Generation of Reducing Sugars from Corncob by Magnetic Carbon-Based Solid Acid Pretreatment Combined with In Situ Enzymatic Hydrolysis

Si Lu
Guangzhou Institute of Energy Conversion

Qiong Wang
Guangzhou Institute of Energy Conversion

Xiaoman Wang
Guangzhou Institute of Energy Conversion

Cuiyi Liang
Guangzhou Institute of Energy Conversion

Juan Fu
Guangzhou Institute of Energy Conversion

Zihan Xu
Guangzhou Institute of Energy Conversion

Zhongming Wang
Guangzhou Institute of Energy Conversion

Zhenhong Yuan
Guangzhou Institute of Energy Conversion

Jun Yue
groningen university, department of chemical engineering

Wei Qi (qiwei@ms.giec.ac.cn)
Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences
https://orcid.org/0000-0002-3000-6951

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Abstract

The efficient conversion of hemicellulose and cellulose in lignocellulosic biomass to reducing sugars remains as one major challenge for the development of biorefinery. In this work, corncob saccharification in the aqueous phase was realized efficiently 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 MMCSA separation). Under the optimized pretreatment conditions (the ratio of corncob, catalyst and water is 1:1:20 (g:g:mL), 160°C for 20 min) and in situ enzymatic hydrolysis (cellulase loading of 20 FPU / g, 24 h), an xylose yield of 88.77% and an enzymatic digestibility of 91.24% were obtained, respectively. Compared with the traditional enzymatic process, the present in situ enzymatic system has advantages of reduced enzyme loading, water consumption, and improved saccharification efficiency. Thus, this study provides a more sustainable and effective method for the saccharification of hemicellulose and cellulose in the corncob to produce reducing sugars.

Introduction

To alleviate the global resource shortage and environmental issues associated with the currently heavy exploitation and utilization of fossil fuel feedstock, the valorisation 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 (Climent et al. 2014; Dong et al. 2019; Isikgor and Becer 2015; Wu et al. 2019). 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 (Ge et al. 2020; Huang et al. 2016). The most commonly adopted method to generate sugars from biomass is its pretreatment combined with the subsequent enzymatic hydrolysis process (Huang et al. 2018; Lin et al. 2020; Schneider et al. 2017).

Various pretreatment methods including physical, chemical, biological and physicochemical ones have been developed to promote lignocellulose enzymatic digestibility (Imman et al. 2015; Ramadoss and Muthukumar 2015; Tan and Lee 2012; Wen et al. 2020; Zhang et al. 2017). Among these, pretreatment by carbon-based solid acids (e.g., prepared by the incomplete carbonization of biomass materials and the subsequent sulfonation) have been identified as a novel catalyst, which are cheaper and exhibit good catalytic performance (Guo et al. 2013). Various kinds of materials were used as precursor to investigate synthesize carbon-solid acid catalyst for pretreatment of lignocellulose through different synthesizing methods, such as lignin-containing spent liquor (Bai et al. 2016), active carbon (Ansanay et al. 2017), sodium lignosulfonate (Li et al. 2018), and microcrystalline cellulose (Qi et al. 2018).

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 (Hara 2010). besides the inherently rich acidic functional groups (-COOH, phenolic -OH) on carbon-based solid acids, the sulfonic acid group (-SO$_3$H) is easily introduced via the sulfonation step (Guo et al. 2013). The phenolic -OH group can 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$_3$H and -COOH) to effectively approach the biomass surface to attack the β1,4-glycosidic bond in order to release reducing sugars (Konwar et al. 2019).

In our recent work (Qi et al. 2019), a magnetic carbon-based solid acid (MMCSA) catalyst was synthesized from micro-crystalline cellulose and ferric chloride by the impregnation-carbonization-sulfonation procedure. This catalyst was applied to pretreat corncob to obtain ca. 75% of xylose yield. An enhanced 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 (Cai et al. 2012; Lin et al. 2020). 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, where the reaction often occurred in ionic liquid (IL) environment (He et al. 2016; Yang et al. 2010). IL was used for lignocellulose pretreatment, then enzyme and buffer were added into the pretreated mixture to complete hydrolysis. Such in situ method simplifies the saccharification process (Ninomiya et al. 2015; Shi et al. 2013). Nevertheless, most IL-tolerant cellulases can only work in specific IL solvents or at low concentrations of IL, limiting the dissolution efficiency of cellulose during the pretreatment and resulting a severe activity delay in the enzymatic hydrolysis (e.g., requiring 72 to 120 h to complete with yields of glucose at only 50–90%) (Ben Hmad and Gargouri 2020; Hu et al. 2016; Zhou et al. 2019). 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 (Wahlström and Suurnäkki 2015).

In this work, the pretreatment conditions were further investigated and optimized based on our previous work (Qi et al. 2019). Then, cellulase was directly added to the pretreated reaction mixture (with the prior separation of MMCSA from the reaction medium) to perform the in situ enzymatic hydrolysis of the corncob residue. In particular, the important effect of in situ enzymatic hydrolysis on enhancing the enzymatic digestibility was investigated in detail. The results have been further compared with the traditional enzymatic hydrolysis process to highlight advantages of the present integrated process.

**Experimental Methods**
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).

Preparation of MMCSA

The catalyst preparation started with mixing of 10 g microcrystalline cellulose (≤ 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-3618K, Guangzhou Yuecheng Factory, China) at 100 °C to evaporate water. The remaining solid was dried in an oven at 105 °C overnight. Thus, obtained 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 washing with hot water (≥ 80 °C) to obtain MMCSA.

The prepared MMCSA is an amorphous carbon consisting of -SO$_3$H, -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 (Qi et al. 2019).

Catalytic pretreatment of corncob by MMCSA

A mixture of corncob, deionized water and MMCSA were measured and loaded into a series of 100 mL autoclaves (Model CJF-0.1, Dalian Tongda Autoclave Reactor Factory, Dalian, China). Each autoclave consisted of a cover and a kettle made of 316 stainless steel (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$_2$SO$_4$ 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.

In situ enzymatic hydrolysis of the pretreated corncob residue

After the pretreatment 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 loadings 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.

**Analytical methods**

The chemical compositions of the natural and pretreated corncobs were analysed according to the standard laboratory analytical procedures (LAP) for biomass analysis, provided by the U.S. National Renewable Energy Laboratory (NREL) (Sluiter et al. 2008). 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

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

where $N$ and $M$ are the mole numbers of sugars in the hydrolysate after the pretreatment and the natural corncob feed, respectively.

The catalytic selectivity for xylose during the pretreatment is defined as

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

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

The enzymatic digestibility for in situ enzymatic hydrolysis of the pretreated residue is calculated as
Enzymatic digestibility = \( \frac{A - C}{B - C} \times 100\% \)  \( (3) \)

where \( A \) denotes the mole number of glucose in the hydrolysate obtained after the enzymatic hydrolysis, \( B \) is the mole number 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

\[
\text{Total sugar yield} = \frac{a}{b} \times 100\% \quad (4)
\]

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

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

\[
\text{Removal rate} = \frac{c}{d} \times 100\% \quad (5)
\]

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

The retention rate of cellulose after the pretreatment is obtained from

\[
\text{Retention rate} = \frac{e}{f} \times 100\% \quad (6)
\]

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

The morphological structure of the natural corncob was characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Japan). The morphological structure of the wet treated corncob was characterized by cold field emission 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 \( 2\theta \) range from 5 ° to 60 ° with a scanning step of 0.0167 °. The crystallinity index (CrI) of the corncob feed or the pretreated residue is calculated by the Segal method as (French 2014; Segal et al. 1959)
Results And Discussion

Catalytic pretreatment of corncob by MMCSA

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. 1(a), the xylose yield increased with increasing reaction time at the early stage of the reaction, indicating the hydrolysis of hemicellulose in the corncob that 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 (Weingarten et al. 2010). 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 demonstrate 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. 1(b). This is explained by the stability of arabinose under hydrothermal and acid conditions (Kootstra et al. 2009).

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. 1(c). The yields of oligosaccharides and hydroxymethylfurfural (HMF) were also extremely low (Table S1), indicating that cellulose was stable during MMCSA pretreatment (Li et al. 2014).

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 remaining experiments in order to obtain the optimized xylose yield.

Effect of catalyst loading

\[
CrI = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (8)
\]

where \(I_{200}\) is the maximum intensity of the crystalline peak at \(2\theta = 22.5^\circ\), and \(I_{am}\) is the minimum intensity near \(2\theta = 18^\circ\) corresponding to the amorphous region.
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 with that, 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 (Imman et al. 2018). 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 (Qi et al. 2018). 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 remaining experimental test (i.e., versus 2.5 g corncob).

**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 (Weingarten et al. 2010). 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) (Li et al. 2014). 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 hemicellulose hydrolysis catalyzed by MMCSA to produce 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.

**Characterization of natural and pretreated corncob**

A component analysis was done of 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 34.70–72.05%, while the proportion of xylan decreased from 32.39–6.50%, 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 $2\theta = 22.5^\circ$ (assigned to the (200) crystalline planes of typical cellulose II structure) increased rapidly, and the $CrI$ increased from 54.74 to 81.30% (Yu et al. 2021). This is due to the fact that the amorphous hemicellulose and partial lignin in corncob were removed during the pretreatment process.
Malgas et al. (2020). The cellulose fraction was well retained, creating a favorable condition for the subsequent enzymatic hydrolysis of the pretreated corncob residue.

### Table 1
Component analysis of natural and pretreated corncob

| Component    | Raw (%) | Pretreated (%) |
|--------------|---------|----------------|
| Glucan       | 34.70   | 72.05          |
| Xylan        | 32.39   | 6.50           |
| Lignin       | 15.20   | 20.23          |
| Cellulose    | 90.92   |                |
| Hemicellulose| 91.22   |                |
| Lignin       | 41.72   |                |

*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.

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. 5(a) 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 was also included for comparison, where the enzymatic digestibility was only 52.43% after hydrolysis at 20 FPU/g for 60 h (Fig. 5(b)), similar to that obtained in our previous study (Qi et al. 2018). Moreover, the experiments were also done by firstly pretreating the natural corncob without MMCSA (i.e., 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 was 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 (Fig. 5(a) and (b)).

Similar trends are also present regarding the total reducing sugar yield (cf. Figure 5(c) 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, where the enzyme 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. (Luo and Zhu 2011; Luo et al. 2011) 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 fiber pore volume, 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. This is only slightly higher than that with the in situ enzymatic hydrolysis (Fig. 5(b)), 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. 6(b) to (d) reveal, 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. 6(a) show. 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 (Wang et al. 2015). The porous and steric structure of the wet residue thus greatly improved the accessibility of cellulase (which is nano-sized) to cellulose, rendering an effective enzyme digestibility in a short time (Alvira et al. 2010; Sun et al. 2016; Zhao and Chen 2013).

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

The in situ enzymatic hydrolysis method has been 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 load 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 (Ge et al. 2020; Solarte-Toro et al. 2020; Wang et al. 2019; Yuan et al. 2019) 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 has 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 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. Secondly, the pretreated residue need not to be detoxified before the in situ enzymatic hydrolysis, indicating that the water consumption is much lower than that traditional ones. This significant reduction effectively reduces wastewater workup load. Thirdly, the pretreatment hydrolysate was directly used for enzymatic hydrolysis after adjusting the pH without adding buffer, which reduces the process steps and is rarely reported.

In summary, the in situ enzymatic hydrolysis based on the pretreatment by MMCSA represents a more efficient and sustainable strategy for the saccharification of lignocellulose towards producing reducing sugars, which has obvious advantages of simplifying the processing step, reducing water consumption and with that the water wastewater treatment, and enhancing the efficiency of enzymatic hydrolysis.

### 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     | Sugarcane bagasse  | Yes                              | Yes    | 72       | 70.2                        |
| 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                        |

**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 pretreatment conditions have been further optimized to obtain a highest xylose yield of 88.77%, corresponding to the use of 2.5 g corncob and 2.5 g catalyst in 50 mL deionized water at 160°C for 20 min. 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. The in situ enzymatic hydrolysis procedure presents clear advantages in terms of reduced saccharification time and water consumption as well as simplified procedures. The results of this work represent a more efficient and sustainable method for the depolymerization of corncob for the comprehensive utilization of lignocellulose towards producing fermentable sugars.

**Declarations**

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**Compliance with ethical standards**

**Conflict of Interest** The authors declare that there are no conflicts of interest.

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Figure 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)
Figure 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)

Figure 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)
Figure 4

X-ray diffraction patterns of corncob before and after the pretreatment
Figure 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)

**Figure 6**

SEM images of natural corncob (a) and wet corncob residues (b-d) after MMCSA pretreatment

**Figure 7**

Schematic diagram of the production of reducing sugars from corncob hydrolysis with the in situ process combining the MMCSA pretreatment with the subsequent enzymatic hydrolysis