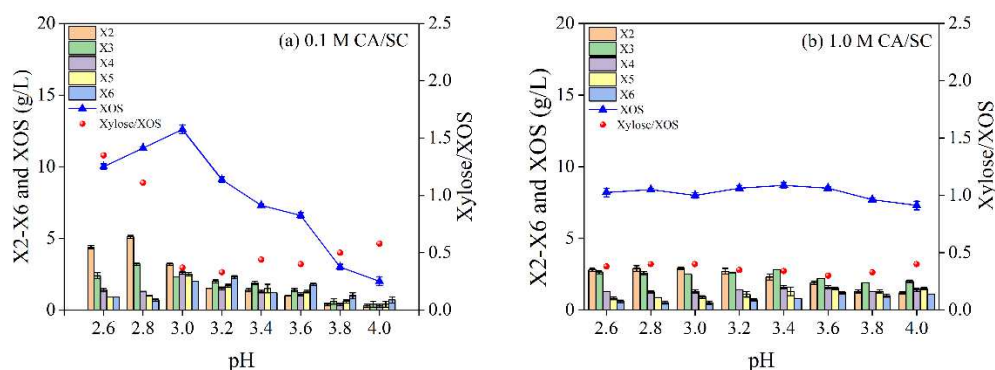


Fig. 3 Effect of CA/SC system pH on XOS and xylose generation from corncob (10%, w/v) at 160 °C for 60 min with pH from 2.6 to 4.0. (a) 0.1 M CA/SC; (b) 1.0 M CA/SC

At fixed CA/SC buffer concentrations, pH showed a pronounced and concentration-dependent effect on XOS production from corncob at 160 °C for 60 min. The pH was adjusted from 2.8 to 4.0 by varying the molar ratio of citric acid to sodium citrate. At 0.1 M CA/SC, the total XOS yield first increased and then decreased with increasing pH, reaching a maximum of 38.3% at pH 3.0, while the xylose yield decreased from 16.3% at pH 2.8 to 12.6% at pH 3.0 (Fig. 3a). Under the more acidic condition of pH 2.8, the higher xylose/XOS ratio of 0.52 indicated more pronounced secondary hydrolysis of XOS. When the pH was further increased from 3.0 to 4.0, both XOS and xylose yields gradually declined, suggesting that xylan depolymerization became insufficient under weaker acidity [14, 31]. A similar trend was observed at 1.0 M CA/SC, but the optimum shifted to a higher pH. The total XOS yield increased from 18.2% at pH 2.8 to 31.9% at pH 3.5, while the xylose yield decreased from 19.3% to 10.9%, and the xylose/XOS ratio decreased markedly from 1.06 to 0.36 (Fig. 3b). Further increasing the pH to 4.0 reduced the XOS yield to 17.6%, indicating that the hydrolysis severity insufficient.

A comparison of the two buffer concentrations showed that the effect of pH cannot be considered independently of buffer concentration. At the same pH values of

2.8 to 3.2, the 1.0 M CA/SC system gave lower XOS yields but substantially higher xylose yields than the 0.1 M system under 160 °C for 60 min (Fig. 3a and b), indicating that the more concentrated buffer promoted excessive secondary hydrolysis under relatively strong acidity. In contrast, at pH 3.5 to 4.0, the 1.0 M system gave higher XOS yields than the 0.1 M system, suggesting that a higher buffer concentration was beneficial for maintaining effective xylan hydrolysis under milder acidic conditions. The oligomer distributions also supported this trend. Lower pH favored the formation of X2 and X3, reflecting more extensive chain cleavage, whereas moderately higher pH allowed the accumulation of X4 to X6, especially in the 0.1 M system (Fig. 3a and b). Overall, these results indicated that XOS production depended on the combined balance between buffer concentration and pH. A lower buffer concentration required a relatively lower pH to achieve efficient hydrolysis, whereas a higher buffer concentration required a milder pH to suppress over-degradation and maximize XOS accumulation. Within the tested range, the most favorable conditions were pH 3.0 at 0.1 M CA/SC with a relatively high XOS yield of 38.3%.

3.2.3 Effect of pretreatment temperature and time on XOS production

Based on the above results, the effects of pretreatment temperature and time on XOS production were further evaluated at a fixed CA/SC buffer concentration of 0.1 M and pH 3.0. At 160 °C, extending the reaction time from 50 to 70 min increased the XOS yield from 36.0% to 45.5%, while the xylose yield increased only moderately from 9.3% to 13.8% (Table 2). However, a further increase to 80 min reduced the XOS yield to 41.3% and increased the xylose/XOS ratio to 0.37, indicating the onset of secondary hydrolysis. A similar but more pronounced trend was observed at 170 °C. The XOS yield increased from 36.0% at 40 min to 45.9% at 50 min and

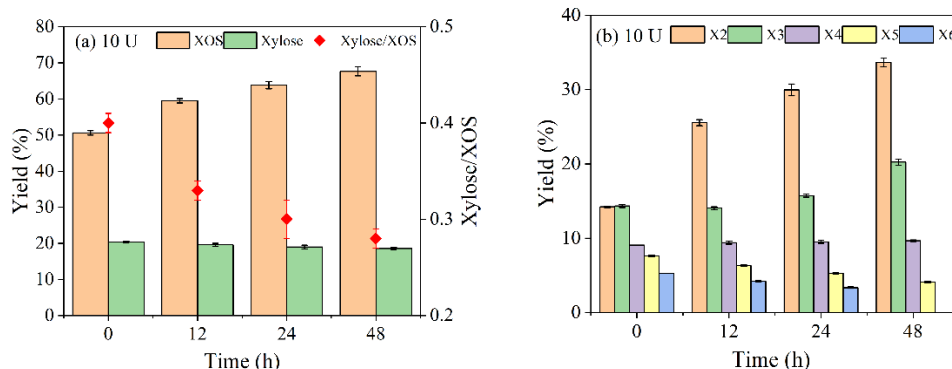
307 **Table 2** Effects of hydrolysis temperature and time on the production of XOS (X2-X6) and by-products (xylose, FA, HMF and furfural) obtained
308 from corncob (10%, w/v) by 0.1 M CA/SC hydrolysis with pH 3.0 at 160–180 °C for 30–80 min

Temperature - time (°C-min)	Yields (%)						Xylose/XOS	FA(g/L)	HMF(g/L)	Furfural(g/L)
	X2	X3	X4	X5	X6	XOS				
160-30	0.3±0.0	0.9±0.1	0.8±0.1	0.8±0.0	0±0.0	2.8±0.2	0.0±0.0	0.4±0.0	0.0±0.0	0.0±0.0
160-40	1.8±0.1	3.5±0.2	2.5±0.2	3.1±0.1	2.1±0.1	13.0±0.4	5.0±0.1	0.4±0.0	0.5±0.0	0.0±0.0
160-50	6.2±0.2	8.9±0.1	7.1±0.1	7.8±0.2	6.1±0.1	36.1±0.3	8.3±0.1	0.2±0.0	0.5±0.1	0.0±0.0
160-60	7.7±0.1	10.0±0.1	7.3±0.2	7.1±0.1	6.2±0.1	38.3±0.2	10.0±0.2	0.3±0.0	0.6±0.2	0.0±0.0
160-70	8.2±0.2	10.9±0.1	8.0±0.1	7.7±0.1	6.5±0.0	41.5±0.3	10.0±0.2	0.2±0.0	0.6±0.1	0.0±0.0
160-80	10.2±0.2	12.3±0.2	8.5±0.1	8.2±0.1	6.3±0.1	45.5±0.3	18.3±0.3	0.4±0.0	0.9±0.2	0.0±0.0
170-30	2.0±0.1	3.5±0.1	3.0±0.1	3.6±0.2	3.2±0.1	15.3±0.2	4.5±0.1	0.3±0.0	0.4±0.1	0.0±0.0
170-40	7.7±0.2	9.6±0.2	7.0±0.1	7.4±0.1	4.3±0.0	35.9±0.3	12.6±0.2	0.4±0.0	0.6±0.1	0.0±0.0
170-50	10.9±0.2	11.1±0.2	9.0±0.1	8.4±0.1	6.4±0.1	45.9±0.3	18.5±0.3	0.4±0.0	0.9±0.2	0.0±0.0
170-60	14.2±0.2	14.3±0.1	9.1±0.1	7.6±0.1	5.3±0.0	50.6±0.2	20.4±0.3	0.4±0.0	0.9±0.1	0.0±0.0
170-70	15.2±0.2	13.0±0.3	6.6±0.1	4.5±0.1	2.9±0.0	42.3±0.4	22.5±0.2	0.5±0.1	1.3±0.1	0.0±0.0
170-80	12.7±0.1	9.9±0.2	5.0±0.1	3.2±0.0	1.7±0.0	32.4±0.2	35.6±0.3	1.1±0.1	1.7±0.3	0.0±0.0
180-30	7.9±0.3	9.8±0.2	7.6±0.1	8.1±0.1	7.0±0.1	40.4±0.4	12.8±0.1	0.3±0.1	0.6±0.1	0.0±0.0
180-40	14.6±0.2	12.0±0.2	6.0±0.1	4.1±0.1	2.6±0.0	39.2±0.2	36.8±0.2	0.9±0.2	1.6±0.2	0.0±0.0
180-50	13.0±0.2	8.9±0.1	3.5±0.1	2.2±0.0	1.4±0.0	29.0±0.3	39.5±0.3	1.4±0.2	2.0±0.2	0.0±0.0
180-60	12.6±0.2	8.3±0.2	3.2±0.1	1.8±0.0	0.8±0.0	26.6±0.2	40.6±0.2	1.5±0.2	1.9±0.1	0.0±0.0
180-70	6.0±0.1	2.6±0.1	0.8±0.0	0.0±0.0	0±0.0	9.4±0.1	36.5±0.3	3.9±0.2	2.8±0.3	0.4±0.0
180-80	6.5±0.3	1.0±0.2	0.0±0.0	0.0±0.0	0±0.0	7.5±0.2	24±0.2	3.2±0.1	2.8±0.2	0.3±0.0

reached a maximum of 50.5% at 60 min, while the xylose yield remained at 15.3% and the xylose/XOS ratio was still relatively low at 0.30 (Table 2). When the reaction time was prolonged to 70 min, the XOS yield decreased to 42.2% and the xylose yield increased to 17.7%, suggesting that over-hydrolysis of XOS became more severe. At 180 °C, the highest XOS yield was only 39.2% at 40 min, after which it declined sharply to 29.0% and 26.7% at 50 and 60 min, respectively, accompanied by a continuous increase in xylose yield from 14.2% to 22.6% (Table 2).

The distribution of individual oligomers also reflected the effect of reaction severity. At 160 and 170 °C, moderate extension of reaction time promoted the accumulation of X2 and X3, while appreciable amounts of X4 to X6 were still retained (Table 2). In contrast, at 180 °C, the yields of X4 to X6 decreased rapidly with increasing time, indicating that higher-degree oligomers were unstable under more severe conditions and were readily converted into lower-degree oligomers and xylose [32]. These results showed that increasing temperature could shorten the time required for xylan depolymerization, but excessive temperature or prolonged treatment accelerated the further degradation of XOS. Among the tested conditions, 170 °C for 60 min was the most favorable for XOS production, giving the highest total XOS yield of 50.5%.

3.3 Effect of endoxylanase hydrolysis on XOS production



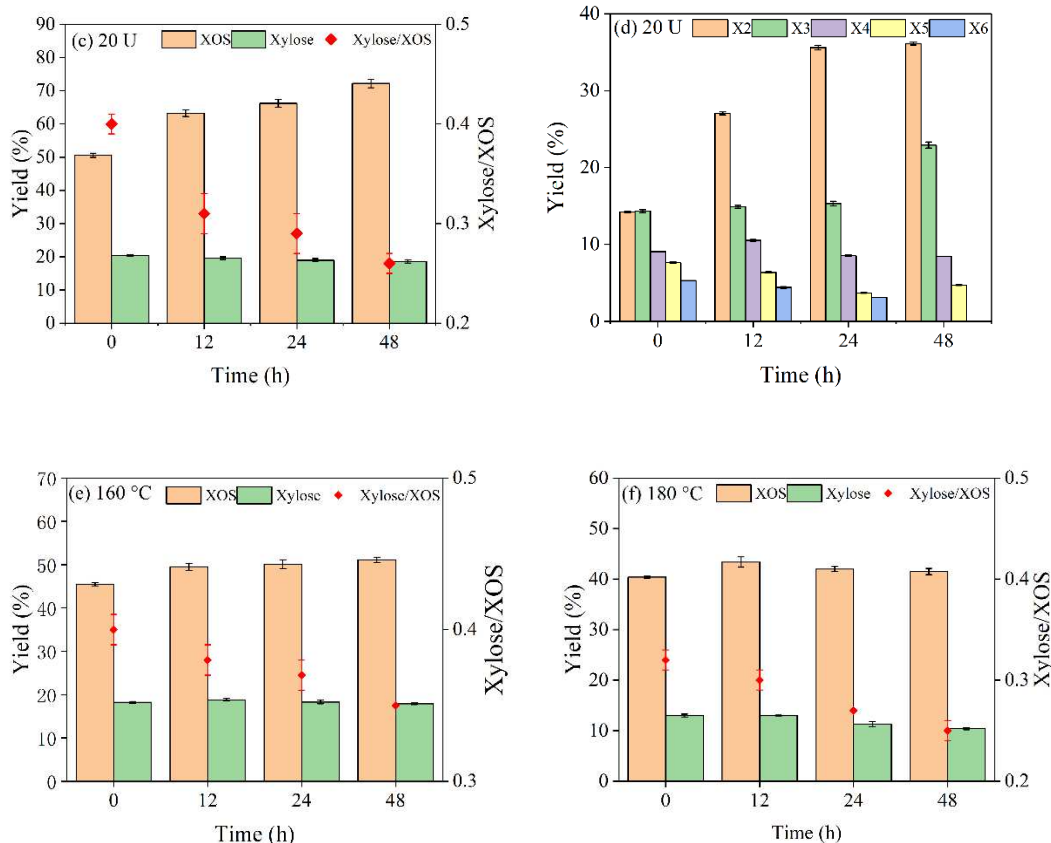


Fig. 4 The yield of XOS (X2-X6) from corncob hydrolyzed by 0.1 M CA/SC with a molar ratio of 1.0 at 160, 170, 180 °C for 40,60,70 min with a pH of 3.0. (a, b) 170 °C, 60 min, 10 U/mL of endoxylanase; (c, d) 170 °C, 60 min, 20 U/mL of endoxylanase.; (e) 160 °C, 70 min, 20 U/mL of endoxylanase; (f) 180 °C, 40 min, 20 U/mL of endoxylanase

To further tailor the XOS profile and improve the yield of low-degree oligomers, the liquid hydrolysates obtained under three representative pretreatment conditions, namely 160 °C for 70 min, 170 °C for 60 min, and 180 °C for 40 min, were subjected to endoxylanase hydrolysis at enzyme dosages of 10 and 20 U/mL for 12, 24, and 48 h (Fig. 4). Among the three hydrolysates, the liquor obtained at 170 °C for 60 min showed the greatest response to enzymatic treatment. At 10 U/mL, the total XOS yield increased from 50.5% to 52.5, 56.8, and 57.6% after 12, 24, and 48 h, respectively (Fig. 4a). At 20 U/mL, the corresponding values further increased to 56.2, 60.2, and 57.2%, with the highest XOS yield of 60.2% obtained at 24 h and a

relatively low xylose/XOS ratio of 0.27 (Fig. 4c). The hydrolysate obtained at 160 °C for 70 min also responded positively, with the XOS yield increasing from 45.5% to a maximum of 51.9% at 20 U/mL for 24 h (Fig. 4e). In contrast, the hydrolysate obtained at 180 °C for 40 min showed only limited improvement, and the highest XOS yield reached 44.0% at 20 U/mL for 24 h (Fig. 4f). This result suggested that the hydrolysate generated under the more severe pretreatment condition already contained a higher proportion of low-degree XOS and xylose, leaving less high-degree oligosaccharide available for further enzymatic conversion.

The changes in oligomer distribution further supported this trend. For all three hydrolysates, enzymatic treatment markedly increased the proportions of X2 and X3, whereas X4 to X6 generally decreased, indicating progressive cleavage of higher-degree oligomers (Fig. 4). This effect was particularly evident for the 170 °C for 60 min hydrolysate, in which X2 and X3 increased to 25.6 and 19.3%, respectively, at 20 U/mL after 24 h (Fig. 4b). In all cases, enzymatic treatment increased the total XOS yield, while the xylose yield increased only slightly, indicating that endoxylanase mainly promoted the conversion of higher-degree oligomers into shorter XOS rather than extensive saccharification to xylose.

HPAEC chromatograms further revealed clear differences in the oligomer distribution of XOS among the CA/SC hydrolysates and the effect of subsequent endoxylanase treatment (Fig. 5). All three pretreatment liquors contained X2 to X6, but the 170 °C for 60 min hydrolysate showed a more evident signal in the DP > 6 region, indicating the presence of residual higher-degree XOS. After endoxylanase hydrolysis, the DP > 6 signals were markedly reduced, accompanied by clear increases in the X2 and X3 peaks. In contrast, the 180 °C for 40 min hydrolysate showed weaker DP > 6 signals (Fig. 5), suggesting that the more severe pretreatment

had already promoted further depolymerization of higher-degree oligomers [33].

These results indicate that endoxylanase mainly converted the residual high-degree soluble oligomers into low-degree XOS, especially X2 and X3.

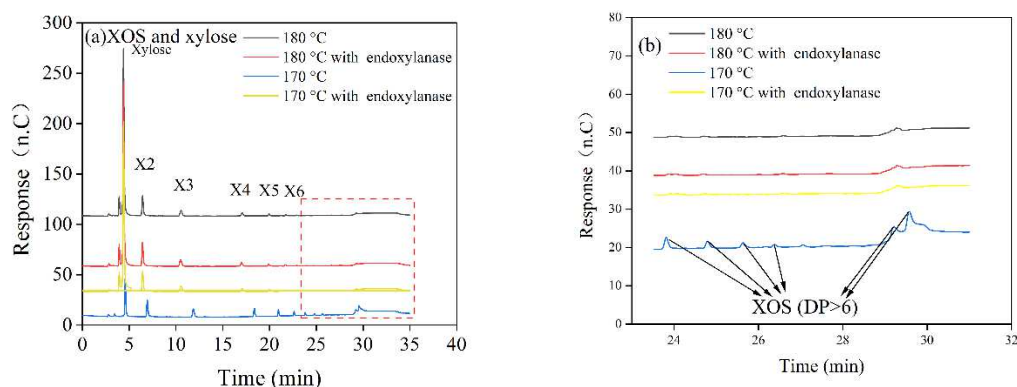


Fig. 5 The HPAEC chromatograms of XOS and xylose from corncob before and after endoxylanase hydrolysis at 170 °C for 60 min, and 180 °C for 40 min. (a) XOS and xylose; (b) XOS (DP>6)

3.5 Chemical composition and structural characteristics of the solid residues

Table 3 The chemical compositions of corncob samples after 0.1 M CA/SC hydrolysis of raw corncob at 160-180 °C for 30-80 min

Temperature (°C)	Time (min)	Glucan (%)	Xylan (%)	Acid- insoluble lignin (%)	Acid- soluble lignin (%)	Solid recovery (%)	Removal (%)		
							Glucan	Xylan	Lignin
160	70	54.9±0.2	14.3±0.3	17.5±0.4	3.8±0.1	59.8±0.2	2.0±0.0	70.3±0.3	25.2±0.1
160	80	53.5±0.3	15.3±0.2	17.3±0.3	3.8±0.1	60.6±0.3	3.2±0.1	68.0±0.2	25.1±0.2
170	40	51.6±0.2	18.9±0.2	13.5±0.2	4.1±0.1	63.9±0.2	1.5±0.1	58.2±0.2	25.8±0.2
170	50	57.0±0.2	13.0±0.1	13.7±0.1	4.0±0.1	57.9±0.1	1.6±0.0	74.0±0.3	26.2±0.1
170	60	57.4±0.2	10.8±0.2	15.6±0.2	4.0±0.0	56.4±0.1	3.4±0.1	78.8±0.2	27.0±0.1
180	30	50.9±0.1	19.2±0.2	14.5±0.2	4.2±0.1	61.4±0.2	6.7±0.2	59.2±0.2	27.3±0.1
180	40	55.9±0.4	12.1±0.3	15.2±0.2	3.9±0.1	55.8±0.1	7.0±0.2	76.7±0.2	28.2±0.1

The chemical composition of the solid residues showed that CA/SC pretreatment selectively removed hemicellulose from corncob while largely preserving cellulose. Raw corncob contained 33.0% glucan, 30.8% xylan, and 17.2% lignin. After pretreatment in 0.1 M citric acid/sodium citrate (CA/SC) buffer at pH 3.0, the glucan content in the solid residues increased to 54.9%, 57.4%, and 55.9% for the residues obtained at 160 °C for 70 min, 170 °C for 60 min, and 180 °C for 40 min, respectively, whereas the xylan content decreased markedly to 14.3%, 10.8%, and 9.2% (Table 3). Correspondingly, xylan removal increased from 70.3% to 78.8% and 81.3% with increasing pretreatment severity. In contrast, glucan removal remained limited at only 2.0%, 3.4%, and 7.0%, indicating good cellulose preservation under all tested conditions (Table 3). Although lignin removal was much lower than xylan removal, increasing only from 25.2% to 28.2%, the lignin content in the residues remained relatively high because extensive hemicellulose removal enriched the remaining solid fraction.

The structural analyses of the representative residue obtained after pretreatment in 0.1 M CA/SC buffer at pH 3.0 and 170 °C for 60 min further supported these compositional changes. XRD showed that the crystallinity index increased from 33.1% for raw corncob to 47.9% after pretreatment (Table 4), indicating the preferential removal of amorphous components, mainly hemicellulose and part of lignin, and the resulting enrichment of the crystalline cellulose fraction. XPS analysis also revealed clear changes in surface chemistry. The O/C ratio increased from 0.4 to 0.5, while the C1 component assigned to C-C/C-H bonds decreased from 41.7% to 37.9% and the C2 component associated with C-O bonds increased from 15.9% to 20.4%. Meanwhile, the C3 component decreased from 12.7% to 7.5% (Table 4).

These changes indicated that CA/SC pretreatment reduced the relative contribution of lignin-rich hydrophobic carbon on the residue surface and exposed more

Table 4 XRD and XPS analysis of raw corncob and corncob hydrolyzed by 0.1 M CA/SC at 170 °C for 60 min.

CA/SC concentration (M)	Crystallinity (%)	O/C	C1 (%)	C2 (%)	C3 (%)
Raw	33.1±0.5	0.4±0.0	41.7±0.1	15.9±0.1	12.7±0.1
0.1	47.9±0.7	0.5±0.0	37.9±0.1	20.4±0.2	7.5±0.1

C1: C–C or C–H bonds; C2: C–O bond; C3: C–O or O–C–O bonds.

Three replicate assays were performed for each experiment and average values are presented

carbohydrate-derived oxygenated structures. The decrease in C3 further suggested the removal of hemicellulose-associated O–C–O and carbonyl-containing groups [34].

Overall, the compositional, XRD, and XPS results consistently showed that CA/SC pretreatment mainly targeted hemicellulose, preserved most of the glucan, and increased the cellulose-rich character of the solid residue.

3.6 Enzymatic saccharification of corncob solid residue

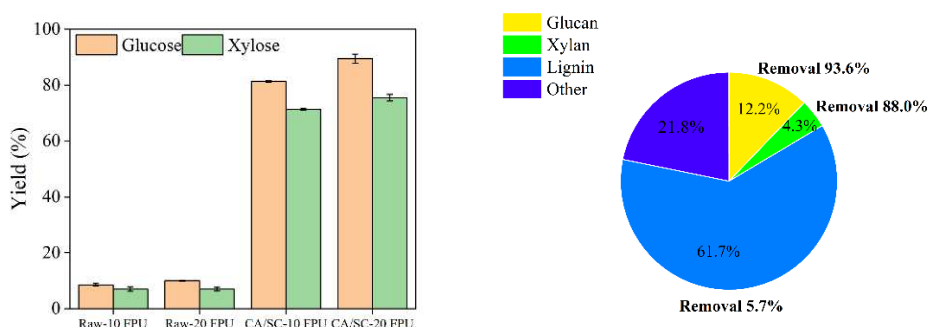


Fig. 6 (a) Yields of glucose and xylose of raw and CA/SC-hydrolyzed corncob hydrolyzed by cellulase hydrolysis at 10 and 20 FPU/g DM and 50 °C for 72 h; (b) The chemical compositions of corncob samples after 20 FPU cellulase hydrolysis at 50 °C for 72 h

Raw-10 FPU: raw corncob with 10 FPU cellulase/g DM;

Raw-20 FPU: raw corncob with 20 FPU cellulase/g DM cellulase;

CA/SC-10 FPU: CA/SC-hydrolyzed corncob with 10 FPU cellulase/g DM cellulase;

The solid residue obtained after the optimal CA/SC pretreatment condition (0.1 M CA/SC buffer at pH 3.0, 170 °C, and 60 min), was further subjected to enzymatic saccharification to evaluate its potential for monosaccharide production. The pretreated residue showed high enzymatic digestibility. When the cellulase loading increased from 10 to 20 FPU/g DM, the glucose yield increased from 81.3% to 89.5%, while the xylose yield increased from 71.4% to 75.5% (Fig. 6a). These results indicated that the CA/SC-pretreated residue was readily hydrolyzed by cellulase, and that increasing enzyme dosage provided only a moderate further improvement, suggesting that the substrate had already become highly accessible after pretreatment. In contrast, raw corncob remained highly recalcitrant under the same enzymatic conditions, giving glucose yields below 20% and only very low xylose yields. This clear difference demonstrates that CA/SC pretreatment markedly enhanced the enzymatic convertibility of corncob. The improvement can be mainly attributed to the selective removal of hemicellulose during pretreatment.

Following enzymatic saccharification, the solid residue became markedly enriched in lignin. During enzymatic hydrolysis, 93.6% of glucan and 88.0% of xylan were removed, whereas lignin removal was only 5.7% (Fig. 6b), indicating that the carbohydrate fraction was extensively hydrolyzed while most of the lignin remained in the residual solid. As a result, the chemical composition of the final residue shifted to 12.2% glucan, 4.3% xylan, and 61.7% lignin (Fig. 6b). This pronounced increase in lignin was result from the preferential removal of polysaccharides during saccharification. The substantial lignin enrichment suggested that the post-saccharification residue could be regarded as an industrial corncob lignin-rich fraction. Such a residue might serve as a potential feedstock for lignin valorization, including its use in phenolic materials, carbon materials, or other lignin-based

products [35-37], thereby improving the overall utilization efficiency of corncob in an integrated biorefinery process.

3.8 Mass balance

The overall mass balance clearly illustrated the fractionation and utilization pathway of corncob in the CA/SC-based process. On the basis of 1,000 g of raw corncob, the feedstock initially contained 333.0 g glucan, 308.0 g xylan, and 1(Zhan et al., 2023)72.0 g lignin. After hydrolysis in 0.1 M, pH 3.0 CA/SC buffer at 170 °C for 60 min, the process generated a xylan-derived liquid fraction and a cellulose-rich solid fraction (Fig. 7). The liquid fraction contained 155.5 g of XOS (DP 2 to 6) and 53.5 g of xylose, while 564.0 g of solid residue was recovered. Compositional analysis showed that this solid residue still contained 323.7 g glucan, 60.9 g xylan, and 110.5 g lignin (Fig. 7), indicating that most of the glucan was retained in the solid phase, whereas a substantial proportion of xylan was solubilized during pretreatment. Further endoxylanase hydrolysis of the CA/SC supernatant upgraded part of the soluble high-degree oligomers into target X2 to X6 products. As a result, the total XOS increased to 185.4 g and the total xylose increased to 57.1 g (Fig. 7). This result indicated that the pretreatment liquor still contained enzyme-convertible xylan-derived oligomers, and that post-hydrolysis with endoxylanase was effective for improving the recovery of low-degree XOS rather than causing extensive conversion to xylose.

The cellulose-rich solid fraction obtained after CA/SC pretreatment was subsequently subjected to cellulase hydrolysis. From the 564.0 g of pretreated solids, 329.2 g glucose and 51.1 g xylose were produced, leaving 169.0 g of residual solids (Fig. 7). This final residue contained 20.6 g glucan, 7.3 g xylan, and 104.2 g lignin. These results showed that most of the retained glucan in the pretreated solids was

further converted into glucose during enzymatic saccharification, while the majority of lignin remained in the residual solid phase. Such a lignin-enriched residue could therefore be regarded as a technical corncob lignin-rich fraction with potential for further valorization.

The results established the CA/SC conjugate acid-base system as an effective platform for XOS-oriented corncob fractionation. A key advantage of this system was that it moderated hydrolysis severity through buffer regulation, thereby promoting xylan conversion to XOS while suppressing excessive degradation relative to citric acid alone. The process also showed good integration at the whole-biomass level, soluble high-degree oligomers in the pretreatment liquor could be further upgraded to target low-degree XOS by endoxylanase, most glucan was retained in the pretreated solids and efficiently converted to glucose by cellulase, and the final residue became enriched in lignin. These features highlighted the potential of the CA/SC system for coordinated utilization of hemicellulose, cellulose, and lignin. At the same time, several limitations remain. Xylose and furfural were still formed under relatively severe conditions, indicating that selective XOS accumulation has not been fully maximized. In addition, lignin removal was moderate, and the downstream separation, purification, and recovery of XOS from the hydrolysate were not addressed in depth. Future work should therefore be focused on further suppressing secondary degradation, improving control over X2 and X3 production, evaluating buffer recyclability and process economics, and exploring higher-value applications of the lignin-rich residue to strengthen the overall feasibility of CA/SC-based corncob biorefining.

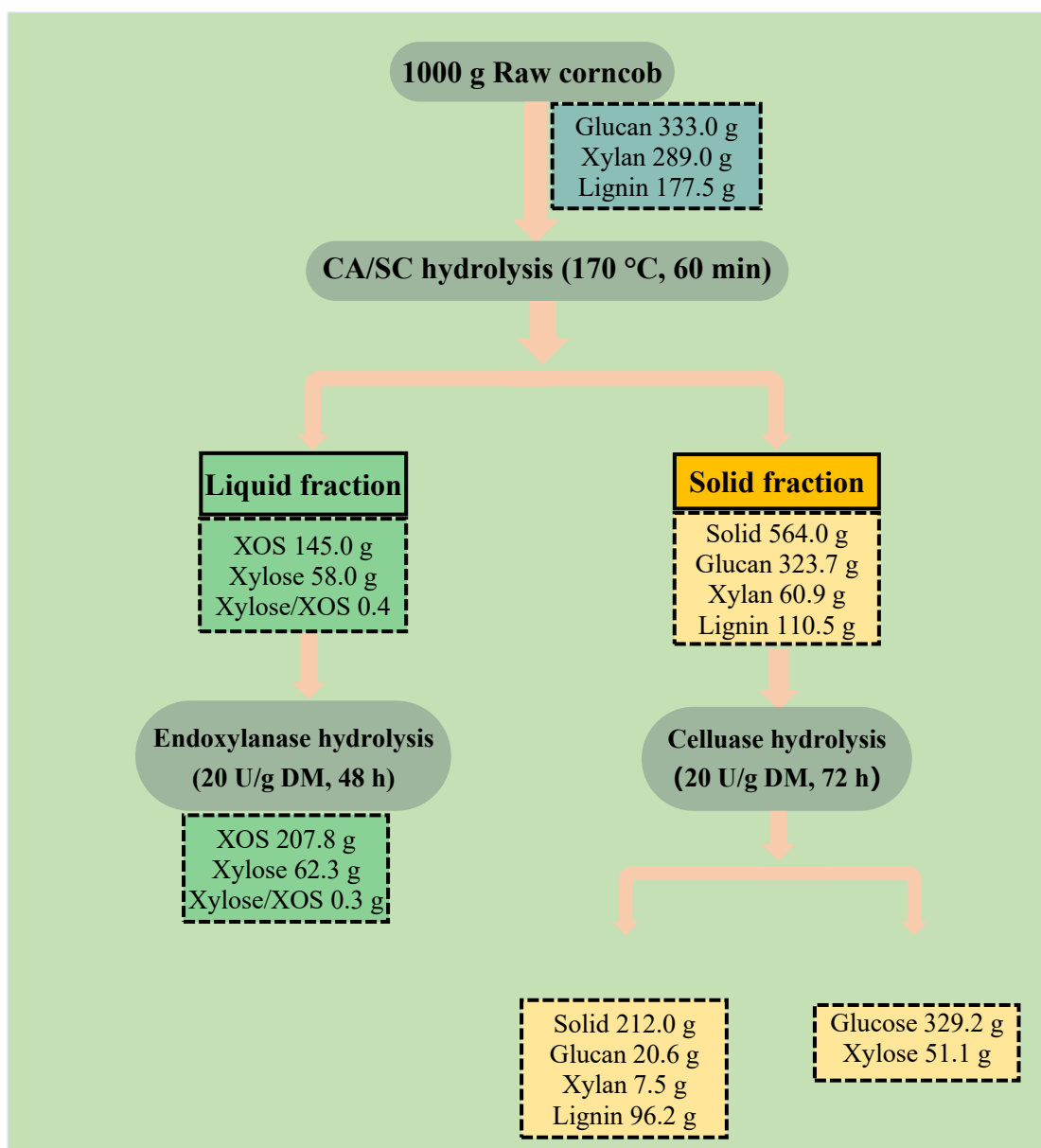


Fig. 7 Mass balance of XOS and monosaccharides production from 1,000 g raw

4. Conclusions

This study demonstrates that the citric acid/sodium citrate conjugate acid-base system is an effective route for XOS-oriented fractionation of corncob. Under the optimal conditions of 0.1 M CA/SC, pH 3.0, 170 °C, and 60 min, the XOS yield reached 50.5% and further increased to 60.2% after endoxylanase hydrolysis. Meanwhile, the pretreatment selectively removed hemicellulose while preserving most glucan, enabling efficient enzymatic saccharification of the solid residue.

507 Overall, the CA/SC system provides a promising strategy for selective XOS
508 production and integrated corncob valorization, although further work is still needed
509 to improve product selectivity and process feasibility.

510 **CRedit authorship contribution statement**

511 **Xiyu Quan**: Investigation, Writing – original draft. **Yajie Shi**: Data curation,
512 Investigation, Writing – original draft. **Hong Liao**: Software, Methodology.
513 **Jie Chu**: Resources, Validation. **Junhua Zhang**: Conceptualization, Funding
514 acquisition, Writing – review & editing.

515 **Declaration of Competing Interest**

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

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