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ORIGINAL ARTICLE

Preparation of reducing sugars from corncob by solid acid catalytic pretreatment combined with in situ enzymatic hydrolysis

Si Lu[1] · Qiong Wang[1] · Xiaoman Wang[1] · Cuiyi Liang[1] · Juan Fu[1] · Zihan Xu[1] · Zhongming Wang[1] · Zhenhong Yuan[1] · Jun Yue[2] · Wei Qi[1]

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Abstract

The efficient conversion of hemicellulose and cellulose into reducing sugars remains as one major challenge for biorefinery of lignocellulosic biomass. In this work, saccharification of corncob in the aqueous phase was effectively realized via pretreatment by magnetic carbon-based solid acid (MMCSA) catalyst, combined with the subsequent in situ enzymatic hydrolysis (occurring in the same pretreatment system after separation of MMCSA). Through the combined two-step hydrolysis of corncob, the total sugar (xylose and glucose) yield of 90.03% was obtained, including a xylose yield of 86.99% and an enzymatic digestibility of pretreatment residue of 91.24% (cellulase loading of 20 FPU/g, 24 h). Compared with the traditional enzymatic hydrolysis of pretreated residue, the presented in situ enzymatic hydrolysis system can reach a comparable enzymatic digestibility in one-third reacting time with a half cellulase loading and save about 31% water consumption, which provides a more sustainable and low-cost method for the saccharification of lignocellulose.

Keywords Biomass · Catalytic pretreatment · In situ enzymatic hydrolysis · Magnetic carbon-based solid acid catalyst · Reducing sugars

1 Introduction

To alleviate the global resource shortage and environmental issues associated with the currently heavy exploitation and utilization of fossil fuel feedstock, the valorization of renewable biomass towards the production of energy, fuels, and chemicals, based on green chemistry and technology, is crucial for a sustainable development of our society and economy [1–4]. Within this context, the efficient and economically viable hydrolysis of polysaccharide components in lignocellulose into reducing sugars is one of the key pathways towards generating biofuels and other biobased (platform) chemicals [5, 6]. The most commonly adopted method to generate sugars from biomass is its pretreatment combined with the subsequent enzymatic hydrolysis process [7–9].

Various pretreatment methods including physical, chemical, biological, and physicochemical ones have been developed to promote lignocellulosic enzymatic digestibility [10–14]. Among these, pretreatment by carbon-based solid acids (e.g., prepared by the incomplete carbonization of biomass materials and the subsequent sulfonation) has been identified as a novel catalyst, which is cheaper and exhibits good catalytic performance [15]. Various kinds of materials were used as precursor to investigate the synthesis of carbon-solid acid catalyst for pretreating lignocellulose through different synthesizing methods, such as lignin-containing spent liquor [16], active carbon [17], sodium lignosulfonate [18], and microcrystalline cellulose [19].

Compared with the conventional solid acids (such as zeolite molecular sieves, ion exchange resins, metal oxides) which usually have only one of Brønsted acid sites and adsorption sites [20], besides the inherently rich acidic functional groups (-COOH, phenolic -OH) on carbon-based solid acids, the sulfonic acid group (-SO_3H) is easily introduced via the sulfonation step [21]. The phenolic -OH group can

✉ Wei Qi
qiwei@ms.giec.ac.cn

[1] Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, CAS Key Laboratory of Renewable Energy, Guangdong Key Laboratory of New and Renewable Energy Research and Development, Guangzhou 510640, China

[2] Department of Chemical Engineering, Engineering and Technology Institute Groningen, University of Groningen, 9747 AG Groningen, The Netherlands

form a strong hydrogen bond with oxygen atom in the β-1,4-glycosidic bond of lignocellulosic biomass, thus creating a linkage with the carbon-based solid acid. This facilitates the Brønsted acid groups (-SO_3H and -COOH) to effectively approach the biomass surface to attack the β-1,4-glycosidic bond in order to release reducing sugars [22].

A kind of magnetic carbon-based solid acid (MMCSA) catalyst was synthesized from microcrystalline cellulose and ferric chloride by the impregnation-carbonization-sulfonation procedure in our previous work [23]. This catalyst was applied to pretreat corncob to obtain ca. 75% of xylose yield directly. After the separation of residue from the pretreatment system and being washed by fresh water, an enzymatic digestibility of the pretreated corncob residue at 95.2% was subsequently achieved (compared with the enzymatic digestibility of 66.6% in the case without pretreatment), with a total sugar yield (xylose and glucose) of 90.4%.

Although the above-mentioned promising pretreatment capability is attainable with carbon-based solid acid catalysts, many deficiencies remain to be solved in the two-step hydrolysis procedure. These typically include multiple workups (e.g., separation, washing, drying and feeding of the pretreated residue for its enzymatic hydrolysis), excessive water usage (since the pretreatment and enzymatic hydrolysis take place in separate aqueous media), and the still long enzymatic hydrolysis time, which limit to a certain extent the economic feasibility of the reducing sugar production towards obtaining biobased fuels and chemicals [8, 24]. To address these issues, not only a further process optimization of separate pretreatment and enzymatic hydrolysis steps is necessary, but also the possibility of a close integration between the two steps should be examined.

A limited research attention has been paid to the in situ enzymatic hydrolysis of lignocellulosic biomass, except the reaction often occurred in ionic liquid (IL) environment [25, 26]. IL was used for lignocellulose pretreatment, and then enzyme and buffer were added into the pretreated mixture to complete hydrolysis. Such in situ method simplifies the saccharification process [27, 28]. Nevertheless, most IL-tolerant cellulases can only work in specific IL solvents or at low concentration of IL, limiting the dissolution efficiency of cellulose during the pretreatment and resulting in a severe activity delay in the enzymatic hydrolysis (e.g., requiring 72 to 120 h to complete with yields of glucose at only 50% to 90%) [29–31]. Although advances have been made in the design and development of IL- and enzyme-compatible systems for the one-pot biomass hydrolysis, the usual high cost of IL limits the economic feasibility of the process [32].

In this work, the pretreatment of corncob by MMCSA was further investigated to enhance the reacting efficiency, and in situ enzymatic hydrolysis of pretreated residue was carried out. Compared with the traditional enzymatic hydrolysis process, the in situ enzymatic hydrolysis of pretreated residue presented obvious advantages of saving water consumption, reducing cellulase loading, enhancing reacting efficiency, and concentrating the total sugar concentration, which provided a more sustainable and effective operating process for the hydrolysis of lignocellulose biomass into reducing sugar.

2 Experimental methods

2.1 Materials

Corncob powder (40–60 mesh) was obtained from a farm in Shandong province, China. It was oven-dried at 80 °C for 12 h before use. Microcrystalline cellulose (Guaranteed Reagent (GR)) and iron (III) chloride (CP) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sulfuric acid (98% (w/w), GR) and sodium hydroxide (AR) were purchased from Guangzhou Chemical Regent Factory (Guangzhou, China). Cellulase (190 FPU/g; one unit of FPU is defined as the amount of cellulase required for producing 1 μmol reducing sugars per minute) was purchased from Imperial Jade Bio-Technology Co., Ltd. (Ningxia, China). Citric acid monohydrate and trisodium citrate dehydrate (AR) were obtained from Damao Chemical Regent Factory (Tianjin, China).

2.2 Preparation of MMCSA

The catalyst preparation started with mixing of 10-g microcrystalline cellulose ($\leq$ 120 meshes) into 1-L $FeCl_3$ solution (10 mmol/L) under a continuous stirring at 400 rpm for 5 h, followed by heating of the mixture in a bench-top electric furnace (Model ES-3618 K, Guangzhou Yuecheng Factory, China) at 100 °C to evaporate water. The remaining solid was dried in an oven at 105 °C overnight. Thus, Fe-impregnated microcrystalline cellulose underwent carbonization (350 °C, 1 h under N_2 atmosphere), followed by sulfonation with sulfuric acid (98% w/w, solid–liquid ratio at 1:10, 130 °C, 10 h), and finally washed with hot water ($>$ 80 °C) to obtain MMCSA.

The prepared MMCSA is an amorphous carbon consisting of -SO_3H, -COOH, and phenolic -OH groups borne on nanographene sheets in a random fashion. The chemical formula of MMCSA is $C_{0.505}H_{0.3014}O_{0.933}S_{0.085}Fe_{0.322}$ and the total acid amount is about 2.82 mmol/g. More details about the preparation process and characterization of MMCSA can be found in our previous work [23].

2.3 Catalytic pretreatment of corncob by MMCSA

A mixture of corncob, deionized water, and MMCSA was measured and loaded into a series of 100-mL autoclaves

(Model CJF-0.1, Dalian Tongda Autoclave Reactor Factory, Dalian, China). The autoclave consists of a cover and a kettle made of 316 stainless steels (equipped with an impeller and a built-in thermocouple). Programmed temperature controllers were used and the actual reactor temperature was measured directly by a thermocouple inserted therein. The reactor with a constant stirring speed of 300 rpm was raised to the specified reaction temperature after 55 min. After the reaction, the reactor was cooled rapidly with cold water at room temperature.

The following pretreatment conditions were screened and optimized in terms of the highest xylose yield, based on the corncob amount of 2.5 g, hydrolysis time (up to 120 min), temperature (130–160 °C), catalyst dosage (0–5 g), and water content (25–75 mL). After the reaction, the supernatant was collected and stored for further products analysis (cf. Section 2.5). In addition, part of the supernatant was treated with H_2SO_4 solution (4%, w/w) in an autoclave (Model GR60DA, Zealway, Xiamen, China) at 121 °C for 60 min in order to depolymerize oligosaccharides into monosaccharides.

The hydrothermal pretreatment of the corncob was further carried out under the same optimized pretreatment condition without the addition of MMCSA catalyst for comparison.

2.4 In situ enzymatic hydrolysis of the pretreated corncob residue

After the pretreatment of corncob under optimal conditions (cf. Section 2.3), MMCSA was separated from the reaction system by an external magnet and the remaining reactant (the hydrolysate and the pretreated corncob residue in a wet state) was transferred into a 100-mL Erlenmeyer flask, in the presence of a cellulase loading of 10 or 20 FPU/g (according to the mass quality of the dried corncob). Trisodium citrate dihydrate was then added to adjust the reaction system pH to 4.8. The hydrolysis was conducted at 50 °C on a shaker at 150 rpm up to 60 h.

2.5 Analytical methods

The chemical compositions of the natural and pretreated corncobs were analyzed according to the standard laboratory analytical procedures (LAP) for biomass analysis, provided by the U.S. National Renewable Energy Laboratory (NREL) [33]. Glucose, xylose, arabinose, and other byproducts (furfural, formic acid, acetic acid, glycolic acid, etc.) in the hydrolysate after being filtered with a 0.45-mL syringe filter were detected by high-performance liquid chromatography (HPLC; Waters 2695, Milford, USA) with a Shodex SH-1011 column. A sulfuric acid aqueous solution (5 mM) was employed as the mobile phase, with a flow rate of 0.5 mL/min and a column temperature of 50 °C.

The yield of sugars (xylose, glucose, or arabinose) after the pretreatment of corncob is defined as [23]

$$\text{Sugar yield}(\%) = \frac{\mathrm{N}}{\mathrm{M}} \times 100\% \tag{1}$$

where N and M are the moles of sugars in the pretreatment hydrolysate and the natural corncob, respectively.

The catalytic selectivity for xylose during the pretreatment is defined as

$$\text{Xylose selectivity}(\%) = \frac{\mathrm{D}}{\mathrm{H}} \times 100\% \tag{2}$$

where D is the mole of xylose in the hydrolysate after the pretreatment and H is the mole of xylan hydrolyzed in hemicellulose during the pretreatment process.

The enzymatic digestibility for in situ enzymatic hydrolysis of the pretreated residue is calculated as [34]

$$\text{Enzymatic digestibility}\ (\%) = \frac{A - C}{B - C} \times 100\% \tag{3}$$

where A denotes the mole of glucose in the hydrolysate obtained after the enzymatic hydrolysis, B is the mole of glucan in the natural corncob, and C is the mole number of glucose in the hydrolysate after the pretreatment.

The total sugar yield is obtained as

$$\text{Total sugar yield}(\%) = \frac{a}{b} \times 100\% \tag{4}$$

where a is the mass quantity of total reducing sugars (glucose and xylose) obtained after the pretreatment and/or enzymatic hydrolysis, and b is the mass quantity of total sugars in the natural corncob.

The removal rate of hemicellulose or lignin after the pretreatment is calculated as

$$\text{Removal rate}(\%) = \frac{c}{d} \times 100\% \tag{5}$$

where c is the mass quality of hemicellulose or lignin removed after the pretreatment and d is that of hemicellulose or lignin in the natural corncob.

The retention rate of cellulose after the pretreatment is obtained from

$$\text{Retention rate}(\%) = \frac{e}{f} \times 100\% \tag{6}$$

where e is the mass quality of cellulose retained in the corncob residue after the pretreatment and f is that of cellulose in the natural corncob.

The morphological structure of the natural corncob was characterized by emission scanning electron microscopy (S-4800, Hitachi, Japan). The morphological structure of the wet treated corncob was characterized by Cryo-scanning electron microscope (S-4800, Hitachi, Japan). The

corresponding crystalline structures were measured by X-ray diffraction (XRD) using an X'Pert Pro MPD (PANalytical, Netherlands, CuKα radiation) operating at 40 kV and 40 mA in the 2θ range from 5 to 80° with a scanning step of 0.0167°. The crystallinity index (*CrI*) of the corncob feed or the pretreated residue is calculated as [35, 36]

$$CrI = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (7)$$

where I_{200} is the maximum intensity of the crystalline peak at 2θ = 22.5°, and I_{am} is the minimum intensity near 2θ = 18° corresponding to the amorphous region.

3 Results and discussion

3.1 Catalytic pretreatment of corncob by MMCSA

3.1.1 Effect of reaction time and temperature

The production of C5/C6 sugars (xylose, glucose, and arabinose) by hydrolysis of corncob using MMCSA as catalyst was studied at a reaction temperature ranging from 130 to 160 °C within a duration up to 120 min. As shown in Fig. 1a, the xylose yield increased with increasing reaction time at the early stage of the reaction, indicating that the hydrolysis of hemicellulose in the corncob resulted in the release of xylose. The peak values of xylose yields were reached in a shorter time with increasing temperature, primarily due to the enhanced overall reaction rate. At prolonged reaction times, the xylose yield tends to decrease especially at relatively high temperatures (above ca. 150 °C) due to the significant build-up of xylose concentration facilitating its degradation. This is in line with the literature findings that the degradation rate of xylose tends to be faster than the rate of xylose formation at the relatively higher temperature [37]. At 160 °C, the xylose yield increased to the highest at 88.77% in around 20 min and almost no xylose oligosaccharides were detected in the hydrolysate. Moreover, the yield of furfural in the hydrolysate was particularly low (cf. Table S1 in the SI). These results demonstrated that MMCSA was highly selective for producing xylose monosaccharides.

The yield of arabinose increases rapidly at the initial stage of reaction and is always on the rise within the reaction time scale investigated below 160 °C, as illustrated in Fig. 1b. This is explained by the stability of arabinose under hydrothermal and acid conditions [38].

In addition, the glucose yield gradually increased with time and only a 16.13% glucose yield was obtained at 160 °C for 120 min as shown in Fig. 1c. The yields of oligosaccharides and hydroxymethylfurfural (HMF) were

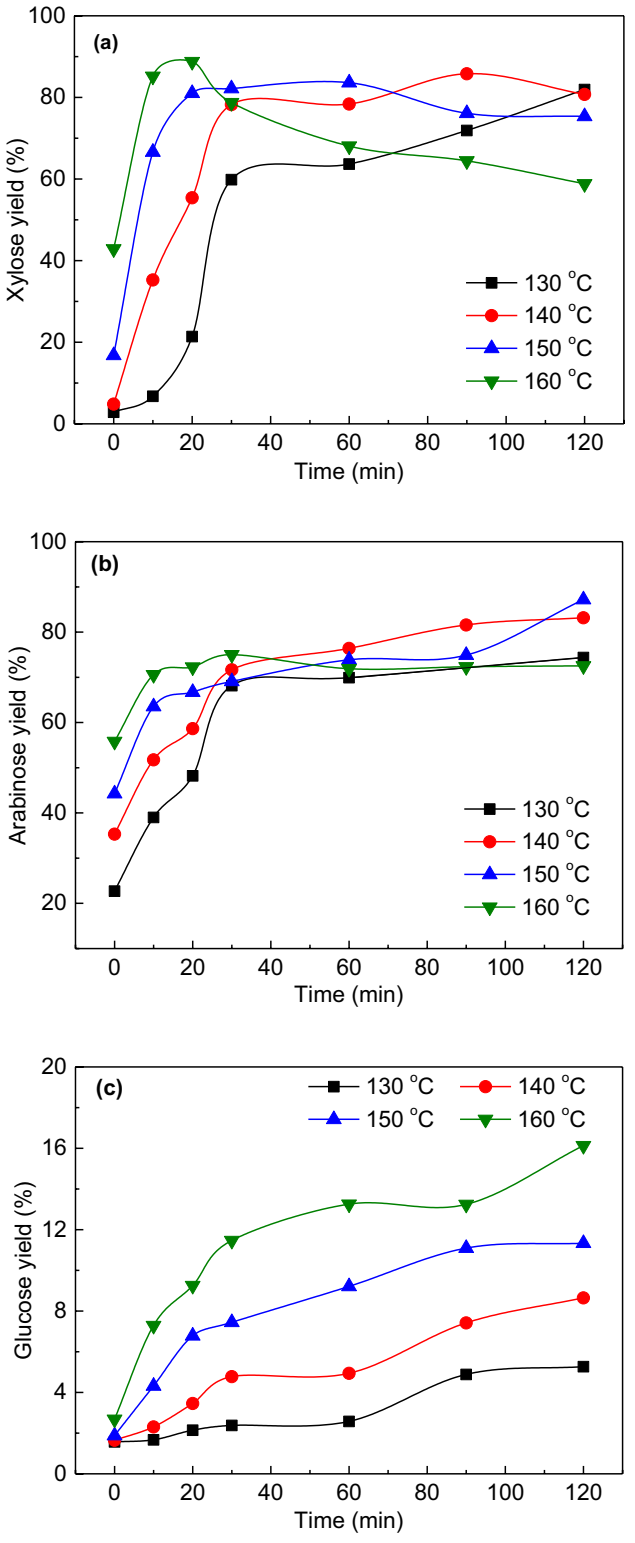

Fig. 1 Effects of reaction time and temperature on the pretreatment of corncob by MMCSA, **a** xylose yield; **b** arabinose yield; **c** glucose yield (reaction conditions: 2.5-g corncob, 2.5-g catalyst, and 50-mL deionized water)

also extremely low (Table S1), indicating that cellulose was stable during MMCSA pretreatment [39].

Given that arabinose and glucose are not the main products in the pretreatment process, the reaction time of 20 min and the reaction temperature of 160 °C were selected in the pretreatment step for the following experiments in order to obtain the optimized xylose yield.

3.1.2 Effect of catalyst loading

The effect of catalyst loading (0–5.0 g) on the hydrolysis of corncob was investigated at 160 °C for 20 min with 2.5-g corncob and 50-mL deionized water. As shown in Fig. 2, the xylose yield is 85.87% at an MMCSA loading of 1.25 g and further increased to 88.77% as the loading increased to 2.5 g. This is explained by the fact that more catalytic active sites in the reaction system were present with the increasing catalyst loading, and subsequently, a higher yield of xylose. The xylose yield was very low (2.49%) when there was no catalyst added, indicating that the hydrolysis contribution from high-temperature liquid water under the current reaction conditions was negligible [40]. However, when the catalyst dosage continued to increase to 5.0 g, the xylose yield decreased to 76.99%, possibly because the excess catalyst led to the presence of sufficient acid sites in the system, which could accelerate the decomposition of xylose in the undesired side reactions [19]. The increase of the furfural concentration in the hydrolysate confirms this speculation, as shown in Fig. S1. Therefore, 2.5 g was selected as the optimum MMCSA catalyst amount for the following experimental test (i.e., versus 2.5-g corncob).

3.1.3 Effect of water content

The water content was varied from 25.0 to 75.0 mL to investigate its effect on the hydrolysis of corncob (2.5 g) by MMCSA (2.5 g) at 160 °C for 20 min. Figure 3 shows that a lowest xylose yield of 63.16% was obtained when 25 mL of water was used. The xylose yield was increased all the way to 88.77% when further increasing water content up to 50 mL, after which it remains approximately unchanged. One explanation for this trend is that xylose undergoes more severe degradation at lower water contents, because the degradation rate of xylose will increase as the concentration of xylose increases [41]. And furfural could be further degraded, e.g., it could be resinized between molecules or undergo condensation reaction with xylose and its intermediates at high temperatures (see the xylose degradation products in Fig. S2) [42].

However, the content of xylooligosaccharides increased with a further increase of the water content above 50 mL, although the total xylose production seems stable, which indicates that the selectivity of MMCSA catalyzing hemicellulose into xylose decreases in the presence of excess water content. Therefore, a moderate water content (50 mL) is beneficial for an optimized sugar yield in the pretreatment step.

In summary, this further investigating process has obvious advantages than our previous work [23], including that an enhanced xylose yield can be obtained under a shorten reaction time and increased ratio of solid to liquid. Compared with the previous work, the reacting temperature was increased from 150 to 160 °C, but the reacting time was shorten from 2 h to 20 min and the ratio of liquid to solid (water to corncob) was decreased from 1:100 (g:mL) to 1:20

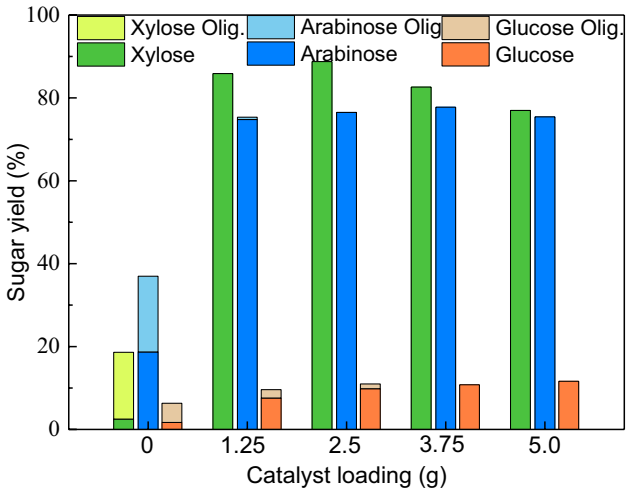

Fig. 2 Effect of catalyst dosage on the sugar yield in the pretreatment of corncob by MMCSA (other reaction conditions: 160 °C, 20 min, 2.5-g corncob, and 50-mL deionized water)

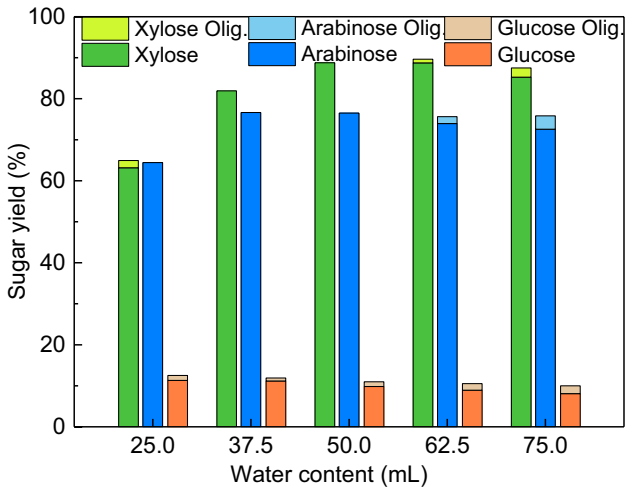

Fig. 3 Effect of water content on sugar yields in the pretreatment of corncob by MMCSA (other reaction conditions: 160 °C, 20 min, 2.5-g corncob, and 2.5-g catalyst)

Table 1 Component analysis of natural and pretreated corncob[a]

Corncob	Corncob components (%)			Retention rate (%)	Removal rate (%)	
	Glucan	Xylan	Lignin	Cellulose	Hemicellulose	Lignin
Raw	34.70	32.39	15.20	90.92	91.22	41.72
Pretreated	72.05	6.50	20.23			

[a]The components were calculated on dried basis and the pretreatment conditions: 160 °C, 20 min, 2.5-g corncob, 2.5-g catalyst, and 50-mL deionized water.

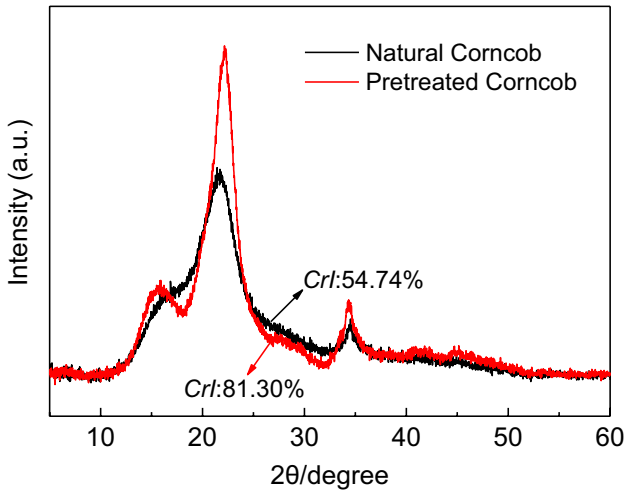

Fig. 4 X-ray diffraction patterns of corncob before and after the pretreatment

(g:mL), which is more consist to the concept of sustainable and green development.

3.1.4 Characterization of natural and pretreated corncob

A component analysis was conducted for the natural corncob and the pretreated corncob (i.e., the residue) under optimal reaction conditions identified above (i.e., 160 °C, 20 min, 2.5-g corncob, 2.5-g catalyst, and 50-mL deionized water). After the pretreatment with MMCSA, the proportion of glucan in the corncob increased from the original 34.70 to 72.05%, while the proportion of xylan decreased from 32.39 to 6.50%, as shown in Table 1. In the meantime, 41.72% of lignin was removed and up to 91.22% of hemicellulose was hydrolyzed, while the selectivity of xylose was as high as 97.16%. Further XRD spectra and crystallinity indices of corncob before and after the pretreatment (Fig. 4) show that upon pretreatment, the diffraction intensity of corncob at $2\theta = 22.5°$ (assigned to the (200) crystalline planes of typical cellulose I structure) increased rapidly, and the *CrI* increased from 54.74 to 81.30% [43]. This is due to the fact that the amorphous hemicellulose and partial lignin in corncob were removed during the pretreatment process [44] while the cellulose fraction was well retained, which might be benefit to the subsequent enzymatic hydrolysis step.

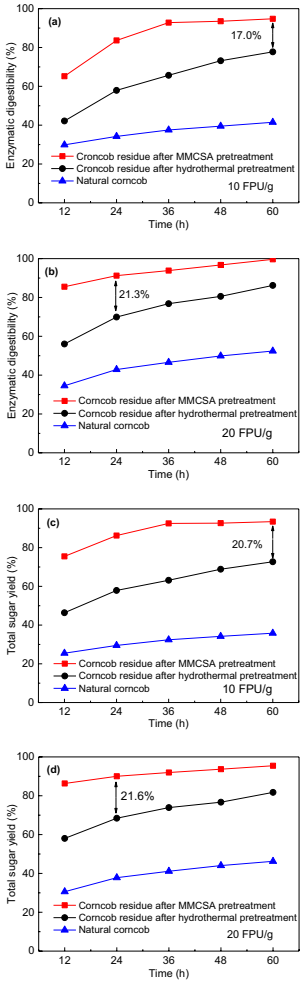

Fig. 5 Enzymatic digestibility (**a**–**b**) and total sugar yield (**c**–**d**) as a function of the reaction time and enzyme dosage in the cases of the in situ enzymatic hydrolysis of the pretreated corncob that is combined with MMCSA or hydrothermal pretreatment, and the cases of the traditional enzymatic hydrolysis of the natural corncob (MMCSA pretreatment conditions: 2.5-g corncob, 2.5-g catalyst, 160 °C, 20 min, and 50-mL deionized water; hydrothermal pretreatment conditions: 2.5-g corncob, 160 °C, 20 min, and 50-mL deionized water)

3.2 In situ enzymatic hydrolysis of the pretreated corncob

To investigate the potential of the direct in situ enzymatic saccharification of the MMCSA-pretreated corncob, cellulase was added to the pretreatment system for enzymatic hydrolysis. As shown in Fig. 5a and b, with a cellulase loading of 20 FPU/g, an over 90% enzymatic digestibility was obtained in 24 h, while with a lower cellulose loading of 10 FPU/g, a similar and high enzymatic digestibility (92.82%) could be also obtained, but at a much longer reaction time (36 h). The results of enzymatic hydrolysis of the natural corncob were also included for comparison, where

the enzymatic digestibility was only 52.43% after hydrolysis at 20 FPU/g for 60 h as shown in Fig. 5b, similar to that obtained in our previous study [19]. Moreover, the experiments were also done by hydrothermal pretreatment of natural corncob (i.e., without adding MMCSA just using water; other conditions being the same), and then the mixture of hydrolysate and residue was collected for enzymatic hydrolysis. The enzymatic hydrolysis results of the corncob residue after such hydrothermal pretreatment without MMCSA were also unsatisfactory (the enzymatic digestibility being around 20% lower than that in the case combining MMCSA pretreatment for a cellulose loading of 10 or 20 FPU/g), although these are better than the results with natural corncob as shown in Fig. 5a and b.

Similar trends are also present regarding the total reducing sugar yield as shown in Fig. 5c and d. It is noteworthy that the total sugar yield after the in situ enzymatic hydrolysis step with 20 FPU/g cellulase for 24 h is as high as 90.03%, implying that xylose produced during the prior MMCSA pretreatment was not (appreciably) consumed in this step. Thus, the above results demonstrate that MMCSA pretreatment combined with the in situ enzymatic hydrolysis technique greatly improves the enzymatic digestibility of cellulose towards an efficient saccharification of lignocellulose.

During the in situ enzymatic hydrolysis, the corncob residue after MMCSA pretreatment was directly digested without further treatment and thus was completely maintained in the wet state. To further elucidate the promising results obtained therein, additional experiments were carried out. After MMCSA pretreatment, the corncob residue and MMCSA were separated from the reaction system, followed by drying the corncob residue at 50 °C for 24 h and then being loaded back into the reaction system for enzymatic hydrolysis (the reaction conditions being the same as in the in situ enzymatic hydrolysis above). As shown in Fig. S3, the in situ enzymatic digestibility of the oven-dried residue is significantly lower than the cases of directly using the wet-state residue, even the enzymatic digestibility is only 78.44% at 40 FPU/g for 24 h. These results clearly demonstrate that maintaining the residue in a completely wet state facilitates an increase in the efficiency of enzymatic hydrolysis. These are in line with the literature results. For instance, Luo et al. [45, 46] have reported the effect of both oven-drying and wet pressing on the enzymatic saccharification of the pretreated lignocellulose and found that these methods could reduce the substrate moisture content and produce irreversible reduction in the pore volume of fiber, which resulted in the fiber hornification and finally reduced the cellulase adsorption ability of the substrate (in other words, the cellulase accessibility to cellulose).

In addition, the wet residue after MMCSA pretreatment was separated, washed with deionized water, and then placed in 0.05-M sodium citrate buffer (pH = 4.8) for enzymatic hydrolysis (other conditions being the same as in the in situ enzymatic hydrolysis). As shown in Fig. S4, in this case, an excellent enzymatic digestibility of 95.94% was also achieved at 20 FPU/g in 24 h. The enzymatic digestibility of pretreated residue is only slightly higher than that with the in situ enzymatic hydrolysis, indicating that the direct use of the hydrolysate (containing among others xylose, lignin, furfural removed from the corncob matrix) during the in situ enzymatic hydrolysis step did not present an appreciable suppression of the activity of cellulase.

During the in situ enzymatic hydrolysis, the wet-state corncob residue obtained from MMCSA pretreatment was used and thus the internal structure of the residue was expected to have larger particle size and pore volume. As Fig. 6b to d reveals, the wet residue has complex types of pores, including micropores and nanopores, with a three-dimensional porous structure. In contrast, the natural corncob showed a flat and dense microscopic surface, and there were almost no pores on the surface, as Fig. 6a. Moreover, few particles (including lignin, glucan, and xylan) were deposited on the surface of wet residues, which avoids the negative effect of steric hindrance in the subsequent enzymatic hydrolysis process [47]. The porous and steric structure of the wet residue thus greatly improved the accessibility of cellulose (which is nano-sized) to cellulase, rendering an effective enzyme digestibility in a short time [48–50].

3.3 The comparison between the in situ enzymatic hydrolysis system and the traditional methods

The in situ enzymatic hydrolysis method is proposed and summarized in Fig. 7, together with a comparison with the traditional enzymatic hydrolysis method. In the traditional enzymatic hydrolysis process, MMCSA pretreatment and subsequent enzymatic hydrolysis (using the dried residue feed) were conducted separately in two pots, and the enzymatic digestibility of the pretreated corncob residue was 82.42% at 72 h under the enzyme loading of 20 FPU/g, as shown in Fig. S5. Meanwhile, some representative studies in the literature were compared and listed in Table 2. Compared with the enzymatic hydrolysis results of the literature [5, 51–53] that were achieved in 72 h (Table 2, entries 1–4), a comparable enzymatic digestibility was obtained by the traditional enzymatic hydrolysis process in this work (Table 2, entry 5). It appears that the hemicellulose and cellulose in the corncob pretreated with MMCSA have achieved almost a complete saccharification by both methods, but the in situ enzymatic hydrolysis method presents obvious advantages over the traditional one.

Firstly, the in situ enzymatic hydrolysis system combined with MMCSA pretreatment can obtain almost the same total sugar yield as in the case for the traditional enzymatic hydrolysis method, but with greatly reduced amount of

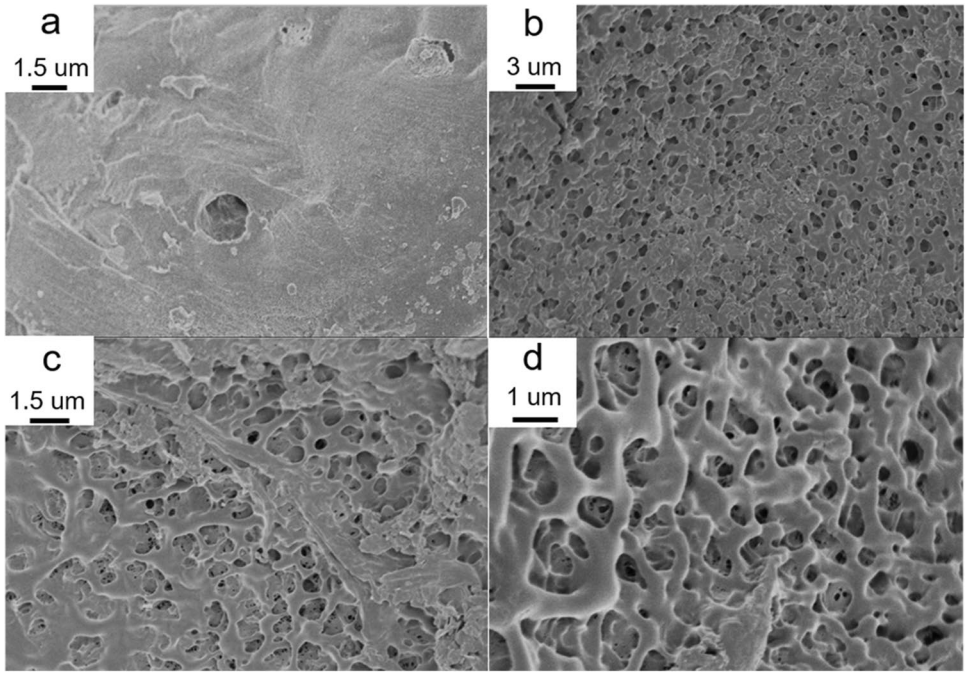

Fig. 6 SEM images of natural corncob (**a**) and wet corncob residues (**b**–**d**) after MMCSA pretreatment

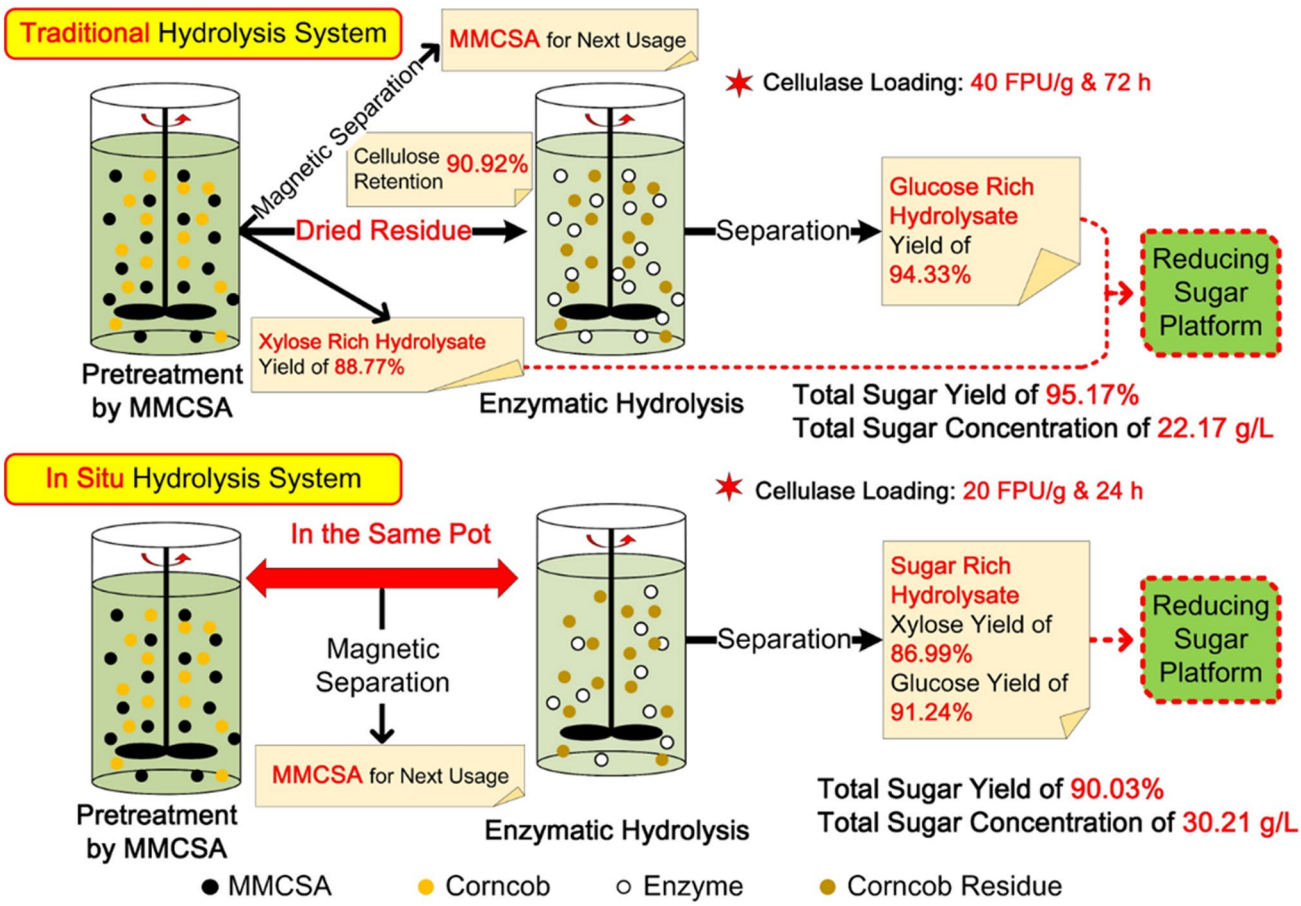

Fig. 7 Schematic diagram of the production of reducing sugars from corncob hydrolysis with the traditional and in situ process

Table 2 Comparison of enzymatic hydrolysis of the pretreated residue by different methods

Entry	Substrate	Residue washed after pretreatment	Buffer	Time (h)	Enzymatic digestibility (%)
1	Coffee-cut stems	Yes	Yes	72	44.8
2	Waste wheat straw	Yes	Yes	72	92.9
3	Tobacco stalk	Yes	Yes	72	86.3
4	Peanut shells	Yes	Yes	72	80.7
5	Corncob (this work)	Yes	Yes	72	82.4
6	Corncob (this work)	No	No	24	91.2

enzyme loading and reaction time, which is more economically feasible. This is supported by the fact that the in situ enzymatic hydrolysis system can achieve an over 90% enzymatic digestibility at only 20 FPU/g of enzyme loading for 24 h. However, it took nearly 72 h to achieve a similar enzymatic digestibility in the traditional enzymatic hydrolysis at a doubled enzyme loading (40 FPU/g), which is similar to the literature results.

Secondly, the in situ enzymatic hydrolysis system could reduce almost 31% water consumption which would otherwise be required in the traditional one (calculations not shown for brevity). This significant reduction effectively reduces wastewater workup load. In the meantime, a higher total sugar concentration (ca. 30 g/L in only 24 h) was obtained than that with the traditional enzymatic hydrolysis (ca. 22 g/L in 72 h over a higher enzyme loading), which is more beneficial for the subsequent high-value utilization such as product purification and sugar fermentation.

Thirdly, the traditional enzymatic hydrolysis requires a separation of the residue from the pretreatment reaction mixture, but in the in situ method, the pretreated residue in the wet state can be directly digested (which reduces the process steps).

4 Conclusions

In this work, the pretreatment of corncob by the magnetic carbon-based solid acid (MMCSA) catalyst has been combined with the subsequent in situ enzymatic hydrolysis to produce reducing sugars (xylose and glucose). The optimum pretreatment condition corresponding to the ratio of corncob, catalyst, and water is 1:1:20 (g:g:mL), and 160 °C for 20 min was constructed to obtain a highest xylose yield of 88.77%. The subsequent in situ enzymatic hydrolysis of the corncob residue in the same pot afforded an enzymatic digestibility of over 90% with a cellulase loading of 20 FPU/g at 50 °C for only 24 h. Compared with the traditional enzymatic hydrolysis process, the presented in situ enzymatic system can reach a comparable enzymatic digestibility in one-third reacting time with a half cellulase loading and save about 31% water consumption, which represents a more efficient and sustainable method for the depolymerization of corncob for the comprehensive utilization of lignocellulose towards producing fermentable sugars.

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