# Ferric chloride aided peracetic acid pretreatment for effective utilization of sugarcane bagasse

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## Abstract

The synergetic impacts of ferric chloride aided peracetic acid (FPA) pretreatment were investigated to enhance the total biomass utilization through effective cellulose conversion and high-quality lignin production. The sugarcane bagasse pretreatment with 2% peracetic acid (PAA) and 0.1 mol/L ferric chloride ($FeCl_3$) effectively removed 51.3% of lignin and 72.2% of xylan while preserving ~97% of cellulose from sugarcane bagasse under mild temperature (90 °C). The FPA pretreated sugarcane bagasse was effectively hydrolyzed with a glucose yield of 313.0 mg/g-biomass, which was 4.5 times higher than the yield of untreated biomass (69.75 mg/g-biomass) and 1.6 and 3.6 times higher than that of individual PAA and $FeCl_3$ pretreated sugarcane bagasse, respectively. The regenerated lignin (FPA lignin) showed great potential for further valorization by preserving the major interunit linkage (up to 86% of β-O-4) without significant carbohydrate contamination and lignin condensation due to its mild reaction conditions. In this study, the combination of PAA and $FeCl_3$ synergistically enhanced the pretreatment efficiency on sugarcane bagasse and resulted in high fermentable sugar and high-quality lignin production.



Keywords: Sugarcane bagasse; Peracetic acid; Pretreatment; Ferric chloride.

## 1. Introduction

Due to the rapid depletion of fossil resources and associated environmental concerns, the demand for alternative and renewable sources has been increased [1]. Because of its abundance supply, sustainability, and renewability, lignocellulosic biomass has been highlighted as a potential feedstock for biofuels, functional materials, and green chemicals [2]. Carbohydrate polymers, especially cellulose in the plant, can be converted into biofuels via diverse biological pathways [3], [4], while non-carbohydrate components like lignin are challenging to convert biologically and even limit the enzyme access to cellulose [5]. In particular, lignin, an aromatic macromolecule mainly comprised of sinapyl, coniferyl, and *p*-coumaryl alcohols, is a major recalcitrance factor in the biological conversion of biomass due to its physical hindrance of the enzyme access to cellulose, enzyme deactivation through non-productive binding to enzymes, and its toxicity to microorganisms [6], [7]. Therefore, it is essential to explore an effective pretreatment of lignocellulosic biomass to reduce this recalcitrance factor for enhancing cellulose conversion.

Sugarcane bagasse is the residue after the extraction of sugarcane juice in the sugar industry. According to the United Nations Food and Agriculture Organization, China is the third biggest producer of sugarcane after Brazil and India (FAO, http://www.fao.org/faostat). Taking the example of Guangxi province in southern China, it accounts for ~65% of sugarcane output in China [8]. At present, sugarcane bagasse is widely utilized for animal feed, papermaking, and burning to supply heat or energy. Sugarcane bagasse is also a viable feedstock for biofuel production because of its high carbohydrate content and yearly output capacity. Sugarcane bagasse is mainly composed of glucan (35–45%), xylan (20–30%), and lignin (15–25%) [9], [10]. Therefore, in terms of the economic and environmental aspects, sugarcane bagasse is a

desirable feedstock in biofuels and biochemical production.

Pretreatment is an essential step for biofuel production from lignocellulosic biomass due to its natural resistance factors [11]. For effective conversion of lignocellulose, many pretreatment strategies have been exploited over the last several decades of research, such as dilute acid [12], [13], alkali [14], [15], ionic liquid [16], [17], deep eutectic solvent [18], [19], acid hydrotrope [20], and organosolv [21], [22] pretreatments. Peracetic acid (PAA) has been used in the pulping and bleaching industries. Peracetic acid (PAA) has a relatively weaker O−O bond dissociation energy (159 $kJ \cdot mol^{-1}$) than hydrogen peroxide (213 $kJ \cdot mol^{-1}$) [23], [24] and can intensively generate radicals. According to the previous studies, homolytic cleavage of the O-O bond in $CH_3C(=O)O-OH$ can generate acetyloxyl and hydroxyl radicals as below (1) [23], [25].

$$CH_3C(=O)O_2H \rightarrow CH_3C(=O)O^{\bullet} + {}^{\bullet}OH \qquad (1)$$

Strong nucleophile radicals can promote lignin decomposition and dissolution by reacting with the nucleophilic sites, including the aromatic ring and the aliphatic side chain of lignin [26]. PAA pretreatment showed effective delignification on biomass in previous studies [27]. However, a long reaction time (e.g., 26.5 h) or relatively high temperature (e.g., 130 °C) was needed to remove lignin effectively, and these severe conditions with peracetic acid caused the unwanted degradation of cellulose. Lewis acids have also been introduced as eco-friendly and economical catalysts for acidic biomass pretreatment due to their nontoxicity and reusability [28]. Lewis acid can form more hydrogen ions ($H_3O^+$) in an aqueous solution, which results in the destruction of biomass structures [29]. The generated $H_3O^+$ has relatively weak acidity. Therefore, Lewis acid pretreatment showed better preservation of cellulose during the pretreatment compared to mineral acids and made it easier to maintain the equipment from corrosion [29], [30]. These hydrogen ions produced by Lewis acids depolymerize hemicellulose

into monosaccharides. For instance, Shen et al. [31] effectively removed hemicellulose from *Eucalyptus camaldulensis* by $AlCl_3$ catalyzed hydrothermal pretreatment. Wei et al. [32] reported that $ZnCl_2$ hydrate pretreatment selectively extracted hemicellulose in eucalyptus as well as converted cellulose I to cellulose II. Similarly, Zhang et al. [33] deconstructed sugarcane bagasse by $FeCl_3$ pretreatment, which resulted in nearly 100% hemicellulose removal. However, in general, Lewis acid-catalyzed hydrothermal pretreatment has a limited delignification impact; therefore, co-solvents have been applied for the further enhancement of biomass pretreatment. The combination of $FeCl_3$ and ethanol pretreatment effectively improved the glucan conversion in sugarcane bagasse by up to 93.8% [34]. Also, Wei et al. [10]reported that $FeCl_3$ catalyzed ethylene glycol pretreatment resulted in 92.3% of cellulose recovery and 63.3% of delignification from sugarcane bagasse at 130 °C. In this study, PAA was applied with $FeCl_3$ to further improve its pretreatment effects on sugarcane bagasse.

For accomplishing a successful biorefinery strategy with lignocellulosic biomass, effective utilization of lignin is also crucial. In particular, the quality of lignin (e.g., high purity, preservation of β-O-4 linkages, less condensation) is as important as its fractionation yield in its subsequent processes for the production of fuels and chemicals [35], [36], [37]. Shuai et al. [38] reported a formaldehyde-activated lignin stabilization through 1,3-dioxane structures with lignin side-chain OH-groups. Catalytic reductive depolymerization of formaldehyde stabilized lignin resulted in a phenolic monomer yield close to theoretical. Similarly, Lan et al. [39] applied acetaldehyde and propionaldehyde to reduce the unwanted lignin condensation and preserve more β-O-4 linkages that result in higher monoaromatic yields from lignin. In this study, $FeCl_3$ catalyzed PAA (i.e., FPA) pretreatment was developed to fractionate high-quality lignin and enhance the biological conversion efficiency of cellulose from sugarcane bagasse under mild

reaction conditions. The impacts of FPA pretreatment on sugarcane bagasse were investigated by the selected characterization and evaluation methods including chemical composition changes, structural properties of cellulose and lignin, and enzymatic digestibility of pretreated biomass based on our previous studies [7], [40]. The degree of polymerization (DP) of cellulose and the cellulose content of cellulose-enriched solids were measured to explain the enhancement of glucose yield via the pretreatment. Also, structural properties of lignin, including molecular weight distribution and the contents of aromatic units and interunit linkages, were analyzed using gel permeation chromatography (GPC) and two-dimensional (2D) $^{1}H$-$^{13}C$ heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) for evaluating the quality of lignin for its post-applications.

## 2. Material and methods

### 2.1. Materials

Sugarcane bagasse (Guangxi, China) was aired-dried, milled to 20-mesh-size by a Wiley mill (MF10, IKA, Guangzhou, China), and stored in a sealed bag until used for the experiments. The fibers have around 26.3% xylan, 39.5% glucan, and 25.0% lignin. Peracetic acid solution (32 wt.%), acetic acid (≥ 99%), ferric chloride ($FeCl_3$), Cellic CTec2 enzyme, and deuterated dimethyl sulfoxide (DMSO-$d_6$) were purchased from Sigma. Other chemicals, including tetrahydrofuran (THF), acetyl anhydride, pyridine, and sodium acetate, were purchased from VWR and Fisher Sci.

### 2.2. Ferric chloride aided peracetic acid (FPA) pretreatment of sugarcane bagasse

Sugarcane bagasse pretreatments were conducted using a 300-mL Parr reactor (Parr Instrument Company) equipped with a temperature sensor and a mechanical stirrer. Air-dried sugarcane

bagasse (10g) was mixed with 100 mL of pretreatment solvent (2 wt.% PAA and/or 0.1mol / L ferric chloride). The pretreatment reaction was conducted at 70 – 130 °C with 250 rpm agitation for 30 – 120 minutes. The applied PAA concentration was determined by our preliminary study (data not shown), and $FeCl_3$ loading was based on the previous study [33]. Once the pretreatment was completed, the Parr reactor was immediately removed from the heating mantle and cooled down to room temperature in the ice bath. The pretreated biomass was recovered by vacuum filtration and further washed with deionized (DI) water until the filter was clear and colorless. The pretreated biomass solids were collected for chemical composition, cellulose degree of polymerization (DP), and enzymatic hydrolysis analysis. The hydrolysate was poured into DI water and stored at 5 °C for 24 h to precipitate lignin. After the precipitation, the lignin samples were obtained by centrifugation, washed with DI water, and lyophilized at −55 °C.

### 2.3. Enzymatic digestibility test

Enzymatic hydrolysis was conducted with untreated sugarcane bagasse and cellulose-enriched solid residues recovered from the pretreatments at a 2% solid loading in sodium acetate buffer (50 mM, pH 4.8) for 72 h. The slurry was agitated at 150 rpm and 50 °C. Enzyme loadings were 20 FPU (filter paper unit)/g dry substrate for Cellic CTec2 (Novozymes). The activity of Cellic CTec2 (90 FPU/mL) was measured as described in the previous study [41]. In brief, 500 μL enzyme was loaded in 1 mL buffer with 50 mg filter paper (1.0 × 6.0 cm) to determine filter-paper cellulase activity. The amount of the generated reducing sugar was measured by 3,5-dinitrosalicylic acid (DNS) assay. The hydrolysates were periodically collected in vials and heated in the block heater at 95 °C for 5 min to deactivate the enzymes and stop hydrolysis. The hydrolysate samples were then centrifuged at 10,000 rpm for 5 min to remove the solids, and the supernatant samples were

filtered through a 0.22 $\mu$m polytetrafluoroethylene (PTFE) filter. At a minimum, all analyses were carried out in duplicates. The test was conducted in duplicate.

### 2.4. Biomass compositional analysis

Biomass compositional analysis was performed with untreated and pretreated sugarcane bagasse according to the standard procedure [42]. Carbohydrate contents in solid residues and hydrolysates generated from enzymatic hydrolysis were analyzed using a high performance liquid chromatography (HPLC, YL 9100, Young-Lin, Seoul, Korea) with a refractive index detector and a Bio-Rad Aminex HPX-87H column at 60 °C. The mobile phase used was 5 mM $H_2SO_4$ with a 0.6 mL/min flow rate. The carbohydrates (i.e., glucose and xylose) contents were determined by the calibration curves with external standards (i.e., pure glucose and xylose). The calculation formula for xylan/lignin removal was displayed as follows:

$$\text{Solid recovery}\,(\%) = \frac{\text{Pretreated dry biomass}\,(\text{g})}{\text{Raw dry biomass}\,(\text{g})} \times 100\,\% \quad (1)$$

$$\text{Glucan recovery} = \frac{\text{Glucan in pretreated solid}\,(\text{g})}{\text{Initial glucan in raw biomass}\,(\text{g})} \times 100\,\% \quad (2)$$

$$\text{Xylan/lignin removal}(\%) = \frac{\text{Xylan/lignin in raw biomass} - \text{Xylan/lignin in pretreated solid}}{\text{Xylan/lignin in raw biomass}} \times 100\,\% \quad (3)$$

### 2.5. Degree of polymerization (DP) of cellulose analysis

The degree of polymerization of cellulose was measured based on a previous report [43]. In brief, holocellulose was isolated from biomass samples (0.6 g) by loading in the mixture of 9.375

g peracetic acid (32%) and 2 g DI water at 25 °C for 24 h. Air-dried holocellulose samples were treated with 17.5% NaOH solution (5 mL) at 25 °C for 2 h and then diluted with an additional 5 mL of DI water for another 2 h of extraction. The sample was centrifugated at 6,000 rpm for 10 min to recover α-cellulose. The recovered α-cellulose was washed with 1% acetic acid (50 mL) and an excessive amount of DI water and freeze-dried. For the GPC analysis, the recovered α-cellulose (8 mg) was derivatized with anhydrous pyridine and phenyl isocyanate at 70 °C for 72 h reaction. After that, the mixture was poured into a methanol/water (7:3) solution to precipitate the cellulose derivatives. The cellulose derivatives were dried in 40 °C vacuum oven. The cellulose molecular weights were analyzed by a GPC system (Waters 2498) with three Waters Styragel columns (HR 0.5, HR 3, and HR 4E) and a UV detector. The GPC was set at 25 °C with 1 mL/ min tetrahydrofuran (THF) as a mobile phase. The cellulose derivative was dissolved in 1 mL of THF solution and then filtered through 0.45 μm PTFE filters. The detector was performed at 280 nm for detecting the cellulose derivatives. Calibration was conducted with polystyrene standards. The molecular weight of the derivatized cellulose repeating unit (519 g $mol^{-1}$) was used to calculate the weight-average degree of polymerization ($DP_w$) of cellulose.

**2.6. GPC analysis for molecular weight distribution of lignin**

The molecular weights and distribution of lignin in untreated and pretreated sugarcane bagasse were analyzed using a GPC. About 2 mg lignin sample was acetylated in acetic anhydride/pyridine mixture (1:1, v/v) for 24 h in a dark environment. At the end of the acetylation, the acetic anhydride/pyridine in the mixture were rotary evaporated with ethyl alcohol. The acetylated samples were dissolved in THF and analyzed using a Waters 2948 GPC system as described in section 2.5. The ball-milled sugarcane bagasse was extracted with 96

vol% of dioxane/water mixture and recovered as control lignin (i.e., milled wood lignin, MWL) from untreated biomass for monitoring the structural changes of lignin during the pretreatment.

### 2.7. NMR analysis for lignin structural properties

The structural properties of each lignin sample were analyzed using a 2D HSQC NMR system. The dried lignin (~25 mg) was dissolved in 0.5 mL of DMSO-$d_6$ solvent (referenced at 39.5/2.49 ppm) and then transferred to the NMR tube (5 mm). The structural properties of lignin samples were analyzed using a Bruker Avance III HD 800 NMR spectrometer equipped with a TCI cryoprobe. The 2D HSQC NMR was conducted with 160 ppm spectral width in the $^{13}C$ dimension with 512 data points, 1.2 s relaxation delay, and 32 scans and 12 ppm spectral width in the $^{1}H$ dimension with 1024 data points. For the lignin NMR data processing and exporting spectra, TopSpin 4.1.0 software was used. The volume integration of specific contours (e.g., $C_\alpha$, $S_{2/6}$, $G_2$, $H_{2/6}$) in the HSQC spectra of each lignin sample was conducted for semi-quantitative analysis of lignin composition (i.e., S/G/H and other aromatic units) and interunit linkages according to the previous study [44].

## 3. Results and Discussion

### 3.1. Biomass composition of FPA pretreated sugarcane bagasse

To understand the synergistic impact of $FeCl_3$ aid on PAA pretreatment, individual pretreatments (i.e., PAA and $FeCl_3$), as well as the combined pretreatment (i.e., FPA pretreatment), were performed with sugarcane bagasse under the same reaction condition (90 °C, 60 min). As **Table 1** presented, PAA pretreatment removed 40.6% of lignin and 58.2% of xylan, and $FeCl_3$ pretreatment selectively removed xylan (43.0%). The decrease of solid recovery was mainly attributed by the fractionation of hemicellulose and/or lignin in the pretreatments. Both

pretreatments preserved cellulose well (98.6% and 93.4% by $FeCl_3$ and PAA, respectively). These pretreatment effects of both methods (i.e., PAA and $FeCl_3$) were consistent with the results in previous studies [45], [46] but not as effective as the literature because of the mild pretreatment temperature (90 °C) in this study. However, further increases in pretreatment temperature caused unexpected cellulose loss in our preliminary study. For this reason, the $FeCl_3$ aided PAA pretreatment (PFA) was applied to improve the pretreatment effects without increasing the process severity. FPA pretreatment resulted in 61.0% solid recovery by better xylan and lignin removals without further glucan loss compared to the single component pretreatments above (38.7% in FPA pretreated biomass vs. 36.9% in PAA pretreated biomass vs. 38.9% in $FeCl_3$ pretreated biomass, glucan contents in untreated biomass dry weight basis). After the FPA pretreatment, the lignin removal was determined to be 57.3%, which is 3.6-fold higher than that of $FeCl_3$ pretreated biomass and 41.1% higher than that of PAA pretreated biomass. The xylan removal was 72.2% by FPA pretreatment, which is 24.1% and 67.9% higher than that of PAA pretreated biomass and $FeCl_3$ pretreated biomass, respectively. The results indicated that the PAF pretreatment effectively reduced the recalcitrance factors in sugarcane bagasse under mild conditions.

For the enhancement of pretreatment effects, different times (30 – 120 min) and temperatures (70 – 130 °C) were also tested with PAF pretreatment. However, as we noticed earlier, further increase of reaction severity (e.g., pretreatment temperature and time) did not significantly improve delignification but notably decreased the total solid recovery caused by unwanted cellulose loss (**Fig. S1**). Also, harsh pretreatment conditions possibly cause unwanted lignin modification and pseudo-lignin formation, which devalue lignin-based co-products [47].

**Table 1.** Biomass composition and cellulose degree of polymerization (DP) of untreated and pretreated sugarcane bagasse. The values of biomass composition are based on the dry weight of untreated biomass. Pretreatment conditions: FPA: 2% peracetic acid and 0.1 mol/L FeCl3, 90 °C, 1 h; PAA: 2% peracetic acid, 90 °C, 1 h; FeCl3: 0.1 mol/L FeCl3, 90 °C, 1 h.

| Pretreatment | Solid recovery (%) | Composition (%) | | | Glucan recovery (%) | Lignin removal (%) | Xylan removal (%) | Cellulose $DP_w$ |
|---|---|---|---|---|---|---|---|---|
| | | Glucan | Xylan | Lignin | | | | |
| Untreated | — | 39.5 ± 0.4 | 26.3 ± 0.6 | 25.0 ± 0.3 | 100 | — | — | 1934 |
| FPA | 61.0 ± 0.8 | 38.7 ± 0.3 | 7.3 ± 0.2 | 10.7 ± 0.1 | 97.9 | 57.3 | 72.2 | 1595 |
| PAA | 70.0 ± 1.1 | 36.9 ± 0.3 | 11.0 ± 0.2 | 14.8 ± 0.3 | 93.4 | 40.6 | 58.2 | 1767 |
| $FeCl_3$ | 79.3 ± 0.9 | 38.9 ± 0.4 | 15.0 ± 0.2 | 21.0 ± 0.7 | 98.6 | 15.9 | 43 | 1674 |

### 3.2. Degree of polymerization (DP) of cellulose in FPA pretreated sugarcane bagasse

In addition to the biomass composition, the weight-average degree of polymerization ($DP_w$) of cellulose was measured to understand the impacts of FPA pretreatment and other pretreatments on sugarcane bagasse (**Table 1**). The $DP_w$ of cellulose isolated from untreated sugarcane bagasse was ~1900, which is comparable to the previous study [48]. The $DP_w$ of cellulose in sugarcane bagasse was slightly reduced by the pretreatments. PAA pretreatment reduced the cellulose $DP_w$ to ~1800, $FeCl_3$ pretreatment reduced to ~1700, and FPA pretreatment lowered it to ~1600. These pretreatments partially depolymerized cellulose to some extent and transformed the biomass more amenable for enzymatic hydrolysis with the increase of reducing ends [49]. Although the FPA pretreated sugarcane bagasse has the lowest cellulose DP, the decrease in cellulose DP was not as significant as other pretreatments in the literature [50], [51], which is possibly due to the mild reaction conditions of this study.

### 3.3. Biological conversion of FPA pretreated sugarcane bagasse

Enhancement of enzymatic digestibility is the major purpose of biomass pretreatment. The production of glucose from untreated and pretreated sugarcane bagasse via enzymatic hydrolysis was presented in **Fig. 1**. Untreated sugarcane bagasse had low glucose release (69.75 mg glucose/g-biomass) due to its natural biomass recalcitrant factors such as lignin and hemicellulose. The effects of these two components in the native cell walls on enzymatic hydrolysis of biomass were investigated in previous studies [52], [53]. The $FeCl_3$ pretreatment did not significantly improve the glucose release, as it resulted in 87.75 mg glucose/g-biomass. PAA pretreated sugarcane bagasse showed a higher glucose release (190.25 mg glucose/g-biomass) than that of $FeCl_3$ pretreated biomass and untreated biomass. FPA pretreated biomass showed the highest glucose release (313.0 mg glucose/g-biomass) after 72 h enzymatic hydrolysis. The amount of glucose released from biomass showed the same order of delignification and xylan removal (**Fig. 1** and **Table 1**). The results indicate that the enhancement of glucose release could be related to the lignin and xylan content in the biomass. In our previous studies, cellulose accessibility was significantly increased by the delignification and the removal of hemicellulose [17] and directly correlated to glucose release [52]. Therefore, the observed results are consistent with previous observations. In addition, deconstruction and removal of lignin can reduce the chance of nonproductive cellulase adsorption and cleave lignin–carbohydrates complexes (LCCs) linkages, which affect the cellulose hydrolysis [54], [55]. As discussed earlier, cellulose DP was also changed by these pretreatments; however, a significant correlation between cellulose DP and glucose release was not observed in this study (**Table 1** and **Fig. 1**).

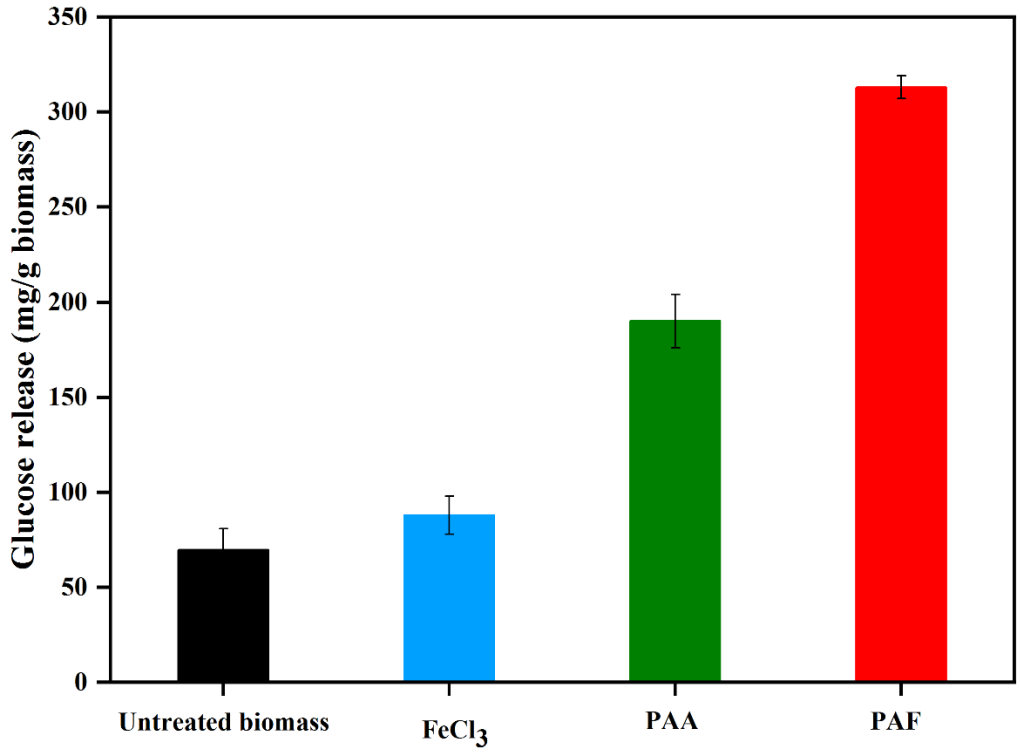


**Fig. 1.** Enzymatic saccharification of the untreated, ferric chloride ($FeCl_3$), peracetic acid (PAA), and $FeCl_3$ aided PAA (FPA) pretreated sugarcane bagasse after 72 hours enzymatic hydrolysis. Pretreatment conditions: $FeCl_3$: 0.1 mol/L $FeCl_3$, 90 °C, 1 h; PAA: 2% peracetic acid, 90 °C, 1 h; FPA: 2% peracetic acid and 0.1 mol/L $FeCl_3$, 90 °C, 1 h.

### 3.4. Characteristics of the FPA lignin

To maximize the utilization of biomass, valorization of the recovered lignin is essential. Characteristics of lignin provide essential information for its post-utilization. Moreover, the preservation of β-aryl ethers in the recovered lignin is important for its valorization. For instance, Lancefield et al. [56] reported that the lignin with high β-O-4 content showed better production than the one with low β-O-4 content in both chemo-catalytic and bio-catalytic conversion approaches. Therefore, the authors suggested mild treatments which can preserve the majority of β-O-4 linkages. Also, Bouxin et al. [57] reported that the β-O-4 linkage content influenced the yield and structure of the monomeric products when depolymerized by a metal-based catalyst.

However, lignin fragments can be condensed and form rigid C-C bonds during the pretreatment and recovery processes [58]. This condensed structure in the lignin can lower the production of monoaromatics and other products; therefore, this modification should be minimized during biomass pretreatment. In this study, aromatic units and interunit linkages as well as molecular weight distribution of lignin were measured to evaluate its quality. Because of the low delignification yield of $FeCl_3$ pretreatment, this lignin was not included in the lignin characterization study.

The structural properties of the fractionated lignin were analyzed by 2D HSQC NMR. The cross-peak assignments of native lignin (MWL) and fractionated lignin samples (i.e., FPA lignin and PAA lignin) were performed according to the previous publications [44]. The aliphatic region ($\delta_C/\delta_H$ 50–90/2.5–6.0 ppm) of the fractionated lignins in **Fig. 2** presents the predominate inter-unit linkages of lignins including β-aryl ethers (A), phenylcoumaran (B), and resinol (C). MWL of sugarcane bagasse had 36% of β-O-4 linkage, 2% of β-5, and 1% of β-β linkage based on total lignin subunit contents. The β-O-4 linkage content in lignin was reduced by the pretreatment conditions. The β-O-4 linkages of PAA and FPA lignin samples were slightly lower than that of MWL, which are 29% and 31%, respectively. However, these lignin samples still preserved 80.6% – 86.1% of β-O-4 linkage in MWL (**Fig. 2** and **Table 2**). This preservation is much higher than in previous studies with 46%–77.4% of the preservation of β-O-4 linkages in the untreated biomass [59], [60]. In this study, C-C bonds like β-5 and β-β linkage contents were notably changed by both PAA and FPA pretreatments.

The chemical shifts of S, G, and H units were observed at $\delta_C/\delta_H$ 103.8/6.70 ($S_{2,6}$), 110.8/6.97 ($G_2$), 114.6/6.95 ($G_5$), 119.0/6.70 ($G_6$), and 127.7/7.18 ($H_{2,6}$), respectively (**Fig. 3**). The cross peaks of *p*-coumarate (PCA) and ferulate (FA) in lignins were observed at $\delta_C/\delta_H$ 130.1/7.48

322 ($PCA_{2,6}$), 144.9/7.52 ($PCA_7$), 111.4/7.33 ($FA_2$), and 144.9/7.52 ($FA_7$). The cross peaks at
323 94.1/6.56 ($T_8$), 98.6/6.21 ($T_6$), 103.7/7.33 ($T'_{2,6}$), and 106.3/7.05 ($T_3$) from tricin (T) in each
324 lignin were also assigned in **Fig. 3**. In previous studies, acid pretreatment caused unwanted lignin
325 condensation [5], [61], [62]; however, mild reaction conditions in this study minimized the side
326 reaction in the fractionated lignin samples by PAA and FPA pretreatments. Interestingly, lignin
327 recovered from PAA pretreatment showed more H unit content compared with the untreated
328 lignin fraction (**Fig. 3** and **Table 2**). This result indicates the possible transformation of S and G
329 units to H units in a peracetic acid environment. Barros et al. [63] also reported demethylation of
330 lignin and quinone structure formation through hydroxylation of lignin by peracetic acid.
331 However, the H unit content was not increased by PAA in the co-existence of $FeCl_3$. Overall, the
332 FPA pretreatment fractionated lignin from sugarcane bagasse showed no significant interunit
333 linkage cleavage and formation of the condensed structures, as shown in **Figs. 2 and 3** and **Table**
334 **2**. Based on the delignification effects and characteristics of lignin, FPA pretreatment showed its
335 great potential to produce high-quality lignin, which is beneficial for depolymerizing into value-
336 added compounds.

337

338 **Table 2.** Aromatic unit and interunit linkage contents of untreated and fractionated sugarcane bagasse lignin.

| Content (%) | MWL | FPA lignin | PAA lignin |
|---|---|---|---|
| Syringyl (S) | 58 | 54 | 53 |
| Guaiacyl (G) | 40 | 44 | 37 |
| *p*-Hydroxyphenyl (H) | 2 | 2 | 10 |
| Ferulate (FA) | 6 | 10 | 5 |
| *p*-Coumarate (PCA) | 50 | 52 | 56 |
| β-O-4 | 36 | 31 | 29 |
| β-5 | 2 | 2 | 3 |
| β-β | 1 | 1 | 1 |

339 Note. Content is calculated as a fraction of total lignin subunits (S + G + H).
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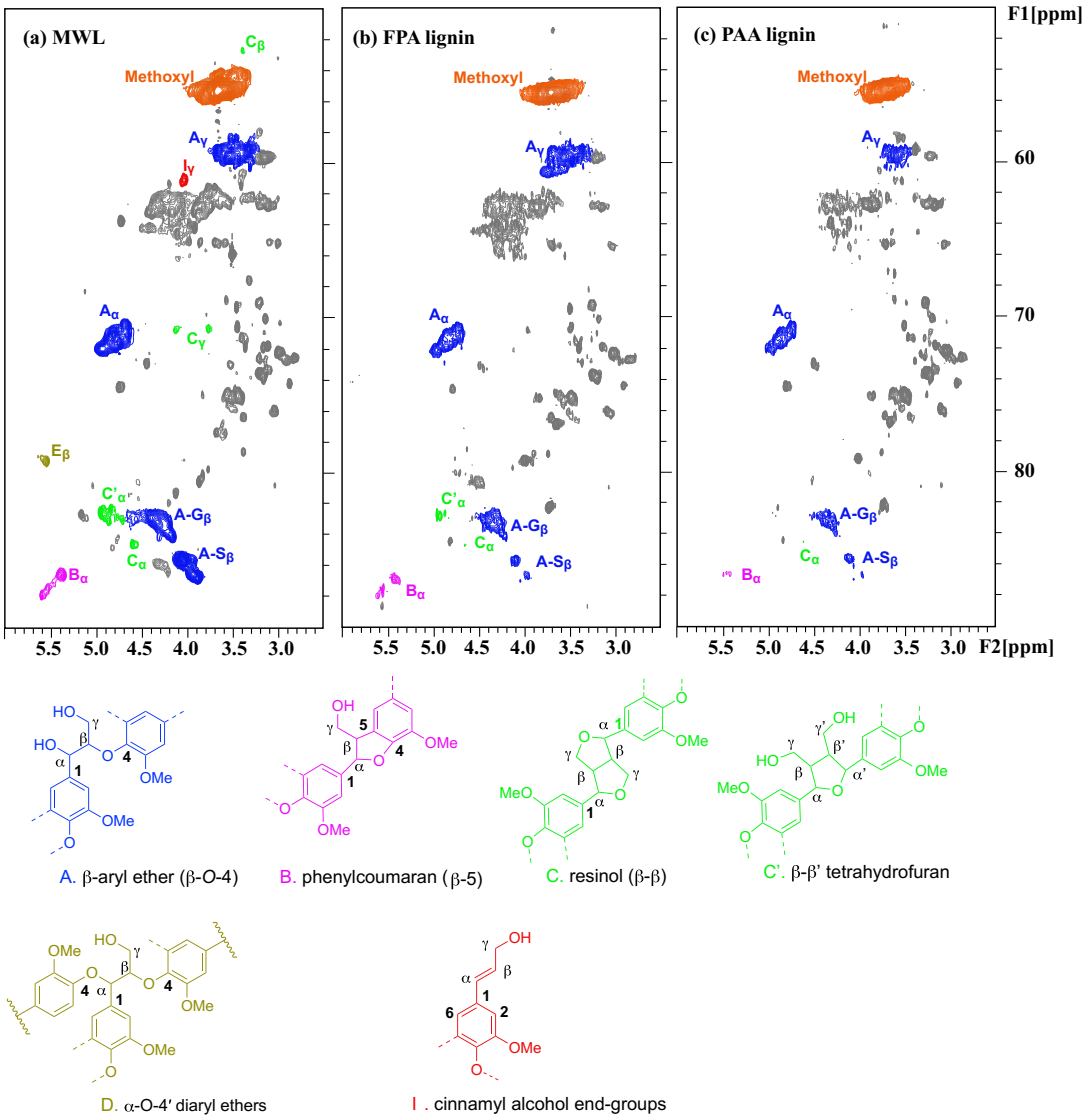


**Fig. 2.** HSQC NMR spectra for aliphatic region of fractionated lignins: (a) MWL, (b) FPA: 2% peracetic acid and 0.1 mol/L $FeCl_3$, 90 °C, 1 h, (c) PAA: 2% peracetic acid, 90 °C, 1 h.

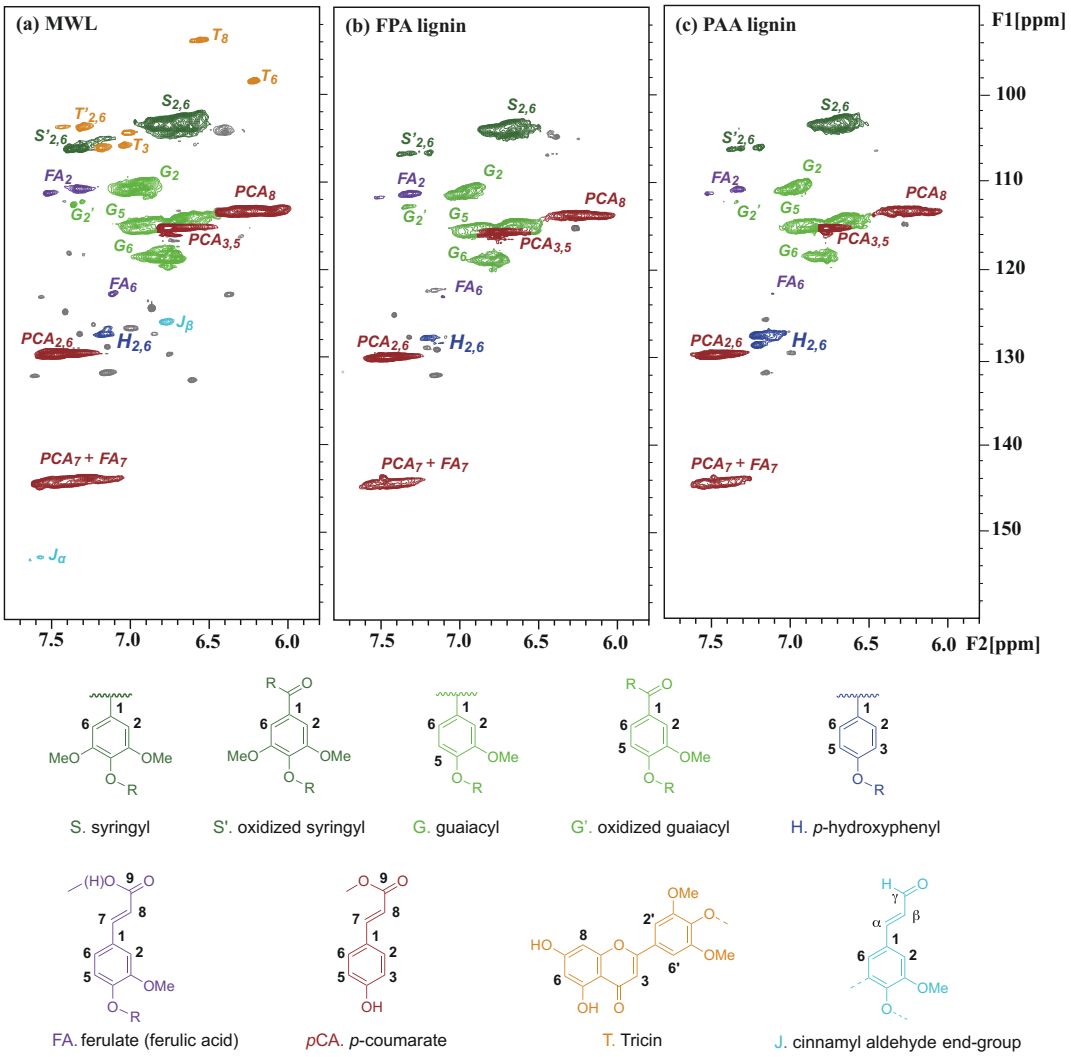


**Fig. 3.** HSQC NMR spectra for aromatic region of fractionated lignins: (a) MWL, (b) FPA: 2% peracetic acid and 0.1 mol/L $FeCl_3$, 90 °C, 1 h, (c) PAA: 2% peracetic acid, 90 °C, 1 h.

The molecular weight distribution of lignin is another property to be used for understanding the pretreatment effects and evaluating the quality of lignin. The weight-average ($M_w$), number-average molecular weight ($M_n$), and polydispersity index (PDI) of MWL and fractionated lignin samples were measured by GPC (**Table 3**). As the β-O-4 linkage cleavage was not significant according to the HSQC spectra in **Fig. 2** and **Table 2**, the $M_w$ of PAA lignin and FPA lignin were retained at similar values. Interestingly, the $M_w$ of PAA lignin was slightly lower, while that of

FPA lignin was higher than the value of MWL. However, the changes in the $M_n$ of these two lignin samples were the opposite. It indicated that the lignin decomposition occurred differently with the addition of $FeCl_3$. However, additional study is necessary to support this hypothesis. Because of these changes in $M_w$ and $M_n$ of lignin samples, the molecular weight distribution (i.e., PDI) was slightly increased after PAA pretreatment (1.73) while decreased after FPA pretreatment with higher PDI (3.32). Overall, FPA pretreatment could fractionate lignin into having similar properties to MWL, especially preserving a large portion of β-O-4 linkage without unwanted condensed structure. These lignin properties are desirable in its subsequential chemical conversion or biomaterial applications [64]; therefore, the investigated PAF pretreatment is promising in future biomass utilization.

**Table 3.** Molecular weight distribution of fractionated lignins.

| Samples | $M_w$ | $M_n$ | PDI |
|---|---|---|---|
| MWL | 6782 ± 56 | 3037 ± 20 | 2.2 |
| FPA | 7069 ± 9 | 2139 ± 12 | 3.3 |
| PAA | 6511 ± 37 | 3767 ± 22 | 1.7 |

## 4. Conclusions

This study shows the synergetic effects of PAA and $FeCl_3$ combined pretreatment on sugarcane bagasse characteristics and its enzymatic conversion into fermentable sugars. The FPA pretreatment resulted in superior effects on delignification and xylan removal compared to individual PAA and $FeCl_3$ pretreatments showed. The sugar release of FPA pretreated sugarcane bagasse was also significantly improved by the aforementioned pretreatment effects. The fractionated lignin showed a well-preserved intact structure such as relatively high β-O-4 linkage

content without condensation due to its mild reaction conditions (90 °C, 60 min), which can improve the lignin conversion efficiency in the subsequential utilization processes. The introduced FPA pretreatment can be a promising pretreatment strategy for the effective utilization of both carbohydrates and lignin in sugarcane bagasse.

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