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$FeCl_3$ Pretreatment of Three Lignocellulosic Biomass for Ethanol Production

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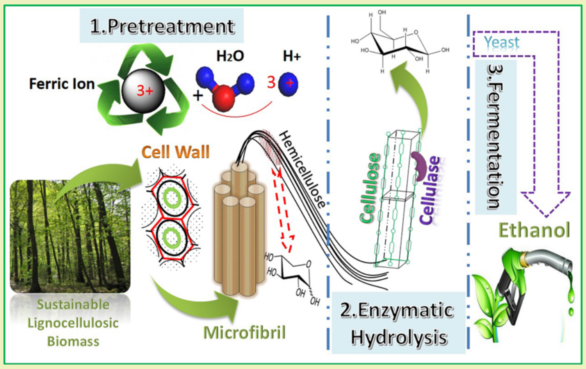
ABSTRACT: Bioethanol from lignocellulosic biomass bioconversion is a promising alternative to fossil fuels. Pretreatment plays an important role in this bioconversion. Recyclable ferric chloride was employed for pretreating three kinds of biomass species, including bagasse, rice straw, and wood fiber, in this study to make a comparison. The results showed that $FeCl_3$ was suitable for pretreating biomass species, especially the rice straw, which contains a high hemicellulose content and has a weak matrix structure. The enzymatic saccharification of rice straw pretreated by $FeCl_3$ could reach 95.1% with the highest ratio (83.3%) of removed hemicellulose to raw cellulose. A favorable conversion (81.9%) of cellulose could be attained; the pretreated rice straw was conducted in high solid loading (20%, w/v) hydrolysis. At the same cost with a lower pretreatment combined severity factor (CSF), $FeCl_3$ pretreated rice straw could achieve a higher conversion than HCl. Meanwhile, $FeCl_3$ could realize more than 90% recovery during the pretreatment, resulting in low environmental pollution. This suggests that $FeCl_3$ pretreatment was competitive for biomass bioconversion.



KEYWORDS: *Lignocellulosic biomass, Pretreatment, Enzymatic hydrolysis, Bioethanol, Suitability*

INTRODUCTION

Production of lignocellulosic ethanol, which is a second generation bioethanol, uses relatively inexpensive, abundant, and renewable agricultural or industrial byproducts, such as bagasse, rice straw, or forestry residues.[1,2] It has less competition for high-quality edible carbohydrates between food and fuel application, compared to first generation bioethanol.[1,2] However, lignocellulose is comprised of carbohydrate polymers (cellulose and hemicellulose) and an aromatic polymer (lignin), which tightly bind to each other mainly by ester and ether linkages between them.[3,4] It has evolved to possess hydrolytic stability and structural recalcitrance to the plant cell walls. In this case, cellulase hydrolysis of lignocellulose, which is one of the crucial and costly steps for bioethanol production, is hard to implement. To extract fermentable glucose, the cellulose in lignocellulosic biomass needs to be exposed and accessible to cellulase.[4,5] Pretreatment is an important tool for achieving this goal.

Meanwhile, pretreating lignocellulosic biomass to realize high yields is vital to commercial success in bioconversion.[4] It not only has a major effect on the cost of prior (e.g., size reduction) as well as subsequent (e.g., enzymatic digestion and fermentation) operations but is among the most expensive processes.[6,7] Hence, much more concern needs to be taken into interactions among these processes to develop economic biomass conversion technologies. Varieties of pretreatments have been exploited, but comparison among them is difficult due to differences in research methodology and used lignocellulosic biomass species.[4,8,9] Besides, their relative attributes differ from each other, even though some of them are competitive. Mostly, pretreatments need to remove or alter hemicellulose or lignin and improve enzymatic accessibility of the substrates.[10,11] Some reports stated that hemicellulose removal had a major influence on cellulose digestion.[10,12,13] Ferric acid pretreatment could efficiently extract the hemicellulose and interrupt ether and ester linkages between lignin and carbohydrates to improve enzymatic accessibility of the substrates but hardly removes the lignin.[14] Moreover, $FeCl_3$ was more readily recycled and less corrosive to equipment than inorganic acids. It had been used for pretreating rice straw,[15] bagasse,[16] and wood fiber[17] separately by some researchers. They obtained a desirable result after they optimized the pretreatment conditions. However, no comparison among them has been made. The suitability of this pretreatment, which is an important parameter to evaluate economic feasibility, has not been comprehended. In addition, only low substrate concentration (such as 2%)[16,17] was carried out in the enzymatic hydrolysis, although high substrate loading to get high glucose concentration is necessary for the sequential fermentation.[18,19] Especially, high glucose concentration in enzymatic hydrolysate means great ethanol contents in the

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Table 1. Compositions of Pretreated Biomass Solid and Byproduct Concentration in Pretreatment Spent Liquor

	solid (%)				spent liquor (g/L)			
sample	yield	glucan	xylan	lignin	xylose	glucose	furfural	HMF
bagasse	58	46.6 ± 2.3	2.15 ± 0.78	41.3 ± 1.3	11.3 ± 0.3	6.77 ± 0.12	5.11 ± 0.15	0.752 ± 0.030
straw	51.3	53.7 ± 3.3	10.2 ± 0.8	30.4 ± 0.9	17.1 ± 0.2	3.18 ± 0.15	6.48 ± 0.47	0.775 ± 0.002
EAWFs	68.9	53.6 ± 0.7	3.58 ± 0.13	38.0 ± 1.5	7.61 ± 0.1	4.36 ± 0.12	3.56 ± 0.22	0.232 ± 0.017

fermentation, resulting in cost reduction of the distillation process of ethanol.[18,19]

In this study, three kinds of lignocellulosic biomass were used for ferric chloride pretreatment to explore the suitability of this pretreatment by investigating their component and structural changes. Different pretreated biomass substrate concentrations (including high solid loading) were applied to enzymatic hydrolysis to access their commercial potential. In the meantime, the economic efficiency of $FeCl_3$ pretreatment would be evaluated by comparing the HCl pretreatment with the same cost.

MATERIALS AND METHODS

Materials. Three biomass species were employed in this study. The sugar cane bagasse as presented by Chen[16] was obtained from Guangxi Guitang Group, Guangxi province, China. The rice straw (consisting of 31.3% glucan, 31.3% xylan, and 15.5% Klason lignin) was obtained from a farm in Guangdong Province, China. The wood fiber, eucalyptus APMP waste fibers (EAWFs), as presented by Chen[17] were provided by a pulp and paper-making industry (Guangxi Jingui Co., China). They were milled by a microplant grinding machine (FZ102, Tianjin Taisite Instrument Co. China), collected through 10-mesh screen, and then air-dried for further use. Chemicals used in this paper were of analytical reagent grade. All experiments were performed in duplicate under the same conditions, and average values were reported.

$FeCl_3$ Pretreatment. The lignocellulosic biomass of 30 g dry weight was loaded in a high pressure reactor (4530 series, Parr Co., U.S.A.) and mixed with ferric chloride solution. The initial concentration of $FeCl_3$ solution with a total volume of 300 mL for the pretreatment was 0.1 mol/L. The mixtures were heated from room temperature to 170 °C and then kept for 30 min. The reactor was immediately cooled by being removed from the heating jacket when the pretreatment was finished. Filtration was then performed for the pretreated samples to separate liquor from the solid parts. The solids after being washed with deionic water were used for enzymatic hydrolysis. Analysis was also conducted for components of washed solids. Collected spent liquors from pretreatment were neutralized using 0.1 mol/L NaOH solution to make Fe (III) precipitate completely. The attained precipitated Fe (III) was then hydrolyzed by 0.1 mol/L HCl solution. The concentration of recoverable ferric ionic can be determined by the phenanthroline method.[20] Meanwhile, the main components in the spent liquors including glucose, xylose, 5-hydroxymethylfurfural (HMF), and furfural were analyzed by ion chromatography (IC) and high-pressure liquid chromatography (HPLC).

The pretreatment severity determined by the combined severity factor (CSF) combined the reaction time, pretreatment temperature, and pH value.[21] The combined severity factor (CSF) is defined as eq 1:

$$\mathrm{CSF} = \log\{t\cdot\exp[(T-100)/14.75]\} - \mathrm{pH} \qquad (1)$$

where t is the pretreatment time in minutes, T is the pretreatment temperature in °C, and pH value is determined by a DELTA 320 pH meter.

Enzymatic Hydrolysis. Pretreated biomass samples with different concentrations were hydrolyzed by cellulase (Celluclast 1.5L) and β-glucosidase (Novozyme 188) in a 100 mL flask, where loadings were 20 FPU and 25 CBU per gram substrate, respectively. The enzymatic hydrolysis of the reaction mixture with a total volume of 50 mL was conducted in a HAc/NaAc buffer (50 mmol/L, pH 4.8) on a rotary shaker (50 °C, 150 rpm). Samples of 0.1 mL were drawn at different time points, incubated in boiling water for 1 min to inactivate the enzymes, and then centrifuged to remove water-insoluble solids. The supernatant of samples was analyzed by the glucose oxidase–peroxidase method (GOPM) for glucose content.[22] The cellulose enzymatic digestibility (CED) of pretreated bagasse is described as follows:

$$\mathrm{CED} = 0.9c_g/10\sigma\beta_g \qquad (2)$$

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 as the glucan content and factors that convert glucose to an equivalent glucan.

Fermentation. One gram of yeast (Angel Yeast Co., Yichang, Hubei, China) was activated in 20 mL of glucose solution of 2% wt at 38 °C for 20 min and then kept at 34 °C for 2 h. The yeast solution was centrifuged to remove the liquor. The resultant precipitate was washed with deionic water. This process was repeated three times. The washed precipitate was then mixed with deionic water to make the total mass reach 20 g. The activated yeast solution of 1 mL was employed in 19 mL obtained enzymatic hydrolyzate for fermentation at 38 °C for 72 h.

Component Analysis of Pretreated Samples. The solid components of treated fibers were determined according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass.[23] All liquor samples from pretreatment for analysis were diluted appropriately with ultrapure water and then filtered through a 0.22 μm film. The quantification of glucose and xylose in the liquors were carried out by IC system (Dionex ICS-3000) with a CarboPac PA20 column at 30 °C. The concentrations of HMF and furfural in the pretreatment solution were determined by HPLC system with a diode array detector (DAD) and C18 column at 30 °C. The eluents (30% methanol and 70% water, vol.) flowed at a rate of 1.0 mL/min.

Ethanol Determination. The concentration of ethanol in the fermentation supernatant was determined by full evaporation head-space gas chromatograph[24,25] with an Automatic Headspace Sampler (DANI HSS86.50) and a gas chromatograph (Agilent 7890A, Agilent Corporation). A closed vial of 22 mL contained 10 μL of supernatant and was placed into an Automatic Headspace Sampler and kept at 105 °C for 5 min to obtain equilibrium. The equilibrium gas of 20 μL was taken from the vial for GC measurement. The GC with a HP-5 capillary column was operated at 40 °C under the carrier gas (nitrogen) with a rate of 25 mL/min. A flame ionization detector was performed at 250 °C under hydrogen and air flowing at 30 and 400 mL/min, respectively. A minimum of three replicates was performed for all analysis.

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

SEM Analysis. SEM was conducted to analyze the microstructural changes and surface characteristics of $FeCl_3$-pretreated biomass species. Samples of untreated and pretreated biomass species were sputter-coated with gold on an ion sputter coater prior to imaging with scanning electron microscope (EVO18, ZEISS Company, German) operating at an accelerating voltage of 10 kV.

■ RESULTS AND DISCUSSION

Component Change of Various Biomass Species after Pretreatment. Component change of lignocellulosic biomass during pretreatment is helpful to assess the effect of pretreatment. Three biomass species were pretreated by the same method to make a comparison. Their main components, including glucan, xylan, and Klason lignin and solid remains, after $FeCl_3$ pretreatment are shown in Table 1. The remained solid masses of all biomass species after pretreatment were less than 70%, and the least remained solid mass was reduce to 51.3% from rice straw. Ferric chloride had an insignificant effect on the removal of lignin. Hence, the mass loss of biomass was probably due to the degradation of carbohydrates in biomass pretreated in the acidic condition.

Table 1 and Figure 1 depict the extent of carbohydrate degradation. Both cellulose and hemicellulose in biomass suffered from decomposition with various levels as shown in Table 1 and Figure 1. All hemicellulose removal based on xylan in untreated biomass was more than 83%, even as high as 93% for bagasse during pretreatment. The sharp decrease in hemicellulose in pretreated biomass had a significant effect on the accessibility of cellulase during enzymatic hydrolysis. Furthermore, removal of hemicellulose enriched the mass fraction of glucan in the pretreated biomass as shown in Table 1, which readily obtained high yield products during the subsequent process. The existing form of removed hemicellulose mainly performed as xylose released in the pretreatment spent liquor. Except for the glucose and xylose in the pretreatment spent liquor, a relatively high yield furfural and a little 5-hydroxymethylfurfural (HMF) were produced. It seemed that ferric acid facilitated the xylose to further degrade as furfural at high temperature during the pretreatment. The greatest concentration of xylose could reach 17.1 g/L released from rice straw in pretreatment spent liquor. This meant that even the ferric chloride pretreatment removed the xylan in bagasse more than in rice straw; the large xylan content in rice straw resulted in a more released concentration of xylose in spent liquor than that of bagasse. Usually, for a specific biomass, hemicellulose removal based on an untreated one had a positive effect on the accessibility of cellulose to enzyme. However, comparison between different species was unfair. In fact, we could not conclude that one acid-pretreated biomass can be more digestible than the other simply in light of higher hemicellulose removal based on raw hemicellulose among different biomass species. For various biomass feedstock, cellulose is surrounded by hemicellulose with different mass fractions. Hence, more removal of hemicellulose based on the surrounded cellulose might expose more target cellulose to the enzyme under inalterable lignin content. In this case, the removed hemicellulose based on glucan in untreated biomass was also calculated in Figure 1. It showed that rice straw underwent actually largest removal of hemicellulose among these biomass species used in this study.

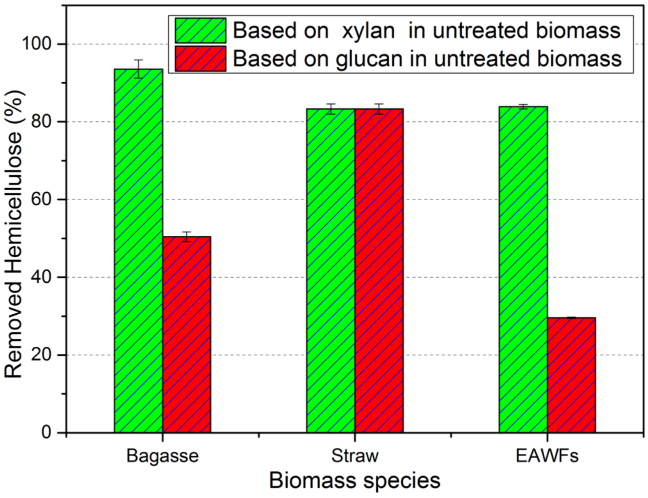

Figure 1. Hemicellulose removal of different biomass species after ferric chloride pretreatment.

Enzymatic Digestibility of Various Biomass Species after Pretreatment. All three kinds of pretreated biomass were used as the substrates for enzymatic hydrolysis. Figure 2 shows the effect of cellulase hydrolysis time on glucose released from different biomass species at low substrate concentration (2%, w/v). At the same pretreatment severity (CSF = 1.94), pretreated straw had a higher hydrolysis rate than bagasse and wood fiber. It could almost achieve the greatest glucose release when hydrolysis was performed at 12 h. This might result from the lower lignin content in pretreated straw than other two species. The highest concentrations of glucose reached 10.0 ± 0.3, 11.9 ± 0.5, and 9.4 ± 0.6 g/L after 72 h enzymatic hydrolysis for bagasse, straw, and EAWFs, respectively. Accordingly, the yields of converting cellulose to glucose were 91.7%, 95.1%, and 81.5%, respectively. In addition, owing to a slightly lower glucan content in pretreated bagasse than that in pretreated rice straw, the cellulase loading (43 FPU) based on glucan for bagasse digestibility was higher than that (37 FPU) for straw. It proved that rice straw was much more digestible than bagasse. Considering their removed hemicellulose based on glucan (50.4%, 83.3%, and 29.6%, respectively) in Figure 1, using hemicellulose removal based on cellulose is much more credible for characterizing the digestibility of different pretreated biomass species. Certainly, it needs to account for enough amounts of samples from diverse resources in future work. In comparison to rice straw pretreated by dilute sulfuric acid, its maximum cellulose enzymatic digestibility reached 85%, with a pretreatment CSF of 2.6.[26] This mainly resulted from the stable hydrogen ion concentration provided by the $FeCl_3$ reversible hydrolysis reaction in water to make the rice straw more reactive.[16] For the SPORL pretreatment (Sulfite Pretreatment to Overcome Recalcitrance

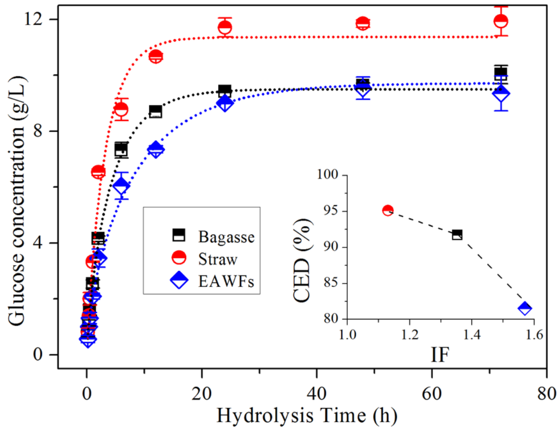

Figure 2. Effect of cellulase hydrolysis time on glucose release from different biomass species by $FeCl_3$ pretreatment.

of Lignocelluloses), at the same CSF of 1.94, the CED of pretreated aspen could reach 91%,[12] lower than the $FeCl_3$ pretreated rice straw and bagasse but higher than pretreated EAWFs. Taking into account hemicellulose removal (34.4%) based on glucan from SPORL pretreatment under this condition, the ratio of removed hemicellulose to raw cellulose consolidated the comparison among various biomass species. In addition, the relationship between the cellulose enzymatic digestibility of different biomass and the inhibition factor (IF) discussed in the previous work[16] is shown in the lower right corner of Figure 2. It shows that the CED of pretreated biomass increased with IF, which meant that IF could also be used for evaluating the suitability of pretreatment. The lowest inhibition factor could reach 1.13, approaching 1 for pretreated straw. It suggested that pretreated straw had much less inhibitors for enzymatic hydrolysis.

Figure 3 shows the effect of substrate concentration on the glucose concentration from different biomass species at different hydrolysis times. In this study, the highest possible substrate concentration was only 20% (w/v) owing to the mixing problem among the hydrolysis mixtures. All of the pretreated biomass hydrolysis displayed the characteristic profiles. The glucose release significantly improved before hydrolysis time at 24 h and then tended to increase smoothly from all the treated biomass. The highest glucose concentration could reach 97.8 g/L from treated rice straw hydrolysis at substrate concentration of 20% (w/v). Correspondingly, only an 81.9% mass fraction of glucan in this pretreated biomass was converted to glucose.

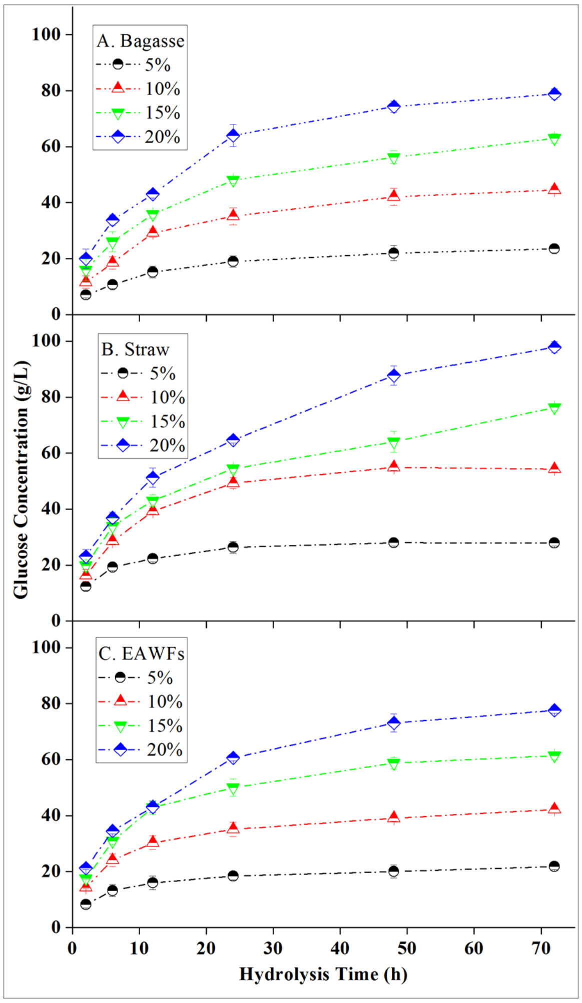

Figure 3. Effect of substrate concentration of enzymatic hydrolysis on the glucose concentration dependent on time.

Several factors can contribute to the low cellulose enzymatic digestibility at high substrate concentration. These may contain the enzyme inactivation, reactivity decrease in the substrate in the course of hydrolysis, nonproductive absorption of the enzyme on lignin, and end product inhibition.[27] Nevertheless, a higher glucose concentration is necessary for the sequential fermentation. Hence, a higher substrate concentration should still be used, even if it has a lower CED. In addition, Figure 4 reveals the effect of substrate concentration of all three pretreated biomass used in the experiment on the cellulose enzymatic digestibility. Generally, at the same enzyme loading, the use of a higher substrate concentration produced hydrolysate with a higher glucose concentration (Figure 3) but resulted in a lower cellulose enzymatic digestibility (Figure 4) for all biomass species. The added trend lines in Figure 4 depict that CED had a good linear relationship with substrate concentration in accordance with other reports.[28] The linearity indicated that a single factor might make a major contribution to the digestible effect. Hodge and co-workers made a conclusion that mass transfer limitation might perform a slight effect on the conversion when substrate concentration was lower than 20%.[29] Furthermore, Kristensen's research had found that end product inhibition (such as high glucose concentration) might be the main reason for low CED at high solid loading when the filter papers were employed in their study.[28] Thus, many studies had conducted simultaneous enzymatic hydrolysis and fermentation to impair end product inhibition.

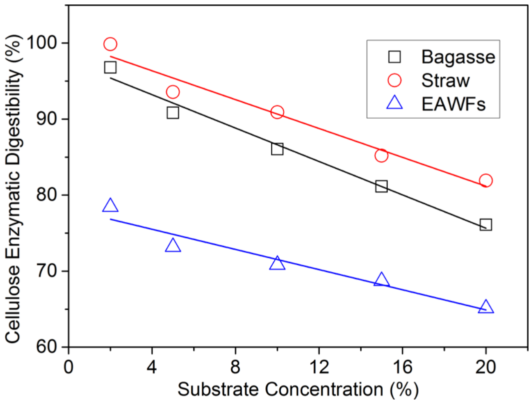

Figure 4. Effect of substrate concentration on the cellulose enzymatic digestibility.

In view of all enzymatic hydrolysis data, a conclusion can be made that pretreated rice straw and bagasse were further readily hydrolyzed than wood fiber was. Especially, rice straw was greatly accessible for cellulase digestion after ferric chloride pretreatment.

Chemical and Physical Changes of Various Biomass Species after Pretreatment. As mentioned in former sections, rice straw after ferric chloride pretreatment was more susceptible to enzymatic digestion than bagasse and wood fiber. The effect of their component content had been investigated. The removal of hemicellulose and lignin had a positive effect on the hydrolysis of lignocellulose by enzymes.

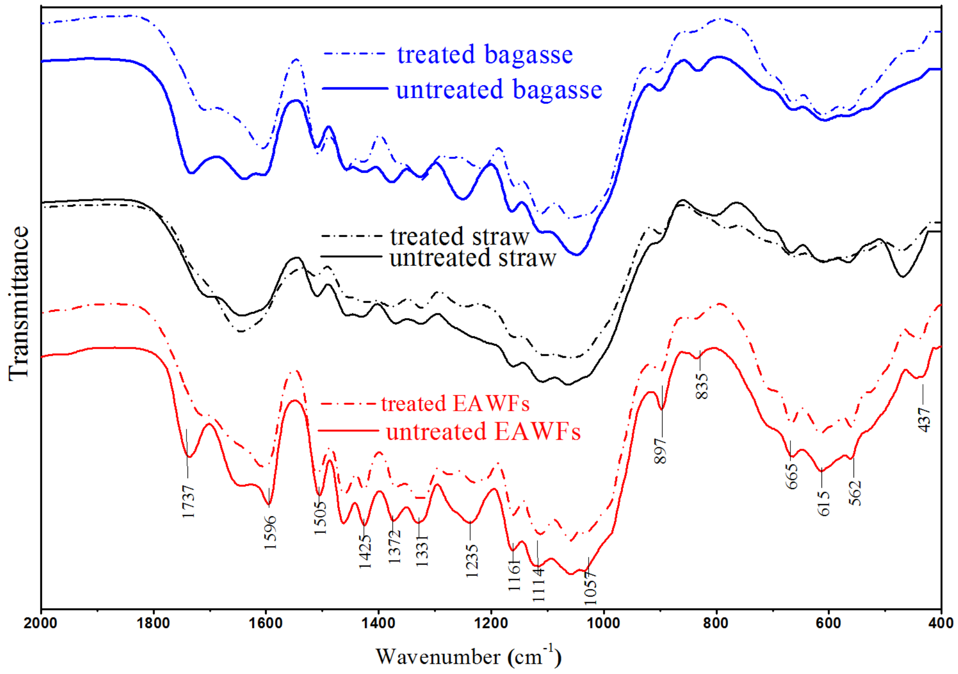

Figure 5. FTIR spectra of various biomass species prior to and after pretreatment.

Nevertheless, the modification of the chemical structure and functional group of biomass compositions also performed an important role in the enzymatic digestibility by changing the productive adsorption of cellulase on substrate.[30] Thus, FTIR spectroscopy was conducted to detect the change of chemical structure and functional group of biomass constituents after ferric chloride pretreatment to probe what differed from each other among these different biomass sources. Figure 5 shows the FTIR spectra of bagasse, rice straw, and wood fibers with or without ferric chloride pretreatment. The FTIR spectrum fluctuated trends of all biomass raw materials were similar, suggesting that they have the same chemical structure and functional group. However, the signals of the ether bond[14,15] at 1235 cm^{-1} and the ester bond[14,31] at 1737 cm^{-1} for spectra of untreated bagasse and wood fiber were both stronger, in comparison to that of untreated rice straw. It meant that the content of ether and ester linkages between lignin and carbohydrates of untreated rice straw was low due to its small lignin content. For this reason, rice straw had a relatively weak matrix structure, and the rice straw was much more readily attacked by the ferric chloride than the other two kinds of biomass. The weakened signals of both of the two spectra peaks among all the treated biomass might contribute to the disruption of the ether and ester linkages between lignin and carbohydrates of biomass or the reduction of lignin. However, the cleavable linkages between lignin and carbohydrates should be the main reason because the lignin was hardly removed based on the lignin content data in the prior discussion. Furthermore, a decrease in the band at 897 cm^{-1}, which demonstrates the presence of predominant β-glycosidic linkages between the sugar units in cellulose and hemicellulose, suggested the change of linkages between sugar units and intermolecular degradation in the hemicellulose structure,[32] which might be attributed to the removal of hemicellulose. In all the cases conducted with $FeCl_3$ pretreatment, it could interrupt the rigid chemical linkages among biomass constituents and remove the hemicellulose surrounding cellulose, facilitating the enzymatic digestion of pretreated biomass.[5]

Additionally, SEM was also employed in the study to observe the physical structure changes of these biomass species for an insight to relationship between CED and physical structure. Figure 6 shows the physical structure change of biomass prior to and after pretreatment. The surface of untreated wood fiber was much flatter, smoother, and more contiguous (Figure 6C) than that of untreated bagasse and rice straw (Figure 6A and B). Especially, the untreated rice straw seemed to have more holes and thin layers. It further conformed that rice straw should be much more susceptible to ferric chloride pretreatment and subsequent enzymatic hydrolysis. More cracks and crimps were observed on the surface of the $FeCl_3$-treated

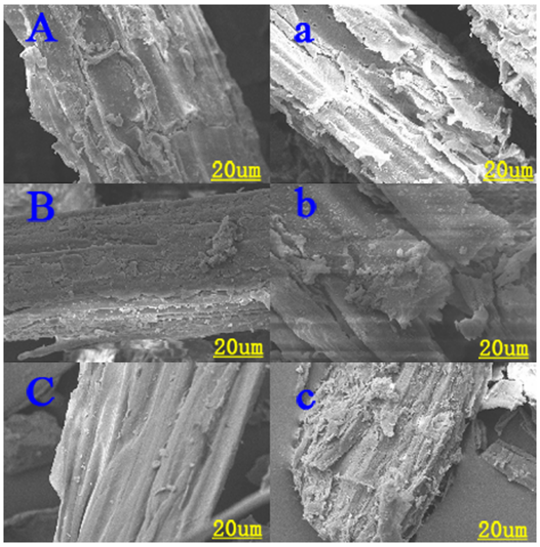

Figure 6. SEM images of bagasse (A and a), rice straw (B and b), and wood fiber (C and c) before and after ferric chloride pretreatment.

biomass (Figure 6a−c). This resulted from the removal of the easily digested hemicellulose, which hindered the contact of the cellulase with cellulose by adsorbing the enzyme and physically blocking the access of enzyme to the cellulose surface. $FeCl_3$ pretreatment could not only cleave recalcitrant structures in the biomass but interrupt orderly hydrogen bonds in crystalline cellulose, which increases the accessibility of cellulase to cellulose. Moreover, $FeCl_3$ pretreatment could damage the intact cell structure and expose the internal structure of the cell, resulting in an increase in the external surface area. Therefore, the pretreated biomass was much more amenable to digestion by cellulase than the untreated one, although the supramolecular structure of biomass was only partially destroyed.

Mass Balance of Converting Rice Straw to Ethanol. Mass balance of processes can provide elementary data to evaluate economic efficiency. In this part, rice straw, due to its high digestibility, was chosen for economic evaluation of ferric chloride pretreatment. As a comparison, hydrochloride acid solution, equivalent to the cost of chemicals consumed in ferric chloride pretreatment, was employed in pretreating the rice straw. During ferric chloride pretreatment, the recoverable ferric chloride should be taken into consideration. It can cut the cost of chemicals consumed in the pretreatment. Hence, the pretreatment chemicals cost needs to exclude the cost of recoverable ferric chloride. Nevertheless, some hydrochloride acids were applied to convert the recoverable precipitated $Fe(OH)_3$ to $FeCl_3$. Thus, the actual cost of ferric chloride pretreatment must include the cost of unrecoverable ferric chloride and consumed hydrochloride acid. The usage and cost of ferric chloride and hydrochloride acid in the ferric chloride pretreatment based on 1 ton of rice straw are summarized in Table 2.

Table 2. Usage and Cost of Chemicals in Ferric Chloride Pretreatment

chemicals	unit price ($/ton)	usage[a] (kg)	actual cost[a] ($)
$FeCl_3·6H_2O$	110	273.2	2.7
32% HCl[b]	200	311.4	62.3
Total			65.0

[a] Based on 1 ton biomass. [b] Used for possible conversion of $Fe(OH)_3$ to $FeCl_3$.

The unit price of chemicals (industrial grade) used in this experiment refer to the data provided by Alibaba Group. The actual cost of ferric chloride was as low as $2.7 per ton biomass, which resulted from the high recovery of ferric chloride (91.0 ± 0.4%). However, a large amount of hydrochloride acid needs to be used to convert the $Fe(OH)_3$ to $FeCl_3$, resulting in high cost ($62.30 per ton biomass). Hence, the total actual cost could reach $65 per ton biomass. To be clear, the total cost of $FeCl_3·6H_2O$ used in pretreatment based on 1 ton biomass only requires $30. Actually, $Fe(OH)_3$, as a recoverable form of $FeCl_3$ in pretreatment, must not be converted to $FeCl_3$ by adding HCl due to its expensive cost. It can be applied to other potential uses. However, in order to compare with the biomass pretreated by $FeCl_3$ at the possible maximum cost, the concentration of hydrochloride acid solution would reach 0.28 mol/L as calculated based on the cost of $65. The HCl pretreatment was conducted under the same temperature and time as the $FeCl_3$ pretreatment. In this case, the HCl pretreatment severity (CSF) could reach 2.99, higher than that of $FeCl_3$ pretreatment. Meanwhile, enzymatic hydrolysis and fermentation were conducted for rice straw pretreated by hydrochloride acid as well as ferric chloride. The total data from converting untreated rice straw to ethanol are plotted in Figure 7 to depict the overall mass balance of the experiment and reveal the effect of ferric chloride and hydrochloride acid pretreatment. Ferric chloride could efficiently degrade hemicellulose and release more xylose than hydrochloride acid did. Therefore, a higher cellulose enzymatic digestibility (95.1%) of pretreated rice straw was obtained owing to greater accessibility of cellulase to the substrate from ferric chloride pretreatment. Certainly, the ultimate ethanol yield was 143 kg (181 L) per 1 ton rice straw from ferric chloride, equivalent to 80.6% theoretical based on untreated rice straw glucan content, more than that (58.6%) from hydrochloride acid pretreatment. In the meantime, a large amount of NaOH solution is needed to neutralize the spent liquor during the HCl pretreatment. Otherwise, it would lead to serious water pollution.

■ CONCLUSIONS

Ferric chloride showed a wide suitability for pretreating biomass species, especially for rice straw, which contains a high hemicellulose content and weak matrix structure. The ratio of removed hemicellulose to raw cellulose seems much more credible for comparing the digestibility of diverse biomass species. With the highest hemicellulose removal (83.3%) based on glucan, a favorable conversion (81.9%) of cellulose could be attained for pretreated rice straw, when conducted in high solid loading (20%) hydrolysis. However, it needs to employ significant amounts of various biomass species to verify the relationship between digestibility and hemicellulose removal based on glucan in future work. Furthermore, $FeCl_3$ pretreatment exhibited greater efficiency and lower pollution than HCl pretreatment.

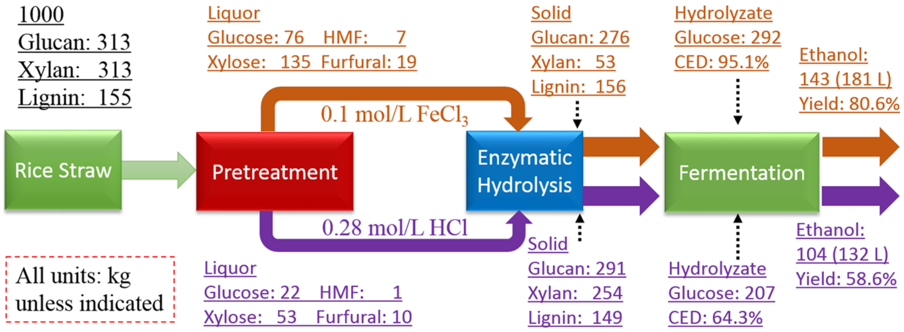

Figure 7. Mass balance for the ferric chloride and hydrochloride acid pretreatment of rice straw with subsequent saccharification and fermentation.

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Notes

The authors declare no competing financial interest.

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