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Highly efficient fractionation of corn stover into lignin monomers and cellulose-rich pulp over $\text{H}_2\text{WO}_4$

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Graphical Abstract
Highlights

- Nearly complete delignification of corn stover was achieved without H₂ at 200 °C.
- H₂WO₄ improved the selectivity of methyl hydroxycinnamates.
- Lignin component was decomposed to monomer through solvolysis.
- Higher temperature and longer reaction time favor for delignification and hemicellulose removal.

ABSTRACT:

In this work, we investigated the fractionation of raw biomass based on a “lignin first” biorefinery strategy that enables the fractionation of corn stover into a stable lignin oil and a cellulose-rich pulp. Lignin fraction was completely disassembled into monomers with a yield of 25.1 wt% in methanol with a H₂WO₄ catalyst at 200 °C for
6 h. Higher temperature (≥220 °C) and longer reaction time (≥6 h) were in favor of higher delignification and hemicellulose removal, but led to lower cellulose retention and lignin monomer yield. Particle size of corn stover has minor impact on the delignification but significant for hemicellulose removal. A two-step mechanism is proposed for the fractionation of corn stover, in which ether and ester bonds between lignin and hemicellulose cleave to form oligomers through methanol solvolysis first, while the depolymerization of lignin oligomers to methyl hydroxycinnamates, e.g. methyl p-coumarate and methyl ferulate, as the major products happens in the catalytic steps.

**KEYWORDS:** Corn stover; Delignification; Lignin first; Tungstic acid, Biomass Retention

1. **INTRODUCTION**

With the diminishing reserves and the increasing consumption of fossil fuel, it is an urgent challenge to develop affordable, environmentally benign and energy efficient process for production of fuels and chemicals from sustainable resources. Lignocellulosic biomass, including grasses, softwoods and hardwoods, has drawn worldwide attention for their noteworthy features like huge abundance, geographical universality, and renewability as well as low cost. As one of the three main components of lignocellulosic biomass together with cellulose and hemicellulose, lignin accounts for 10–35% by weight, but up to 40% by energy in dry biomass due to its lower O/C ratio[1-3]. Lignin, an amorphous tridimensional polymer, consists of various phenylpropanoid units such as p-coumaryl (H), coniferyl (G) and sinapyl (S) alcohols, which are predominantly linked by either C-O or C-C bonds. Specifically, β-O-4, α-O-4, β-β, 5-5 and β-5 bonds are major phenolic building blocks of lignin. In
principle, an ample benzene rings in the structure of lignin makes it an ideal feedstock for production of diverse aromatic compounds. In the 2nd generation (2G) biomass conversion process, due to its aromatic nature, complexity, irregularity and heterogeneity, lignin is often regarded as a major hindrance to the enzymatic hydrolysis of cellulose and hemicellulose and the consequent fermentation step, though many biomass pretreatment strategies have been designed for efficient isolation of lignin. Large amounts of organic solvents are needed to solubilize both lignin and hemicellulose, for instance organosolv process, and those solvents must be removed by high energy-consuming distillation. On contrary, in the pulp/paper industries, strong alkalis are employed to cleave both ester and ether bonds between hemicelluloses and lignin macromolecules that produce a large volume of alkaline wastewater and Kraft lignin. The latter is generally being recognized as a waste byproduct and used for heat supply[1, 2, 4]. Moreover, pretreatment of lignocellulosic biomass with strong mineral acids, mainly H_2SO_4, is reported to hydrolyze the polysaccharides, leaving Klason lignin in the residue. Such technical lignins are condensed through the cleavage of reactive β-O-4 bonds by inversely forming the robust C-C bonds, thus the chemical inertness of lignin increases[5]. Many strategies including pyrolysis[6], hydrolysis[7, 8], oxidation[9-13], hydrogenolysis[14-16] and alcoholysis[1, 4, 17-31] have been explored for technical lignin depolymerization. For instance, Ford and coworkers disassembled organosolv lignin into a series of cyclohexyl derivatives with a Cu-doped porous metal oxide in supercritical methanol at 300 °C without external hydrogen[22]. Barta and coworkers have investigated the hydrogenolysis of Kraft lignin in supercritical methanol under a H_2 atmosphere at 320 °C over various supported bimetal catalysts with an aromatic monomers yield of 35% and illuminated the role of metals and supports[32]. In contrast, Hensen and coworkers used ethanol for organosolv lignin and obtained aromatics as the dominate monomeric compounds and revealed the role of ethanol and metal oxides catalyst[25-27]. Li and coworkers reported a series of Mo-based catalysts for complete depolymerization of Kraft lignin into aromatic monomers and diverse aliphatic
alcohols and esters without char formation in supercritical ethanol[1, 17-19, 21] and managed to clarify the mechanism in their processes[18].

Compared with technically isolated lignin, the number of cleavable β-O-4 bonds is higher in native lignin, which is easier to depolymerize with high aromatic monomer yields. Thus ‘lignin-first’ strategy is at forefront of the lignocellulosic biorefinery to preserve lignin’s precious β-O-4 bonds in the fractionation step. In the lignin-first strategy, the native lignin is solvolytically fractionated from whole biomass in the first step by leaving polysaccharides residue in the pulp. Subsequently, the catalytic depolymerization of fragmented lignin into aromatic monomers can be achieved in the second step. By applying this method, high lignin monomers yield has been reported by many research groups. For instance, Song et al. have reported the valorization of native birch wood over Ni/C catalyst in presence of methanol as a H-donor solvent at 200 ºC with total selectivity of propylguaiacol and propylsyringol about 90% at a lignin conversion of ~54%[33]. Despite lower conversion of lignin, they proposed the simultaneous solvolysis and hydrogenolysis depolymerization mechanism for the first time. In parallel, Ferrini et al. fractionated the poplar wood to lignin oil and carbohydrate pulp in presence of 2-propanol with water solvents through H transfer process over Raney Ni catalyst[34, 35]. Hensen research group investigated the delignification of woody biomass to 54 wt% aromatic and long-chain aliphatic products in supercritical ethanol (as solvent and H-donor) over CuMgAl mixed oxide catalyst[36]. They also tested Pd/C and metal triflates (as a Lewis acid co-catalyst) system for birch wood delignification with a phenolic monomer yield of about 50% and demonstrated the role of Pd/C catalyst and acid co-catalysts respectively with related model compounds [37, 38]. Similarly, the group of Abu-Omar used a bimetallic Zn/Pd/C catalyst, where Zn(II) acted as a Lewis acid co-catalyst, to convert C–O bonds of poplar WT-717 into phenolic products (yield = 54-61 wt%) at 225 ºC for 12 h [39, 40]. Most of the published research works have focused on the fractionation of hardwoods biomass, which has higher S/G monolignol ratios (about 3), resulted in higher proportion of β-O-4 bonds and lower proportion of refractory C-C bonds. In contrast, softwoods and agricultural wastes have much lower
S/G ratio as 0.01[41] and 0.62[42] respectively along with higher proportion of C-C bonds, which make the fractionation of those biomass more challenging. In particular, limited work on the fractionation of agricultural wastes like corn stover was reported, and therefore more work to optimize the process for agricultural wastes delignification is highly needed. Recently, Abu-Omar et al. have achieved almost total delignification of miscanthus over Ni/C catalyst and obtained an aromatic compounds yield of 29%[43]. Meantime, Anderson et al. investigated the reductive catalytic fractionation of corn stover over a Ni on acidified carbon at 200 °C resulted in lignin oil yields of 55% with sugar retention values >90%[42]. To best of our knowledge, no reports available so far on the fractionation of corn stover over (non-)noble metal-free catalyst.

In our previous study, H₂WO₄ was examined for selective conversion of guaiacol to tert-butylphenols, wherein WO₃ and Brönsted acid sites on H₂WO₄ play an important role in the conversion of guaiacol and formation of tert-butylphenols with high selectivity, respectively[44]. In this work, we demonstrated the fractionation of corn stover, a highly abundant herbaceous biomass, via “lignin first” biorefinery approach in presence of methanol over H₂WO₄ without the addition of external H₂. The influences of reaction parameter and corn stover particle size besides reaction pathways are discussed for fractionation of corn stover.

2. EXPERIMENTAL SECTION

2.1. Materials

The analytical reagent grade solvents including methanol (MeOH) and dichloromethane (DCM) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Tungstic acid (>99% purity) and anisole (>99.97% purity) were obtained from Sigma-Aldrich. All these chemicals were directly used as received. Corn stover was provided by a farm of Ninghe District, Tianjin, China.
2.2. Corn stover compositional analysis and characterization

Compositional analysis of corn stover was performed by the standard NREL laboratory analytical procedure[9]. Briefly, corn stover was scrubbed with alcohol pad to remove the sediment that present on the surface and smashed to pass through a 120-mesh screen. Then, the powder was treated with water and ethanol in a Soxhlet extractor for 12 h respectively to remove the extractives such as fatty acids, wax, salts, and free sugars, and then was dried overnight at 120 °C. The extractives-free corn stover powder was then digested in 72 wt% sulfuric acid with frequent interval stirring of every 5 min for 1 h at 30 °C. Deionized water was added into the slurry to achieve a 4% acid concentration. Subsequently, the slurry was heated in an autoclave at ~120 °C for another 1 h. Next, the mixture was filtered to separate the solid phase containing the insoluble lignin, known as Klason lignin, and ash from the sugar-rich aqueous solution. The solid phase was washed several times with water and dried overnight at 105 °C to determine the residue content, which was then ashed to determine the ash content and hence determine the Klason lignin content. The sugar-rich aqueous solution was diluted with deionized water and subjected to high performance liquid chromatography (HPLC, Agilent 1200 series HPLC) equipped with a refractive index detector to quantify the sugar content resulting from corn stover and thus determine the cellulose and hemicellulose contents. A Bio-Rad Aminex HPX-87C Column was used for sugar content analysis at 65 °C with a flow rate of 0.5 mL min\(^{-1}\) of 5 mmol L\(^{-1}\) H\(_2\)SO\(_4\) aqueous solution as the mobile phase.

Thermogravimetric analysis (TGA) of corn stover was performed with STA449 F3, Netzsch in an Ar flow (50 mL min\(^{-1}\), STP) from room temperature to 800 °C at a heating rate of 5 °C min\(^{-1}\), and then the temperature was kept at 800 °C for 1 h. The scanning electron microscope (SEM) micrographs of corn stover and reaction residue were taken with A S4800 microscope with acceleration voltage of 3 kV.

2.3. Activity measurement
Typically, the extractives-free corn stover (2 g), methanol (100 ml) and tungstic acid (0.5 g) were placed in a 250 mL stainless-steel autoclave reactor (Anhui Kemi Machinery Technology Co., Ltd). The temperature of the reaction was varied from 180 to 260 °C at a stirring speed of 600 rpm. When the desired reaction time was reached, the reactor was quenched with water to room temperature. After releasing the pressure, the autoclave was opened, and the reaction mixtures were collected and combined with the solution obtained from washing the autoclave with DCM. The cellulose-rich residue and liquid products were separated through suction filtration. The filter cake was washed with DCM for three times to make sure all the organic-soluble products were collected. Then, DCM was removed by vacuum evaporation to yield the total liquid products. A liquid-liquid extraction was conducted in a biphasic solution consisting of approximately 10 mL water and 10 mL DCM to separate the dissolved sugar from organic-soluble lignin products. The DCM layer was separated, and the water was extracted two additional times with 5 mL of DCM, while the DCM layers were combined and extracted once with 10 mL of water. The DCM was then removed by vacuum evaporation to yield the DCM-soluble products. The water–soluble products yield was determined as the total liquid products yield minus the DCM-soluble products yield. The lignin oil yield was determined as the DCM-soluble products yield minus the aliphatic compounds yield dissolved in DCM, which explained in detailed at section 2.4, and the lignin oil yield was used to assess delignification degree. Delignification degree and recovery can be calculated using following equations:

\[
\text{Delignification degree (wt%)} = \frac{\text{Weight of lignin oil}}{\text{Weight of corn stover} \times \text{Klason lignin weight percentage in corn stover}} \times 100\% \quad (1)
\]

\[
\text{Recovery of all products during corn stover fractionation (wt%)} = \frac{\text{Weight of liquid products} + \text{weight of carbohydrate-rich residue}}{\text{Weight of corn stover}} \times 100\% \quad (2)
\]
2.4. Product analysis and characterization

The lignin oil dissolved in DCM was injected into gas chromatograph (GC, Agilent 7890), which equipped with an HP-5 capillary column (Agilent, 30 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID) for quantitative analysis with anisole as an internal standard. The GC parameters used for the analysis were injector temperature 280 °C; detector temperature 300 °C; split ratio 50:1. The column was initially set at 50 °C and heated at a rate of 10 °C min⁻¹ to 180 °C, kept for 1 min, then heated at a rate of 5 °C min⁻¹ to 240 °C and maintained at 240 °C for another 1 min. The product quantification was performed on a gas-chromatography-mass spectroscopy (GC-MS, GC-Agilent 6890A, and MS-Agilent 5973C) with He as the carrier gas. The temperature ramping rate of GC-MS was the same as with GC. The yield of lignin monomers was calculated based on mass of Klason lignin.

Lignin monomer yield (wt%) = \frac{\text{Weight of each lignin monomer}}{\text{Weight of corn stover} \times \text{Klason lignin weight percentage in corn stover} \times 100\%} \tag{3}

2.5. Carbohydrate analysis

The carbohydrate retention was calculated based on the amount of carbohydrate in the corn stover and cellulose-rich residue after reaction, which can be determined in familiar procedure with corn stover compositional analysis. Cellulose retention and hemicellulose removal rates can be calculated using following equations:

Cellulose retention (wt%) = \frac{\text{Weight of cellulose in the residue}}{\text{Weight of corn stover} \times \text{cellulose weight percentage in corn stover} \times 100\%} \tag{3}
Hemicellulose removal rate (wt%) = \( \frac{1 - \text{Weight of hemicellulose in the residue}}{\text{Weight of corn stover} \times \text{hemicellulose weight percentage in corn stover}} \) \times 100\% \quad (4)

2.6. MOLDI-TOF Mass Spectrometry

The DCM-soluble lignin oil obtained at different temperatures (180-240 °C) over \( \text{H}_2\text{WO}_4 \) was analyzed with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Mass spectra were acquired on an Auto III MALDI-TOF (Applied Bruker Daltonics, USA). The instrument was operated at an accelerating voltage of 20 kV. A 200 Hz pulsed ND: YAG laser (355 nm) was used for MALDI-TOF. Positive ion MALDI-TOF MS spectra were collected from the signal average of 1500 laser shots in the linear detection mode.

2.7. 2D-HSQC-NMR

The 2D-HSQC-NMR spectra were obtained with a Bruker Ascend 400 MHz spectrometer. Prior to analysis, DCM solvent in the lignin oil was firstly removed through vacuum evaporation at 35 °C, and then the samples were dissolved in 500 μL CDCl₃.

3. RESULTS

3.1. Corn stover characterization

Table S1 shows the composition of corn stover. Specifically, corn stover is composed of 32.9% of cellulose, 22.8% of hemicellulose, 14.8% of lignin, 5.4% of ash and 24.0% of others, which consists of mainly protein and fats. After pretreatment with water and ethanol, protein and fats were almost completely removed, with a composition of 43.2% of cellulose, 29.1% of hemicellulose, 19.4% of lignin, 6.1% of ash and 1.1% of others (Table S1). Figure S1 illustrates the TG-DTG curve of corn stover in Ar. Corn stover begins to decompose at 210 °C. The weight loss rate reaches
the maximum at 315 °C. The weight keeps almost stable when the temperature reaches about 500 °C, leaving only carbon and ash in the solid.

3.2. Catalytic reaction results

As shown in Table 1, H₂WO₄ exhibited a promising activity for corn stover fractionation in methanol at 200 °C with the delignification degree of 98.5% and cellulose retention of 82.2%. The hemicellulose fraction (78.7%) was converted into sugar derivatives that dissolved either in water or DCM. Through analysis of the obtained DCM-soluble products, a total 11 kinds of products can be detected with GC-MS, as illustrated in Figure 1. The structures of the identified products were also shown with their corresponding number labels. Corn stover has more content of p-coumaric and ferulic acids, which are the special monomers with the content of about 10-20% and mostly bound through ester bonds to the lignin matrix [41, 42], and thus methyl p-coumarate (peak 10) and methyl ferulate (peak 11) were obtained as the dominant products, with the yields of 17.2 and 3.8 wt% respectively (Table 2, Entry 2), via transesterification with methanol solvent over H₂WO₄ [42]. Besides, some aliphatic compounds (~10.0 mg) mainly methyl glycolate and methyl lactate were also obtained in the product distributions that appeared before 4 min in TIC (Figure 1). For comparison, a blank experiment without H₂WO₄ (Table 1, Entry 1) was also carried out under the same reaction conditions. The delignification degree and total monomer yield decreased sharply to 69.1% and 17.1 wt% respectively. Moreover, cellulose retention and hemicellulose removal rate were very low as compared with that of H₂WO₄ catalyzed reaction. Similarly, monomer distribution also differed, for instance yield of 4-allylsyringol (1.4 wt%) was higher, from the result with H₂WO₄. These results clearly showed that H₂WO₄ was an effective catalyst not only for the delignification process, but also for improving the selectivity towards methyl p-coumarate and methyl ferulate (Table 2).

3.3. Effects of temperature
Table 3 summarized the results of corn stover fractionation in methanol with or without catalyst at different temperatures. The trend of delignification degree, hemicellulose removal rate and cellulose retention were also shown in Figure 2(a), 2(b) and 2(c). As reaction temperatures raised from 180 °C to 260 °C, both delignification degree and hemicellulose removal rate were increased, whereas the rate of cellulose retention was declined, on both the H$_2$WO$_4$-free and H$_2$WO$_4$-catalyzed reactions. However, due to the partial sugar solubilization, as observed by Anderson et al. [42], delignification degree (>100%, Figure 2(a)) as well as lignin oil yield (>387 mg, Table 3) exceeded than the theoretical limit at higher temperature (>220 °C). Char formation was also started to observe when the reaction temperature was beyond 240 °C, which agreed with the previous work with lignin or its model compounds in methanol[20, 27].

Table 4 summarized the monomer yields in methanol on H$_2$WO$_4$ and H$_2$WO$_4$-free catalyst at different temperatures. For both with and without H$_2$WO$_4$, the yields of three major products namely methyl $p$-coumarate, methyl ferulate and 4-ethyl guaiacol raised from 180 °C to 200 °C, though their yields decreased and inversely increased the undesired condensed products from 220 °C to 260 °C. The yield of etherified product of methyl $p$-coumarate (0.2 to 1.2 wt% on H$_2$WO$_4$-free catalyst) raised specifically at the temperature range of 200-260 °C. Methyl $p$-coumarate was decreased, while 4-ethyl phenol appeared at higher temperatures (240-260 °C).

Aliphatic compounds yield in organic phase raised promptly with H$_2$WO$_4$ from 220 °C (12.3 mg) to 240 °C (56.4 mg), though they only formed beyond the supercritical temperature of methanol (239 °C) with a yield of 2.2 mg at 240 °C and 30.4 mg at 260 °C without catalyst.

3.4. Effects of reaction time and particle size

To optimize the fractionation process of corn stover, the effects of reaction time were also investigated. As shown in Table 5, more than 91% delignification degree could be achieved in 1 h. Prolonged reaction time from 8 to 24 h slightly increased the delignification process. After reaction for 24 h, the delignification degree was 100.5%
owing to the part of the sugar solubilization of corn stover. As shown in Table 6, the lignin monomers yield increased upon increasing reaction time. In more details, the total lignin monomers yield were steadily raised from 13.4 wt% in 1 h to 25.1 wt% in 6 h; on further increasing the reaction time (>8 h), decrease of lignin monomer yield was observed until 24 h. Notably, 4-allylsyringol was obtained as one of the major products (at 1 h) with 2.6 wt% yield that diminished gradually (until 8 h) and completely disappeared after 12 h.

In addition, the effects of corn stover particle sizes (0.0125 to 5 mm) were also examined. Table S2 and Table S3 show that the corn stover particle size had almost no influence on the degree of delignification and the production of monomers, though hemicellulose removal rate was greatly reduced to 49.8% from 78.7% by changing the particle size of corn stover from 0.0125 to 5 mm, respectively.

3.5. MALDI-TOF of lignin oil

MALDI-TOF MS results give the insight on the change of the molecular weight of the lignin fragments. Figure 4 displayed the MALDI-TOF MS of lignin oil samples that obtained from different reaction temperature in the range of 180-260 °C over H2WO4. When the reaction temperature between 180 to 200 °C, the fragments with the m/z of ~50-200 were identified. With increasing reaction temperature to 220-260 °C, however, the undesired condensation reactions dominate on the 4-allylsyringol and 4-ethylphenol/guaiacol monomers, and thus their products yield decreased and/or vanished (Table 4).

3.6. 2D-HSQC-NMR of lignin oil

To gain structural information of lignin inter-unit linkages, the lignin oil samples at different reaction temperature (Figure 5) were also done by 2D-HSQC-NMR spectra. Figure 5(a-c) exhibited prominent signals corresponding to β-O-4 ether linkages (A). As shown in Figure 5(a), for the lignin oil obtained at 200 °C without catalyst, cross signal of methoxyl group (OMe) was found at δc/δH 56.02/3.78 ppm. The Cα-Hα
correlations in β-O-4 of structure A (Aα) were observed at δ\textsubscript{C}/δ\textsubscript{H} 72.64/4.96 ppm, while the C\textsubscript{β}-H\textsubscript{β} correlations (Aβ) were observed at δ\textsubscript{C}/δ\textsubscript{H} 81.1/4.22 ppm. However, with increasing reaction temperature from 180 to 200 °C, both Aα and Aβ signals corresponding to the β-O-4 ether bond were decreased and were completely vanished after 240 °C (Figure 5(b-d)). Medium signals for resinol substructures (B) appeared at δ\textsubscript{C}/δ\textsubscript{H} 86.09/4.86 ppm for C\textsubscript{α}-H\textsubscript{α} correlations (Bα) and δ\textsubscript{C}/δ\textsubscript{H} 56.00/3.09 ppm for the C\textsubscript{β}-H\textsubscript{β} (Bβ), besides phenylcoumaran substructures (Cα) with weak signals (Figure 5(b)) was observed only to lignin oil, which was obtained at 180 °C with H\textsubscript{2}WO\textsubscript{4}.

4. DISCUSSION

“Lignin first” biorefinery strategy for corn stover was investigated by varying different parameters such as reaction temperature, time, particle size of corn stover in presence and absence of H\textsubscript{2}WO\textsubscript{4} catalyst. Without tar or char, nearly complete delignification of corn stover along with cellulose pulp were achieved in methanol over H\textsubscript{2}WO\textsubscript{4} at 200 °C for 6 h. Both reaction temperature and time have significant effects on the degree of delignification and the monomer yield, although the particle size of corn stover had no influence on them. In this process, owing to its high solubility in organic solvents, lignin was fractionated from corn stover in methanol through solvolysis, prior to hemicellulose and cellulose fractionation, in the lignocellulose valorization process. It is widely accepted that bulk lignin is fragmented into smaller segments via solvolysis in the first step. Subsequently, the fragmented lignin species underwent depolymerization to form monomers and oligomers on the catalyst surface in the second step [18, 33, 45]. As confirmed with 2D-HSQC-NMR (Figure 5), the β-O-4 ether linkages in lignin oil were significantly diminished with increase of reaction temperature (from 180 to 200 °C) and completely disappeared to form small monomers after >200 °C. Moreover, H\textsubscript{2}WO\textsubscript{4} also promotes the solvolysis reaction by continuously consuming the formed oligomers into monomers.

In fact, lower alcohols such as methanol and ethanol as H-donor solvents always exhibit remarkable activity for solvolysis reaction in lignin valorization[11, 18, 45-
Scheme 1 gives the proposed corn stover reaction pathway of the first step in methanol. Methanol can be dissociated to form H\(^+\) and CH\(_3\)O\(^-\) ions. The ester bonds between lignin and hemicellulose were broken through an “addition-elimination” process, while the ether bonds were broken via the S\(_2\)2 substitution reaction. Besides the linkages between hemicellulose and lignin, some of the ester and ether bonds within lignin units were also broken, forming organic-soluble oligomers. High temperature favored the dissociation of methanol and improved the solvolysis and its subsequent reactions. Nevertheless, the cellulose retention declined at >220 °C, which may be due to the decomposition of cellulose on the acid sites of H\(_2\)WO\(_4\). To further confirm, the obtained cellulose residues over the different reaction temperature (from 180 to 260 °C) in presence of H\(_2\)WO\(_4\) were analyzed with scanning electron microscopy (SEM) to compare their microstructure and micromorphology with that of corn stover. After treating corn stover with methanol over H\(_2\)WO\(_4\) at 180 °C (Figure 3(b)) and 200 °C (Figure 3(c)), the fibrous structures were almost retained as similar as to corn stover (Figure 3(a)). However, the fibrous structure was started to be destroyed at 220 °C (Figure 3(d)), and completely changed to small fragments at >220 °C. This phenomenon was also in accordance with the TGA result, as shown in Figure S1, which suggested the decomposition of corn stover fraction occurred at about 220 °C.

As shown in Scheme 2, the monomers were formed through the depolymerization of lignin fraction in the second step. The lignin underwent a dehydration-hydrogenolysis reaction\([48, 49]\) to break the β-O-4 ether bonds to form lignin monomers such as methyl p-coumarate, methyl ferulate and 4-allylsyringol. Song et al. \([33]\) demonstrated that the existence of active hydrogen atoms in methanol could saturate the α-β C=C bond over Ni/C without the addition of external hydrogen. Due to the electron receptor effect of ester groups, the side α-β C=C bond of methyl p-coumarate and methyl ferulate for hydrogenation were not as active as that of their corresponding decarboxylated products of 4-ethylphenol and 4-vinylguaiacol, respectively (Scheme 2). On the other hand, Arnold et al. \([50]\) demonstrated that thermal decarboxylation reaction proceed via the α-β C=C on carboxylate group.
Interestingly, the side α-β C=C bond of 4-vinylphenol/guaiacol was fully saturated and converted into 4-ethylphenol/guaiacol without the addition of H₂WO₄ catalyst, while main products namely methyl p-coumarate and methyl ferulate were still largely remained in the product distributions. Moreover, H₂WO₄ is likely to be suppressed the decarboxylation reaction, and thus enhanced the selectivity of methyl hydroxycinnamates. 4-Allylsyringol is likely to be recondensed over H₂WO₄ or at higher temperature to form phenolic derivatives.

The total monomer yields were decreased at >200 °C (Table 4, Entry 8-10), which may be attributed to the condensation reaction between the formed small monomers on the acid sites. MALDI-TOF MS of lignin oil confirms that the molecular weight distribution pattern of m/z shifted to higher range of m/z with increasing reaction temperature from 200 to 260 °C (Fig. 4). This was attributed mainly to the condensation reaction between the formed small monomers of 4-allylsyringol or 4-ethylphenol/guaiacol over acid catalyst [5]. However, the yield of etherified methyl p-coumarate increased (1 wt%) until the reaction temperature of 240 °C, which suggested the alkylation reaction on methyl p-coumarate. 4-Ethyl phenol appeared only in the higher temperature range of 240-260 °C through the similar mechanism with the formation of 4-ethylguaiacol. Side C=C bond hydrogenation of methyl p-coumarate occurred mildly at 260 °C without any hydrogenation catalyst. This phenomenon suggested that the active hydrogen atom from methanol became more reactive in higher temperature especially beyond its supercritical temperature (239 °C). Aliphatic compounds yield in organic phase raised promptly with H₂WO₄ from 220 °C to 240 °C, which indicates the conversion of polysaccharide into organic-soluble products. However, on further increasing temperature to 260 °C, these compounds were further decomposed into smaller molecules in gas phase and thus decreased the aliphatic compounds yield (Table 4, Entry 8-10).

5. CONCLUSION

In summary, corn stover fractionation and its subsequent conversion of lignin fraction to monomers were achieved simultaneously with high efficiency through
“lignin first” concept. The highest degree of delignification of 98.5% along with lignin monomers yield of 25.1 wt% and cellulose retention of 82.2% were achieved in methanol at 200 °C for 6 h over H₂WO₄ without the addition of external hydrogen gas. Methanol served as solvents for lignin first approach as well as H-donor for hydrogenation of monomers containing α-β C=C bonds in the side chain. Ether and ester bonds between lignin and hemicellulose of corn stover were cleaved to form lignin oligomers through methanol solvolysis reaction, while H₂WO₄ promotes the depolymerization of lignin oligomers into methyl p-coumarate and methyl ferulate predominately without tar or char formation. Both TGA and SEM results suggested that the decomposition of corn stover or its fractions was occurred at about 220 °C, thus the lignin monomers yield and cellulose retention were decreased in the temperature range >220 °C. However, increasing the particle size of corn stover had no influence in delignification process but hindered the removal of hemicellulose.

Credit author statement:
Yongdan Li initiated the research program and has been the supervisor of the project as well as the first author.
Hong Chen supplied a part of the funding and guided the experimental design and has been the advisor of the first author.
Saravanan Kasipandi polished the manuscript and responded the referee’s comments.
Zewei Ma carried out the research and wrote the first version of the manuscript.
Zhe Wen, Linhao Yu, and Kai Cui joined the experiments and contributed the ideas of the research.

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

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REFERENCE

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<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Total Liquid Products Mass (mg)</th>
<th>Lignin Oil Mass (mg)</th>
<th>Delignification Degree (%)</th>
<th>Cellulose Retention (%)</th>
<th>Hemicellulose Removal Rate (%)</th>
<th>Residue Massb (mg)</th>
<th>Recovery (%)</th>
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<td>-</td>
<td>410</td>
<td>269</td>
<td>69.1</td>
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aReaction conditions: 2000 mg corn stover, 500 mg catalyst, 100 mL methanol, 600 rpm, 6 h. bMass of the residue excluded catalyst and moisture.
Table 2. Yields of lignin monomer in methanol at 200 °C

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>H (%)</th>
<th>G (%)</th>
<th>S (%)</th>
<th>Irregular</th>
<th>Total Monomer (wt%)</th>
<th>Aliphatic Compound in Organic Phase (mg)</th>
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Reaction conditions: 2000 mg corn stover, 500 mg catalyst, 100 mL methanol, 600 rpm, 6 h.
### Table 3. Fractionation of corn stover at different reaction temperature$^a$

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<th>Entry</th>
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<th>Total Liquid Products Mass (mg)</th>
<th>Lignin Oil Mass (mg)</th>
<th>Residue Mass$^b$ (mg)</th>
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$^a$Reaction conditions: 2000 mg corn stover, 500 mg H$_2$WO$_4$, 100 mL Methanol, 600 rpm, 6 h. $^b$Mass of the residue excluded catalyst and moisture.
<table>
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<th>Entry</th>
<th>Catalyst</th>
<th>Temperature (°C)</th>
<th>H (wt%)</th>
<th>G (wt%)</th>
<th>S (wt%)</th>
<th>Irregular others (wt%)</th>
<th>Total Monomer (wt%)</th>
<th>Aliphatic Compounds in Organic Phase (mg)</th>
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*Reaction conditions: 2000 mg corn stover, 500 mg H₂WO₄, 100 mL Methanol, 600 rpm, 6 h.*
Table 5. Fractionation of corn stover at different reaction time

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (h)</th>
<th>Total Liquid Products Mass (mg)</th>
<th>Lignin Oil Mass (mg)</th>
<th>Delignification Degree (%)</th>
<th>Residue Mass (mg)b</th>
<th>Recovery (%)</th>
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<td>975</td>
<td>97.6</td>
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aReaction conditions: 2000 mg corn stover, 500 mg H$_2$WO$_4$, 100 mL methanol, 200 °C, 600 rpm.
bMass of the residue excluded catalyst and moisture.
Table 6. Effects of reaction time on the lignin monomer yields$^a$

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (h)</th>
<th>H</th>
<th>G</th>
<th>S</th>
<th>Irregular</th>
<th>Total Monomer (wt%)</th>
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<td>0.5</td>
<td>3.5</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$Reaction conditions: 2000 mg corn stover, 500 mg H$_2$WO$_4$, 100 mL methanol, 200 °C, 600 rpm.
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Scheme 2. Proposed reaction pathway of depolymerization of lignin fraction in methanol over H$_2$WO$_4$ catalyst in the second step.