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Enhanced Cellulase Hydrolysis of Eucalyptus Waste Fibers from Pulp Mill by Tween80-Assisted Ferric Chloride Pretreatment

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ABSTRACT: Pretreatment combining $FeCl_3$ and Tween80 was performed for cellulose-to-ethanol conversion of eucalyptus alkaline peroxide mechanical pulping waste fibers (EAWFs). The $FeCl_3$ pretreatment alone showed a good effect on the enzymatic hydrolysis of EAWFs, but inhibited enzyme activity to some extent. A surfactant, Tween80, added during $FeCl_3$ pretreatment was shown to significantly enhance enzyme reaction by eluting enzymatic inhibitors such as iron(III) that are present at the surface of the pretreated biomass. Treatment temperature, liquid−solid ratio, treatment time, $FeCl_3$ concentration, and Tween80 dosage for pretreatment were optimized as follows: 180 °C, 8:1, 30 min, 0.15 mol/L, and 1% (w/v). Pretreated EAWFs under such optimal conditions provided enzymatic glucose (based on 100 g of oven-dried feedstock) and substrate enzymatic digestibility of EAWFs of 34.8 g and 91.3% after 72 h of enzymatic hydrolysis, respectively, with an initial cellulase loading of 20 FPU/g substrate.

KEYWORDS: *waste fibers, ferric chloride pretreatment, Tween80, substrate enzymatic digestibility*

INTRODUCTION

Eucalyptus, a type of fast-growth tree grown in tropical or subtropical regions around the globe, represents a tremendous potential natural resource, providing fiber for pulp and paper-making industries.[1] Meanwhile, alkaline peroxide mechanical pulping (APMP) is a promising pulping method because of its advantages of high pulping yields, applicability to a wide source of materials, simplified process parameters, and reduced pollution.[2] During the APMP process for eucalyptus, there is a significant portion of waste fibers that cannot be used for making papers due to their short length. In general, eucalyptus waste fibers from an APMP plant accounts for 10% of the wood raw material. Conventional treatments of these waster fibers are mainly land-filling and burning, which severely pollute the environment and result in a severe underutilization of resources.[3] The obtained waste fibers of the eucalyptus by APMP processing still contain a high content of carbohydrates, but their physical structure is thin and very flexible. Thus, these features indicate that eucalyptus APMP waste fibers (EAWFs) can be readily pretreated for cellulose-to-ethanol conversion, which has proven to be a promising utilization of biomass.[4]

Biomass pretreatment to reduce the size of a raw material or modify the structure of biomass components is an important tool for practical cellulose-to-ethanol conversion processes.[5] In recent years, varieties of biomass pretreatments have been reported, such as uncatalyzed steam explosion, liquid hot water, dilute acid, inorganic salt, lime, and ammonia to remove or alter hemicellulose or lignin and improve enzymatic accessibility of the substrates. Sun and Cheng[6] have reviewed the advantages and limitations of a number of pretreatments. Among these pretreatment methods, dilute acids, such as H_2SO_4 and HCl, are regarded as powerful and effective agents for biomass pretreatment,[5,6] resulting from the efficient removal of hemicellulose from the biomass, improving the enzymatic accessibility of cellulose. However, inorganic acids are difficult to recycle and corrosive to equipment, resulting in commercial impracticality for biomass bioconversion. Similarly, ferric chlorides, performing as strong Lewis acids in water, are reported to efficiently remove hemicellulose from the biomass like inorganic acids do for biomass.[7] Moreover, $FeCl_3$ is more readily recycled and less corrosive to equipment than inorganic acids. Liu et al.[7] also reported that pretreatment with $FeCl_3$ could not only efficiently remove hemicelluloses but break down ether and ester linkages between lignin and carbohydrates in the raw material. However, during $FeCl_3$ pretreatment, the majority of lignin, byproducts, and iron ions remain in the pretreated solid residue, which may inhibit cellulase activity and reduce hydrolysis efficiency.[7−10] If these inhibitors can be removed after $FeCl_3$ pretreatment, the enzymatic digestibility of lignocellulosic materials may be enhanced. In this case, surfactant could be used in the pretreatment, as surfactant could lower the surface tension of water and change the interfacial property between different phases, probably increasing the inhibitors' solubility in water. There is also some research that has reported the reduction of the amount of lignin remaining on the pretreated material and acceleration of enzymatic hydrolysis by adding a nonionic surfactant to the pretreatment system.[11,12]

Therefore, pretreating EAWFs with $FeCl_3$ has been preliminarily performed to investigate its potential to pretreat EAWFs for cellulose-to-ethanol conversion. Then nonionic surfactant Tween80 was added in the $FeCl_3$ pretreatment to assess the enhanced effect. In the present research, we also detected changes in the crystallinity of pretreated EAWFs by X-ray diffraction (XRD), in functional groups of pretreated EAWFs by Fourier transform infrared spectroscopy (FTIR), and in the iron ions enclosed in pretreated EAWFs by X-ray

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Table 1. Recoveries of Solid and Glucan, and Glucan Content in Pretreated EAWFs at Varying $FeCl_3$ Pretreatment Alone Conditions

temperature, °C	ratio, v/w	time, min	$FeCl_3$, mol/L	initial pH	solid recovery, %	glucan content, %	glucan recovery, %
160	8	20	0.1	1.80	75.1	52.3 ± 1.7	90.5
170					74.4	49.0 ± 0.4	83.9
180					71.8	52.9 ± 1.3	87.5
180	6	20	0.1	1.80	72.4	48.9 ± 0.9	81.6
	8				71.8	52.9 ± 1.3	87.5
	10				71.6	52.4 ± 1.2	86.4
180	8	0	0.1	1.80	75.2	51.7 ± 1.3	89.5
		10			72.4	52.4 ± 0.3	87.3
		20			71.8	52.9 ± 1.3	87.5
		30			67.6	52.5 ± 1.0	81.8
		40			69.5	56.0 ± 0.9	89.7
180	8	30	0	7.62	89.3	44.2 ± 1.2	90.9
			0.05	2.02	74.2	53.4 ± 1.1	91.3
			0.1	1.80	67.6	52.5 ± 1.0	81.8
			0.15	1.68	62.5	55.5 ± 0.4	79.9
			0.2	1.58	58.5	54.5 ± 0.9	73.4

photoelectron spectroscopy (XPS) to provide a mechanism for Tween80-assisted $FeCl_3$ pretreatment.

MATERIALS AND METHODS

Materials. Eucalyptus APMP waste fibers were provided by a pulp and paper-making industry (Guangxi Jingui Co., China) without any other treatment. The glucan, xylan, and lignin contents of untreated EAWFs were 43.4%, 15.3%, and 25.5%, respectively. Chemicals used in this study were of analytical reagent grade. All experiments were performed in duplicate under the same conditions, and average values were reported.

Pretreatment of EAWFs. $FeCl_3$ pretreatment was carried out in a stirred autoclave (4530 series, Parr Co., USA) with a total volume of 1 L. The initial concentrations of $FeCl_3$ and nonionic surfactant (Tween80) for the pretreatment were chosen to be from 0 to 0.2 (mol/L) and 0 to 0.1% (w/v), respectively. Thirty grams of EAWFs was loaded in the reactor and mixed with the chemical agent solution in a liquid/solid ratio from 6:1 to 8:1 (v/w). Both solution and EAWFs were initially at room temperature and then heated at a rate of 6 °C/min to the set temperature. The pretreatment was done at three temperature levels in the range 160−180 °C at times ranging from 0 to 40 min. The reactor was immediately removed from the heating jacket and allowed to cool when the pretreatment was completed. The treated fibers were rinsed with water to prepare them for the enzymatic hydrolysis. The solid recovery and glucan content in the pretreated biomass were analyzed.

Determination of Glucan in Treated Fibers. Glucan contents of treated fibers were determined according to the National Renewable Energy Laboratory (NREL, Golden, CO, USA) analytical methods for biomass.[13] The glucan content of the treated fiber was determined based on monomer content measured after a two-step acid hydrolysis procedure to fractionate the fiber. A step with 72% (w/w) H_2SO_4 at 30 ± 3 °C for 60 ± 5 min was first used. In the second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 °C for 1 h. This hydrolysis liquid was then analyzed for glucan content by the glucose oxidase−peroxidase method (GOPM).[14] The glucan content (β_g) in pretreated fibers was calculated using eq 1.

$$\beta_g = 0.9c_gV/300(1 - w) \quad (1)$$

where c_g (g/L) is the concentration of glucose in the supernatant and w is the moisture of pretreated fibers. The factor that converts glucose to an equivalent glucan and the volume (V) of the hydrolysate are respectively 0.9 and 86.73 mL.

Enzymatic Hydrolysis. Pretreated fibers of 2% w/v were hydrolyzed by cellulase (Celluclast 1.5L) with an enzyme loading of 20 filter paper units (FPU)/g substrate in a 100 mL flask, and β-glucosidase (Novozyme 188), at an enzyme loading of 25 cellobiase units (CBU)/g substrate, was used to supplement the β-glucosidase activity of cellulose. The enzymatic hydrolysis of the reaction mixture (50 mL) was performed in a 0.05 M HAc/NaAc buffer (pH 4.8) at 50 °C on a rotary shaker at 150 rpm. Aliquots of 0.3 mL were taken at time points of 2, 6, 12, 24, 48, and 72 h, kept in boiling water for 1 min to inactivate the enzyme, and then centrifuged to remove water-insoluble solids. The supernatant of the samples was analyzed by GOPM for glucose concentration. The substrate enzymatic digestibility (SED) of pretreated fibers was described as follows:

$$\text{SED} = 0.9c_g/10\sigma\beta_g \quad (2)$$

where c_g (g/L) is the concentration of glucose in the supernatant and σ (%, w/v) is the substrate concentration. The parameters of β_g (%) and 0.9 are, respectively, defined from eq 1.

Meanwhile, the enzymatic hydrolysis glucose yield (EHGY) of EAWFs was defined as in eq 3.

$$\text{EHGY} = \text{SED} \times \text{glucan recovery} \quad (3)$$

Crystallinity Measurements. The crystallinity of untreated and treated EAWFs was determined by X-ray diffraction using a Bruker D8-Advance diffractometer (Bruker, Karlsruhe, Germany) with Cu radiation (k = 0.15418 nm); the operation voltage and current were maintained at 40 kV and 40 mA, respectively. 2θ ranged from 5° to 60° in steps of 0.04° at time intervals of 0.2 s. The crystallinity index (CrI) of biomass was defined as the percentage of crystalline material and was calculated based on eq 4.[15,16]

$$\text{CrI} = (I_{002} - I_{am})/I_{002} \times 100\% \quad (4)$$

in which I_{002} is the intensity of the 002 peak for the maximum crystalline portion at 2θ = 22.8°, and I_{am} is the intensity for the amorphous portion of biomass at 2θ = 18.7°.

FTIR Analysis. Dried untreated and treated EAWF of 5 mg was mixed with 500 mg of KBr in an agate mortar and pressed into discs using an HY-12 tablet press (Tianjin, China). The samples were analyzed by a Thermo Nicolet FTIR spectrometer (USA) by obtaining spectra between 400 and 4000 cm^{-1} and analyzing them using OPUS software (Bruker, Germany).

XPS Analysis. The chemical elements of the surfaces of the pretreated EAWFs were analyzed using an X-ray photoelectron spectroscopy/ESCA (Kratos Ltd., England) with an Al Kα X-ray source (150 W). The pressure in the analysis chamber was maintained at approximately 5 × 10^{-9} Torr during each measurement. The pass energies of high- and low-resolution XPS spectra were 40 and 160 eV, respectively. All binding energies were referenced to the neutral C 1s peak at 284.6 eV for charge compensation. Fe 2p spectra were

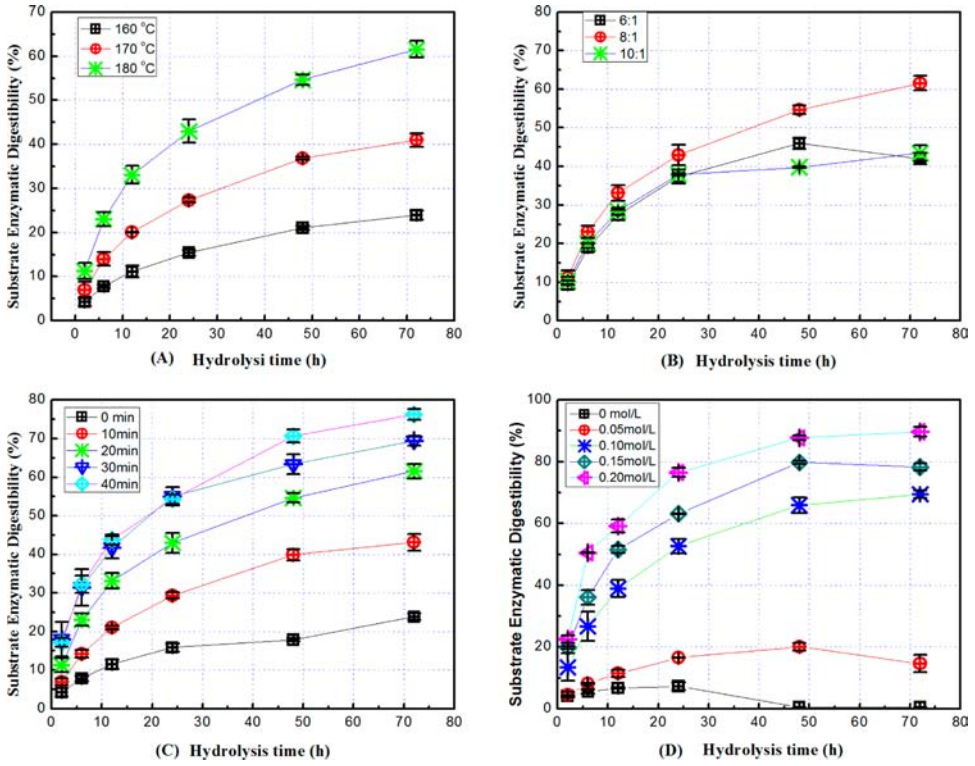

Figure 1. Effect of $FeCl_3$ pretreatment alone conditions on the SED of pretreated EAWFs: (A) effect of pretreatment holding temperature on the SED; (B) effect of pretreatment liquid−solid ratio on the SED; (C) effect of pretreatment holding time on the SED; (D) effect of $FeCl_3$ concentration on the SED.

obtained from low-resolution XPS spectra measurements for determining the iron content.[17]

RESULTS AND DISCUSSION

Effect of $FeCl_3$ Pretreatment on Glucan Recovery of EAWFs. Generally, the biological route for conversion of biomass to bioethanol involved three steps: pretreatment, hydrolysis, and fermentation. Fermentation of glucose to ethanol is actually a mature, commercially robust, and highly optimized technology, while the hydrolysis is difficult to implement because of the recalcitrance of lignocellulosic biomass. In this regard, pretreatment must be applied for the biomass to overcome its recalcitrance. In general, pretreatment breaks down the linkages among components or releases some constituents in the biomass so that the hydrolysis of polysaccharides can be carried out efficiently. $FeCl_3$ pretreatment can break the linkages and degrade hemicellulose in straw materials, thus exposing cellulose for easy attack by cellulase. However, the glucan, which is required to be preserved in biomass solids as much as possible, also encounters degradation during the $FeCl_3$ pretreatment process. Therefore, it is necessary to investigate the effect of $FeCl_3$ pretreatment on glucan recovery of EAWFs for maximizing economic benefit.

The glucan content in pretreated EAWFs and solid and glucan recovery of EAWFs at different $FeCl_3$ pretreatment conditions are summarized in Table 1. It can be seen from Table 1 that the solid recoveries of EAWFs mostly ranged from 70% to 90% after pretreatment, which implied that biomass pretreated at different conditions could confront mass loss, indicating the removal of some constituents (mainly hemicellulose) in the feedstock after acid pretreatment.[6] Furthermore, even though all the glucan content in pretreated EAWFs had a relative increase in comparison to that (43.4%) in the raw material, glucan recoveries of EAWFs after pretreatment accounted for less than 90% of potential glucan in raw materials. The reduction of glucan suggested that $FeCl_3$ pretreatment could also decompose glucan, consistent with previous reports.[7,18] From Table 1, it was also found that pretreatment conditions including temperature, holding time, and liquid/solid ratio showed no significant effect on the glucan recovery of EAWFs pretreated at the same concentration (0.1 mol/L) of $FeCl_3$. However, a sharp decrease in glucan recovery could be observed with the increase of $FeCl_3$ concentration and the reduction of pH values of the pretreatment solutions. Moreover, when EAWFs were pretreated with 0.20 mol/L $FeCl_3$ at a pH value of 1.58, the lowest glucan recovery (73.4%) was obtained. The results demonstrated that the concentration of $FeCl_3$ played a more important role in the degradation of glucan in EAWFs than any other pretreatment conditions because $FeCl_3$ dissolved in water undergoes hydrolysis. The higher the $FeCl_3$ concentration, the more hydrogen ions that are produced based on the $FeCl_3$ hydrolysis. Carbohydrate was easy to degrade in the acidic environment, attributed to $FeCl_3$ hydrolysis. Hence, the usage of $FeCl_3$ must be controlled very well to achieve a competitive glucan recovery. In addition, despite pretreatment conducted at the harshest condition, the glucan recovery of EAWFs was improved to 73.4%, in contrast to the glucan recovery of 49.8% reported in the literature.[7,18] This may be attributed to the different intrinsic properties of the feedstock between EAWFs and corn stover because the specific pretreatment method had distinct actions on diverse biomass.

Effect of $FeCl_3$ Pretreatment on Enzymatic Hydrolysis of EAWFs. The high glucan recovery during pretreatment did

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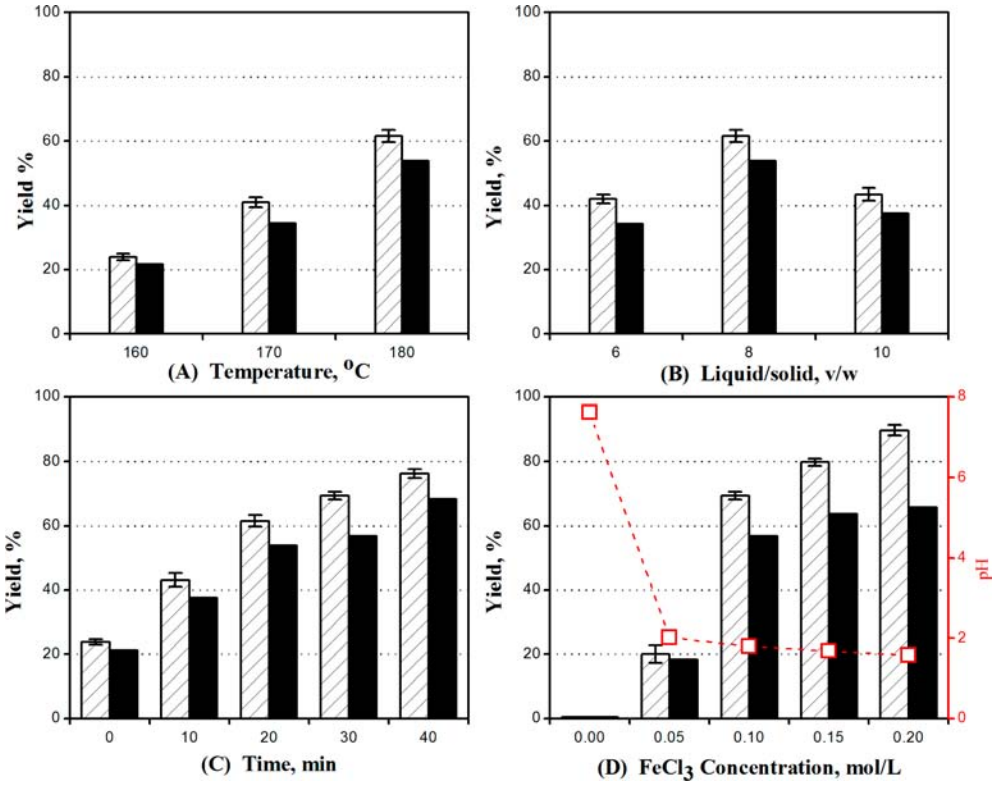

Figure 2. SED (hatched) and EHGY (black) after 72 h of enzymatic hydrolysis of EAWFs pretreated by $FeCl_3$ alone at different conditions and pH values (red □) of different $FeCl_3$ concentration solutions.

not represent a high final glucose yield because enzymatic digestibility of glucan had a profound effect on it. Therefore, enzymatic hydrolysis experiments were conducted to explore the influence of each pretreatment condition on substrate enzymatic digestibility in this study. The effect of $FeCl_3$ pretreatment conditions on SED is depicted in Figure 1.

It can be seen from Figure 1 that increasing hydrolysis time from 24 h to 72 h led to only a 4.7−18.6% increase of substrate enzymatic digestibility of EAWFs from pretreatments except for that with hot water, in which the concentration of $FeCl_3$ is zero. The slight increments demonstrated that most of the cellulose was converted to glucose within the initial 24 h cellulase hydrolysis of pretreated EAWFs. In contrast, the SED of EAWFs pretreated with hot water dropped from 7.3% to approximately zero with an increase of the enzymatic hydrolysis time from 24 h to 72 h (Figure 1D), owing to the glucose digested by the growth of microorganisms without the addition of antibiotics such as tetracycline and cycloheximide.[19,20] This phenomenon hardly occurred in enzymatic hydrolysis of EAWFs pretreated with $FeCl_3$, which was attributed to the antimicrobial activity of iron ion adsorbed on the pretreated EAWFs.[21] Moreover, all pretreatment conditions had obvious influences on the SED of pretreated EAWFs, exhibiting significant differences on glucan recovery.

Figure 1A showed that the SED of EAWFs pretreated at the same $FeCl_3$ concentration, pretreatment holding time, and liquid/solid ratio was improved with the rise of pretreatment holding temperature. The SED for 72 h hydrolysis of EAWFs pretreated at 180 °C was 1.5-fold and 3-fold, respectively, as great as that at 170 and 160 °C. The dramatic increases indicated that even though pretreatment temperature did not affect the glucan recovery during the pretreatment process, it had a considerable effect on enzymatic hydrolysis of pretreated EAWFs, accounted by the fact that higher pretreatment temperature could result in the degradation of hemicellulose and modification of lignin, thus increasing accessibility of the cellulase.[22] But a temperature higher than 180 °C was not necessary nor recommended because of higher temperature implying more energy consumption and inhibitors of fermentation such as furfural and hydroxymethylfurfural. Hence, 180 °C was recommended as the optimum pretreatment temperature for EAWFs.

Figure 1B illustrates the relationship between the pretreatment liquid/solid ratio and SED of pretreated EAWFs after 72 h of enzymatic hydrolysis. The SED of EAWFs pretreated at a liquid/solid ratio of 8:1 outperformed that pretreated at other ratios (Figure 2B). The reality that weak cellulose conversion was obtained for EAWFs pretreated at the low pretreatment ratio of 6:1 was ascribed to poor homogeneity of the reaction, while the SED of EAWFs pretreated at a ratio of 10:1 was worse than that at a ratio of 8:1, possibly due to more adsorption (more than 11.1%) on the pretreated biomass by ferric ion, which is an enzymatic hydrolysis inhibitor. In addition, a high pretreatment ratio of 10:1 wasted water and chemicals. It is important that pretreatment involve minimal water addition to reduce energy demands and produce an acceptable sugar and consequently final product concentration. This economizes on not only the direct cost of pretreatment itself but also that for upstream and downstream operations.[23] Therefore, the pretreatment ratio of 8:1 was optimal.

Figure 1C depicts that the cellulose conversion of pretreated EAWFs under the same enzymatic hydrolysis condition increases quickly before 20 min and then had a slight increase with the increase of pretreatment holding time. This trend suggested that the longer the pretreatment time, the more the degradation of hemicellulose would occur to increase

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Table 2. SED and EHGY after 72 h of Enzymatic Hydrolysis Resulting from Tween80-Assisted $FeCl_3$ Pretreatment at Different Conditions

$FeCl_3$, mol/L	Tween80%, w/v	solid recovery, %	glucan content, %	glucan recovery, %	SED, %	EHGY,[a] %
0	0	89.3	44.2 ± 1.2	90.9	0.5 ± 0.0	0.4
	0.5	90.0	42.1 ± 2.1	87.4	0.5 ± 0.1	0.4
	1	90.8	45.0 ± 0.2	94.1	1.2 ± 0.1	1.1
0.05	0	74.2	53.4 ± 1.1	91.3	14.6 ± 2.7	13.4
	0.5	75.0	52.4 ± 1.0	90.5	23.6 ± 1.2	21.4
	1	76.7	52.7 ± 1.6	93.2	27 ± 2.4	25.2
0.1	0	67.6	52.5 ± 1.0	81.8	69.4 ± 1.2	56.8
	0.5	67.3	55.8 ± 2.7	86.5	72.5 ± 1.6	62.7
	1	67.4	53.8 ± 0.4	83.5	77.5 ± 1.1	64.7
0.15	0	62.5	55.5 ± 0.4	79.9	78.3 ± 1.1	62.6
	0.5	61.1	54.5 ± 2.2	76.7	91.3 ± 1.1	70.0
	1	62.6	54.8 ± 1.3	79.0	91.3 ± 1.2	72.1
0.2	0	58.5	54.5 ± 0.9	73.4	89.7 ± 1.6	65.9
	0.5	58.6	56.6 ± 0.8	76.4	91.8 ± 1.7	70.1
	1	59.1	54.2 ± 1.5	73.9	90.9 ± 0.0	67.2

[a]EHGY is calculated based on the potential glucose composition of the feedstock.

accessibility of cellulase on cellulose, similar to the effect of temperature on the feedstock, as described above. However, if pretreatment takes longer than 30 min, cellulose may also undergo severe degradation, which would decrease the total economical profit. Hence, in this case, 30 min could be regarded as the relatively favorable pretreatment holding time.

Figure 1D demonstrated the influence of ferric chloride concentration on the SED of EAWFs. When EAWFs were pretreated without $FeCl_3$ and with $FeCl_3$ of 0.05 mol/L, a rather low SED (less than 20%) of pretreated EAWFs was obtained. However, once the $FeCl_3$ concentration was raised to 0.1 mol/L, the SED of pretreated EAWFs had a tremendous improvement, reaching 61.6%. In addition, the highest SED of 89.7% could be obtained for EAWFs pretreated with 0.2 mol/L $FeCl_3$ solution. Pretreatment with higher $FeCl_3$ concentrations could induce greater cellulose conversion for EAWFs after cellulase hydrolysis, which suggested that ferric chloride could destroy EAWFs' tight structure by efficiently decomposing the hemicellulose,[7,18] but higher $FeCl_3$ concentrations provided a lower pH value. For instance, when the $FeCl_3$ concentration was 0.20 mol/L, the pH value of the pretreatment solution was 1.58. Stronger acidic conditions negatively affected the glucan recovery and manufacturing equipment.

Furthermore, taking both glucan recovery and SED into consideration, the enzymatic hydrolysis glucose yield based on the potential glucose in untreated EAWFs was calculated as shown in Figure 2. It illustrated that the effect of pretreatment conditions, except for $FeCl_3$ concentration and the initial pH value of the pretreatment solution, on EHGY was similar to that on SED of pretreated EAWFs. When the $FeCl_3$ concentration and initial pH value varied from 0.1 to 0.2 mol/L and 1.8 to 1.58, respectively, the increment (20.3%) of SED was larger than that (9.1%) of the EHGY for pretreated EAWFs. The slight increment of EHGY was due to the reduction of glucan recovery of EAWFs under pretreatments with $FeCl_3$ solution of high concentration and low pH value. In addition, only 65.8% for the greatest EHGY of pretreated EAWFs was acquired, corresponding to enzymatic glucose (EG) of 28.6 g based on 100 g of oven-dried raw material. In conclusion, even though a great SED of EAWFs pretreated with a high $FeCl_3$ concentration was attained, the final enzymatic hydrolysis glucose yield seemed to have no competition because of the losses of glucan in the EAWFs during pretreatment. Hence, it is not an efficient approach to improve the SED by adding more $FeCl_3$.

Enhanced EHGY of EAWFs by Tween80-Assisted $FeCl_3$ Pretreatment. The nonionic surfactant Tween80 was used in $FeCl_3$ pretreatment for facilitating cellulase hydrolysis of EAWFs. Primarily, pretreating EAWFs with Tween80 and $FeCl_3$ was carried out to evaluate the enhanced effect of Tween80 on the pretreatment with $FeCl_3$. The concentrations of Tween80 and $FeCl_3$ used in the pretreatment for EAWFs were taken into consideration. The other pretreatment conditions including temperature, liquid/solid ratio, and holding time adopted in this pretreatment were 180 °C, 8:1, and 30 min, respectively.

The substrate enzymatic digestibility and enzymatic hydrolysis glucose yield of EAWFs after Tween80-assisted $FeCl_3$ pretreatment are summarized in Table 2. When the $FeCl_3$ concentration in the pretreatment of EAWFs ranged from 0.05 to 0.15 mol/L, the SED and EHGY could be improved with an increase of Tween80 concentration. However, Tween80 itself did not affect the pretreatment, as shown in Table 2, where EAWFs pretreated with only Tween80 solution yielded little glucose after cellulase hydrolysis, which performed the same as the treatment with hot water. When the $FeCl_3$ concentration was 0.2 mol/L, the SED of pretreated EAWFs changed only slightly with an increase of Tween80 dosage. Actually, EHGY based on the potential glucose in the feedstock is more important when we consider the SED of the biomass. Hydrolyzing EAWFs pretreated by using 0.15 mol/L $FeCl_3$ combined with 1% Tween80 could release 72.1% EHGY, 10% higher than the EHGY (62.6%) of EAWFs pretreated with the same $FeCl_3$ concentration without Tween80. It is obvious that Tween80 added in the $FeCl_3$ pretreatment could significantly promote enzymatic hydrolysis of EAWFs. In this experiment, we found that even though the highest SED (91.8%) in pretreated EAWFs after 72 h hydrolysis was obtained from waster fibers pretreated by combined 0.2 mol/L $FeCl_3$ and 0.5% Tween80, the EHGY of these treated EAWFs was only 70.1%. This could be because the reduction of glucan in EAWFs under severe pretreatment conditions decreased the overall enzymatic glucose yield despite the high SED. In conclusion, Tween80 could improve the enzymatic digestibility

without sacrificing glucan recovery, leading to a high enzymatic glucose based on the raw material.

To assess the feasibility of Tween80-assisted $FeCl_3$ pretreatment performed for EAWFs, the EG of pretreated EAWFs was compared with that of pretreated eucalyptus from other pretreatment methods. EAWFs pretreated by Tween80-assisted $FeCl_3$ could release 34.8 g of glucose after cellulase hydrolysis, more than that from pretreatment systems including dilute acid, alkaline, hot liquid water, and steam explosion, which EG values were 32.2, 30.4, 34.3, and 25.3 g, respectively.[24−27] However, EG from Tween80-assisted $FeCl_3$ pretreatment did not match that (39.0 g) from SPORL pretreatment reported by Zhu et al.[28] Even though a higher glucose yield could be gained from the pretreatment system performed by Zhu,[28] chemicals such as SO_2 released during the pretreatment process were difficult to handle. Therefore, in terms of enzymatic hydrolysis, Tween80-assisted $FeCl_3$ pretreatment is an efficient and promising pretreatment technique for EAWFs.

Effect of Tween80 on the Crystallinity of EAWFs. The crystallinity of the biomass material has been considered a major factor that affects enzymatic hydrolysis.[29] The crystallinity is influenced by the composition of the biomass, in which hemicellulose and lignin are regarded as amorphous portions, while cellulose is considered to be crystalline.[30] Many previous studies have reported that biomass pretreatment could increase the CrI by removing amorphous substances in the biomass, improving enzymatic hydrolysis.[31,32]

Therefore, XRD was conducted to investigate whether or not Tween80 enhanced the enzymatic digestibility by raising the crystallinity of EAWFs. The CrI of EAWFs pretreated by $FeCl_3$ alone, Tween80-assisted $FeCl_3$, and hot water are calculated as shown in Table 3. The CrI of pretreated EAWFs were higher

Table 3. Effect of Fe(III) on the Surface of Pretreated EAWFs and CrI on the SEG and EG

		atomic ratios[a] (%)				
pretreatment	CrI (%)	C	O	Fe	SED (%)	EHGY[b] (%)
hot water	57.3				0.5 ± 0.0	0.4
$FeCl_3$	62.6	73.76	26.12	0.12	78.3 ± 1.1	62.6
$FeCl_3$ with Tween80	63.0	73.77	26.17	0.05	91.3 ± 1.1	72.1

[a]Atomic ratio is based on the element at the pretreated EAWFs' surface. [b]EHGY is calculated based on the potential glucose composition of the feedstock. CrI of untreated EAWFs was 56.3%.

than that (56.3%) of the untreated ones, while the CrI showed little change between pretreatment with and without Tween80 (Table 3). The results demonstrated $FeCl_3$ pretreatment could improve the CrI of EAWFs, but had no significant effect on that by adding Tween80. In comparison to the SED (0.4%) of pretreated EAWFs with low CrI, an increase in SED (78.3%) with high CrI could be observed, consistent with the opinion of Bak.[32] However, greater cellulose conversion (91.3%) could be obtained for EAWFs pretreated by adding Tween80, despite a similar CrI to that from $FeCl_3$ pretreatment alone. This meant that the improvement in SED of EAWFs pretreated by adding Tween80 was not attributed to the CrI of the biomass.

Effect of Tween80 on the Chemical Structure of EAWFs. The modification of the chemical structure and functional group of biomass compositions influences the enzymatic digestibility by changing the productive adsorption of cellulase on the substrate.[33] Thus, FTIR spectroscopy was used to determine the change of chemical structure and functional group of EAWF constituents after pretreatment by $FeCl_3$ with/without Tween80 for exploring the reason for the enhancement of the SED by addition of Tween80 during pretreatment.

Figure 3 shows the FTIR spectra of untreated, hot water-treated, $FeCl_3$-treated, and Tween80-assisted $FeCl_3$-treated

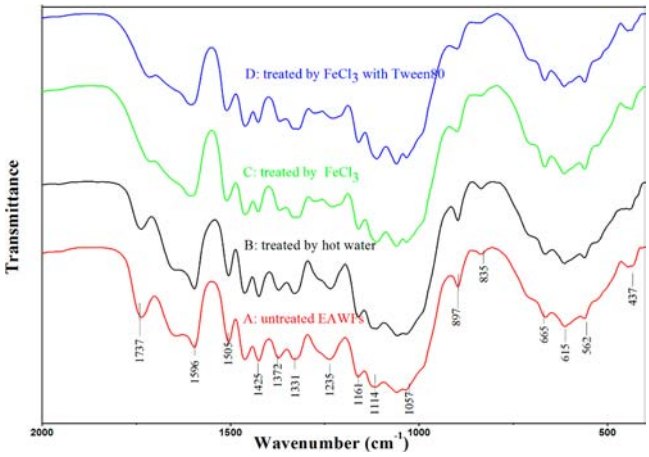

Figure 3. FTIR spectra of untreated and treated EAWFs: (A) untreated EAWFs; (B) hot water pretreatment; (C) $FeCl_3$ pretreatment; (D) Tween80-assisted $FeCl_3$ pretreatment.

EAWFs. The FTIR spectrum of EAWFs pretreated by hot water was similar to the untreated ones, suggesting hot water hardly decomposed the EAWFs. In comparison to the FTIR spectra of untreated EAWFs, the signals of the ether bond at 1235 cm^{-1} [7,18] and the ester bond at 1737 cm^{-1} [7,34] for $FeCl_3$-treated EAWFs both became weaker. This indicated that $FeCl_3$ pretreatment might cleave the ether and ester linkages between lignin and carbohydrates of EAWFs or reduce the lignin percentage in the biomass. However, the lignin content of treated EAWFs was 40.9%, higher than that (25.5%) of untreated EAWFs. Hence, this pretreatment only disrupts the linkages between lignin and carbohydrates of EAWFs and does not remove lignin. A small sharp band at 897 cm^{-1} demonstrates the presence of predominant β-glycosidic linkages between the sugar units in cellulose and hemicellulose.[35] The decrease of this peak for $FeCl_3$-treated EAWFs suggested the change of linkages between sugar units and intermolecular degradation in the hemicellulose structure. Enhanced adsorption peaks of the C−O−H stretching of primary and secondary alcohols at 1057 cm^{-1} [36] suggested that the glucan content in pretreated EAWFs increased because of the removal of hemicellulose in the feedstock by $FeCl_3$ pretreatment. All cases performed with $FeCl_3$ pretreatment showed that it could destroy the rigid matrix of EAWFs, facilitating the cellulase hydrolysis of the pretreated biomass. However, the FTIR spectrum of EAWFs pretreated by $FeCl_3$ with Tween80 was almost the same as that from pretreatment without Tween80. This result further confirmed that Tween80 had little effect on the degradation of the biomass, indicating Tween80 did not affect the SED of EAWFs by changing the chemical structure during the pretreatment.

Effect of Tween80 on the Removal of Iron at the EAWF Surface. As the enhanced SED was not contributed by the change of internal factors such as CrI and chemical

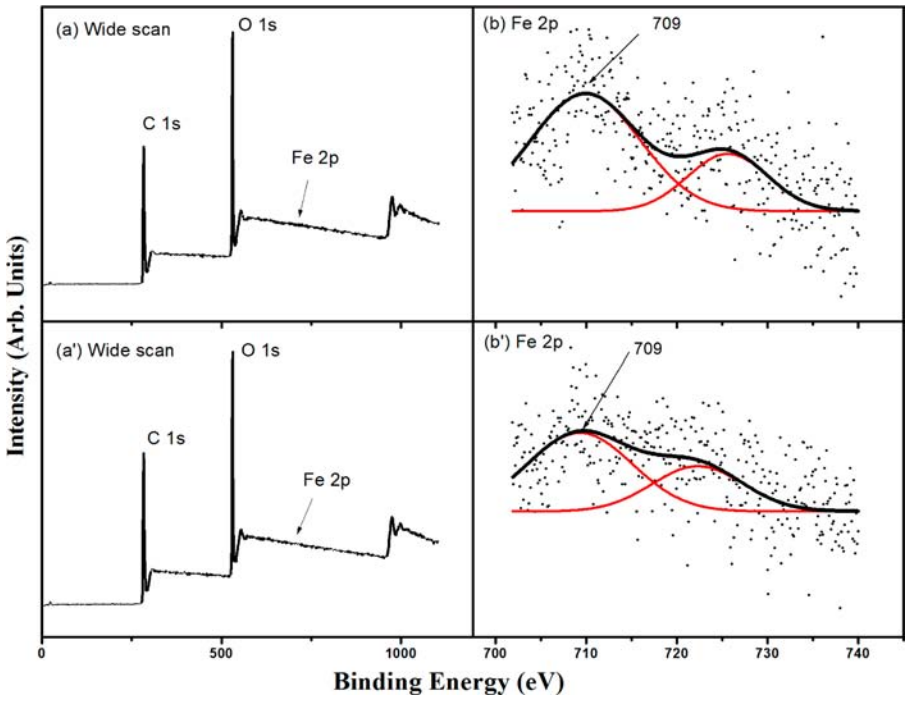

Figure 4. XPS wide scan, Fe 2p core-level spectra of the EAWFs pretreated with Tween80 (a′ and b′) and without Tween80 (a and b).

composition of EAWFs, XPS was conducted to determine the residual content of iron(III) on the pretreated biomass surface, which has been proven to inhibit cellulase hydrolysis.[10,37]

Figure 4a′ and a show the XPS wide scan spectra of the EAWFs pretreated with and without Tween80, respectively. From the wide scan, the C 1s and O 1s spectrum peak, respectively at the binding energy of 284 eV and 531 eV, could be observed, but not the peak of Fe 2p.[17] Then the Fe 2p core-level spectrum was determined, as shown in Figure 4b′ and b, to confirm the existence of iron. The rather weak spectrum of iron could be detected, even though it was difficult to see because of the noise. Hence, individual Fe 2p peaks were extracted by a curve-fitting process from the diffraction intensity profiles. Integrating the wide and core-level spectra, the atomic ratio of C:O:Fe for the EAWFs pretreated with and without Tween80 was calculated, as shown in Table 3. The same ratio of C:O indicated that pretreatment with and without Tween80 had a similar effect on the degradation of the biomass. The contents of Fe were merely 0.12% and 0.05% (Table 3), corresponding to 1.8 and 0.72 mmol/L iron ion in the hydrolysate when the pretreated EAWFs were used for cellulase hydrolysis in this experiment. However, the addition of a Fe(III) concentration of just 1 mmol/L provided additional significant reduction in the SED of the substrate cellulose.[37] Nevertheless, the content of Fe (0.05%) at the surface of the EAWFs pretreated by $FeCl_3$ with addition of Tween80 was less than that (0.12%) without Tween80. In this case, the SED of EAWFs pretreated by $FeCl_3$ with addition of Tween80 was greater by 14% than that without Tween80. Hence, we could infer that the pretreatment with Tween80 had some effect on the elimination of iron, thus enhancing the enzymatic hydrolysis of the biomass. Besides, hydrophobic degradation products, which may also inhibit the cellulase, could be easy to extract by the addition of Tween80. However, byproducts were difficult to determine, as they were diverse from biomass pretreatment. Therefore, the effects of other byproducts from pretreatment performed on cellulase hydrolysis will be discussed in later work to further explore the mechanism of surfactant pretreatment. Generally, addition of Tween80 in the $FeCl_3$ pretreatment facilitated cellulase hydrolysis of the pretreated EAWFs.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) González-García, S.; Moreira, M. T.; Feijoo, G.; Murphy, R. J. Comparative life cycle assessment of ethanol production from fast-growing wood crops (black locust, eucalyptus and poplar). *Biomass Bioenergy* **2012**, *39*, 378−388.

(2) Xu, E. C.; Zhou, Y. J. Synergistic effects between chemical mechanical pulps and chemical pulps from hardwoods. *Tappi J.* **2007**, *6*, 4−9.

(3) Das, T. K.; Jain, A. K. Pollution prevention advances in pulp and paper processing. *Environ. Prog.* **2001**, *20*, 87−92.

(4) Kumar, R.; Mago, G.; Balan, V.; Wyman, C. E. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresour. Technol.* **2009**, *100*, 3948−3962.

(5) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673−686.

(6) Sun, Y.; Cheng, J. Y. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* **2002**, *83*, 1−11.

(7) Liu, L.; Sun, J.; Li, M.; Wang, S.; Pei, H.; Zhang, J. Enhanced enzymatic hydrolysis and structural features of corn stover by $FeCl_3$ pretreatment. *Bioresour. Technol.* **2009**, *100*, 5853−5858.
(8) Mooney, C. A.; Mansfield, S. D.; Touhy, M. G.; Saddler, J. N. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresour. Technol.* **1998**, *64*, 113−119.
(9) Kumar, R.; Wyman, C. E. Cellulase adsorption and relationship to features of corn stover solids produced by leading pretreatments. *Biotechnol. Bioeng.* **2009**, *103*, 252−267.
(10) Tejirian, A.; Xu, F. Inhibition of cellulase-catalyzed lignocellulosic hydrolysis by iron and oxidative metal ions and complexes. *Appl. Environ. Microb.* **2010**, *76*, 7673−82.
(11) Kurakake, M.; Ooshima, H.; Kato, J.; Harano, Y. Pretreatment of bagasse by nonionic surfactant for the enzymatic hydrolysis. *Bioresour. Technol.* **1994**, *49*, 247−251.
(12) Kim, H. J.; Kim, S. B.; Kim, C. J. The effects of nonionic surfactants on the pretreatment and enzymatic hydrolysis of recycled newspaper. *Biotechnol. Bioprocess Eng.* **2007**, *12*, 147−151.
(13) NREL. Biomass analysis technology team standard biomass analytical procedures: determination of structural carbohydrates and lignin in biomass, Golden, CO, 2008. http://www.nrel.gov/biomass/analytical_procedures.html.
(14) Lin, J.; Pillay, B.; Singh, S. Purification and biochemical characterization of β-glucosidase from a thermophilic fungus, *Thermomyces lanuginosus*-SSBP. *Appl. Biochem.* **1999**, *30*, 81−87.
(15) Segal, L.; Creely, J. J.; Martin, A. E.; Conrad, C. M. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Text. Res. J.* **1959**, *29*, 786−794.
(16) Park, S.; Baker, J. O.; Himmel, M. E.; Parilla, P. A.; Johnson, D. K. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol. Biofuels* **2010**, *3*, 10.
(17) Figueira, M. M.; Volesky, B.; Mathieu, H. J. Instrumental analysis study of iron species biosorption by Sargassum biomass. *Environ. Sci. Technol.* **1999**, *33*, 1840−1846.
(18) Lü, J.; Zhou, P. Optimization of microwave-assisted $FeCl_3$ pretreatment conditions of rice straw and utilization of *Trichoderma viride* and *Bacillus pumilus* for production of reducing sugars. *Bioresour. Technol.* **2011**, *102*, 6966−6971.
(19) Yang, B.; Wyman, C. E. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnol. Bioeng.* **2004**, *86*, 88−95.
(20) Rawn, C. D.; Van Etten, J. L. Mechanism of antibacterial antibiotic sensitivity in *Pythium ultimum*. *J. Gen. Microbiol.* **1978**, *108*, 133−139.
(21) Cheng, C.; Doyle, M. P.; Luchansky, J. B. Identification of *Pseudomonas fluorescens* strains isolated from raw pork and chicken that produce siderophores antagonistic towards foodborne pathogens. *J. Food Protect.* **1995**, *58*, 1340−1344.
(22) Donohoe, B. S.; Decker, S. R.; Tucker, M. P.; Himmel, M. E.; Vinzant, T. B. Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment. *Biotechnol. Bioeng.* **2008**, *101*, 913−925.
(23) Lynd, L. R.; Wyman, C. E.; Gerngross, T. U. Biocommodity engineering. *Biotechnol. Prog.* **1999**, *15*, 777−793.
(24) Wei, W.; Wu, S.; Liu, L. Enzymatic saccharification of dilute acid pretreated eucalyptus chips for fermentable sugar production. *Bioresour. Technol.* **2012**, *110*, 302−307.
(25) Park, J.; Kang, M.; Kim, J. S.; Lee, J.; Choi, W.; Lee, J. Enhancement of enzymatic digestibility of Eucalyptus grandis pretreated by NaOH catalyzed steam explosion. *Bioresour. Technol.* **2012**, *123*, 707−712.
(26) Yu, Q.; Zhuang, X.; Yuan, Z.; Wang, Q.; Qi, W.; Wang, W.; Zhang, Y.; Xu, J.; Xu, H. Two-step liquid hot water pretreatment of *Eucalyptus grandis* to enhance sugar recovery and enzymatic digestibility of cellulose. *Bioresour. Technol.* **2010**, *101*, 4895−4899.
(27) Emmel, A.; Mathias, A. L.; Wypych, F.; Ramos, L. P. Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion. *Bioresour. Technol.* **2003**, *86*, 105−115.
(28) Zhu, J. Y.; Verrill, S.; Liu, H.; Herian, V.; Pan, X.; Rockwood, D. On polydispersity of plant biomass recalcitrance and its effects on pretreatment optimization for sugar production. *Bioenergy Res.* **2011**, *4*, 201−210.
(29) Li, C.; Knierim, B.; Manisseri, C.; Arora, R.; Scheller, H. V.; Auer, M.; Vogel, K. P.; Simmons, B. A.; Singh, S. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. *Bioresour. Technol.* **2010**, *101*, 4900−4906.
(30) Jeoh, T.; Ishizawa, C. I.; Davis, M. F.; Himmel, M. E.; Adney, W. S.; Johnson, D. K. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnol. Bioeng.* **2007**, *98*, 112−122.
(31) Kim, S.; Holtzapple, M. T. Effect of structural features on enzyme digestibility of corn stover. *Bioresour. Technol.* **2006**, *97*, 583−591.
(32) Bak, J. S.; Ko, J. K.; Han, Y. H.; Lee, B. C.; Choi, I.; Kim, K. H. Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation pretreatment. *Bioresour. Technol.* **2009**, *100*, 1285−1290.
(33) Börjesson, J.; Engqvist, M.; Sipos, B.; Tjerneld, F. Effect of poly(ethylene glycol) on enzymatic hydrolysis and adsorption of cellulase enzymes to pretreated lignocellulose. *Enzyme Microb. Technol.* **2007**, *41*, 186−195.
(34) Sun, X. F.; Xu, F.; Sun, R. C.; Wang, Y. X.; Fowler, P.; Baird, M. S. Characteristics of degraded lignins obtained from steam exploded wheat straw. *Polym. Degrad. Stab.* **2004**, *86*, 245−256.
(35) Xiao, B.; Sun, X. F.; Sun, R. Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. *Polym. Degrad. Stab.* **2001**, *74*, 307−319.
(36) Guo, G.; Chen, W.; Chen, W.; Men, L.; Hwang, W. Characterization of dilute acid pretreatment of silvergrass for ethanol production. *Bioresour. Technol.* **2008**, *99*, 6046−6053.
(37) Liu, H.; Zhu, J. Y.; Fu, S. Y. Effects of lignin−metal complexation on enzymatic hydrolysis of cellulose. *J. Agric. Food Chem.* **2010**, *58*, 7233−7238.