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Chemical Composition of Native and Ammonia Fiber Expansion Pretreated Rice Straw-Unextracted versus Extractives-free Material

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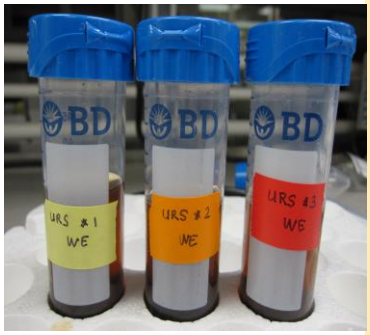
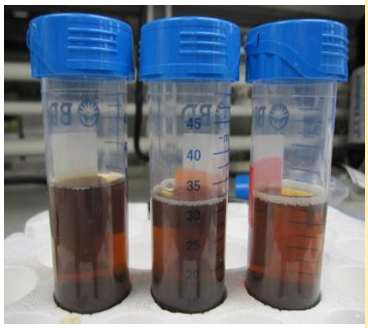
Graphical abstract





Abstract

Characterization of chemical composition for lignocellulosic biomasss (LCB) is essential in the conversion process of LCB to bioenergy and biochemicals. Accurate quantification of chemical composition allows for better determination of the LCB conversion and product yield in LCB processing. However, the presence of the extractable material is stated to strongly influence the compositional analysis of the LCB. This study was conducted to analyze and compare the chemical composition of (A) native-whole unextracted rice straw of (1) untreated rice straw (URS) and (2) AFEX pretreated rice straw (AC1RS and AC2RS) as well as (B) the extractives-free rice straw of (1) URS and (2) AFEX pretreated rice straw (AC1RS and AC2RS). The effect of the AFEX pretreatment on the composition of rice straw was determined using the extractives-free material of URS, AC1RS and AC2RS. The actual reported composition of native-whole rice straw of URS and AFEX pretreated rice straw were made based on the corrected values of the extractives-free rice straw of URS and AFEX pretreated rice straw. The rice straw was analyzed for structural constituents such as glucan, xylan, arabinan and Klason lignin as well as non-structural constituents. The results demonstrated that the extractives in native-whole unextracted rice straw significantly interfere with the analysis of Klason lignin. The lignin content of the rice straw was overestimated if the extractives were not removed prior to the compositional analysis. The extractives in rice straw statistically did not affect the carbohydrate analyses. However, some soluble sugars were removed from the rice straw during the extraction process.

Keywords: Lignocellulosic biomass; rice straw; compositional analysis; extractives

Abstrak

Pencirian komposisi kimia biojisim berlignoselulosa (*LCB*) amat penting didalam proses penukaran *LCB* kepada biotenaga dan biokimia. Kuantifikasi komposisi kimia yang tepat membolehkan penentuan penukaran *LCB* dan hasil produk didalam pemprosesan *LCB* dilakukan dengan lebih baik. Walaubagaimanapun, kehadiran bahan boleh ekstrak dinyatakan mampu memberi pengaruhi yang kuat terhadap analisa komposisi *LCB*. Kajian ini dilakukan untuk menganalisa dan membandingkan komposisi kimia (A) jerami padi penuh tidak terekstrak daripada (1) jerami padi tidak terawat (*URS*) dan (2) jerami padi terawat *AFEX* (*ACIRS* dan *AC2RS*) dan juga komposisi kimia (B) jerami padi bebas bahan boleh ekstrak daripada (1) *URS* dan (2) jerami padi terawat *AFEX* (*ACIRS* dan *AC2RS*). Kesan prarawatan AFEX ke atas komposisi jerami padi ditentukan menggunakan jerami padi bebas bahan boleh ekstrak daripada URS, ACIRS dan AC2RS. Komposisi sebenar jerami padi penuh URS dan jerami padi terawat AFEX ditentukan berdasarkan pelarasan semula nilai komposisi jerami padi bebas bahan boleh ekstrak daripada URS dan jerami padi terawat AFEX. Analisa komposisi jerami padi tertumpu kepada juzuk berstruktur seperti glukan, xilan, arabinan dan lignin Klason berserta juzuk tidak berstruktur yang lain. Hasil keputusan menunjukkan bahan boleh ekstrak didalam jerami padi penuh tidak terekstrak amat mempengaruhi analisa lignin Klason. Kandungan lignin jerami padi penuh terlebih anggar sekiranya bahan boleh ekstrak yang terdapat didalam jerami padi penuh tidak dibuang terlebih dahulu sebelum analisa komposisi dilakukan. Analisa statistik menunjukkan bahan boleh ekstrak tidak mempengaruhi analisa karbohidrat. Walau bagaimanapun, terdapat gula boleh larut yang terekstrak daripada jerami padi semasa proses pengekstrakan.

Kata kunci: Biojisim berlignoselulosa; jerami padi; analisa komposisi; bahan boleh ekstrak

1.0 INTRODUCTION

Agricultural residues of lignocellulosic biomass (LCB) such as rice straw, wheat straw and bagasse, are composed of cellulose (C6H10O5)x, hemicelluloses such as xylan and arabinan (C5H8O4)m, and lignin [C9H10O3˙(OCH3)0.9-1.7]n. The respective compositions are approximately 35-40% for cellulose, 25-35% for hemicellulose and 12-18% for lignin.[7,18,19] These LCBs, however, differ in their structural complexity in terms of the type of arrangement and spatial distribution of constituent monomeric subunits.[18] The compositions and percentages of hemicellulose and lignin vary from one plant species to another, therefore, it is difficult to arrive at generalizations concerning the chemical structure and abundance of these polymers.[12]

Accurate quantification of biomass chemical composition enables evaluation of process conversion, product yield and process economics. It improves the understanding of the economics and environmental impacts of biomass conversion processes by providing values and uncertainties for use in process and life-cycle models.[9,22] Therefore, it is very critical and important to develop and implement a suitable and systematic standard analysis method in performing the compositional analysis.

In this study, we used a collection of standard laboratory analytical procedures specifically for the compositional analysis of LCB developed by National Renewable Energy Laboratory (NREL). One of the sequences in compositional analysis of LCB is the extraction process, and it is used to extract the extractable material, known as the extractives, that vary so much in the biomass chemical composition. These extractives always need to be removed prior to compositional analysis for structural components in biomass, and failure to remove these materials can cause significant error in mass closure.[9] This study was conducted to analyze and compare the chemical composition of native native-whole unextracted rice straw of untreated rice straw (URS) and AFEX pretreated rice straw (AC1RS and AC2RS) as well as the extractives-free rice straw of URS and AFEX pretreated rice straw (AC1RS and AC2RS). The effect of the AFEX pretreatment on the composition of rice straw was also determined using the extractives-free material of URS, ACIRS and AC2RS.

2.0 MATERIALS AND METHODS

Based on NREL procedures, the compositional analysis of the native rice straw and anhydrous ammonia pretreated rice straw, involved ash and protein analysis, sequence of extractions with distilled water followed by 95% ethanol (190 proof), two-stage acid hydrolysis and gravimetric filtration of the acid hydrolysate. These protocols were performed on the (1) native-whole unextracted material of URS and AFEX pretreated rice straw (AC1RS and AC2RS) as well as on the (2) extractives-free material of URS and AFEX pretreated rice straw (AC1RS and AC2RS). Solvent-extracted rice straw or known as the extractives-free material was prepared by extracting the native-whole URS and AFEX pretreated rice straw (AC1RS and AC2RS) sequentially with water and ethanol according to NREL protocols. The compositions of the (1) native-whole unextracted material of URS and AFEX pretreated rice straw (AC1RS and AC2RS) as well as on the (2) extractives-free material of URS and AFEX pretreated rice straw (AC1RS and AC2RS) were characterized based on the structural constituents such as glucan, xylan, arabinan and Klason lignin as well as the non-structural constituents such as ash and extractives.

2.1 Preparation of rice straw

The rice straw in Sungai Burung, Selangor was cut using sickle at the height of 5 cm to 10 cm from the ground level, cleaned from dirts and soil, and dried naturally in the open air for 2 to 3 days. The Foss mill (Eden Prairie, MN) with 5 mm sieve was used to grind the 5 cm large particle of native rice straw to produce the 5 mm small particle of rice straw and were stored at 4 °C until further use.

2.2 Ammonia Fiber Expansion Pretreatment of Rice Straw

Two optimized AFEX pretreatment conditions from a previous study, identified as AFEX C1 and AFEX C2, were used to pretreat the rice straw.[10] Rice straw of the predetermined moisture level was loaded into a bench-top high-pressure Parr reactor with a 2000 mL capacity (PARR Instrument Co., IL) and liquid ammonia was slowly charged to the reactor. The reactor temperature was raised and maintained at the desired temperature for a given residence time and pressure, as reported before.[2]

2.3 Compositional Analysis

Compositional analysis was performed on native rice straw (URS) and AFEX pretreated rice straw (AC1RS and AC2RS) using milled rice straw of 5 mm according to Laboratory Analysis Protocol (LAP) developed by the National Renewable Energy Laboratory (Golden, Colorado USA).[10] The URS and AFEX pretreated rice straw (AC1RS and AC2RS) were extracted with water and 95% ethanol using an ASE2000 (Accelerated Solvent Extractor, DIONEX, CA) to remove the extractives before quantifying the structural carbohydrates and lignin in the acid hydrolysis step. Crude protein was calculated based on nitrogen content in the biomass. A Skalar Primacs SN Total Nitrogen Analyzer (Breda, Netherlands), was used to estimate the nitrogen content in the biomass using the Dumas method.

2.4 High Performance Liquid Chromatography (HPLC) for Carbohydrate Quantification

HPLC analysis was to use to quantify the monomeric sugars that reflected the amount of carbohydrate in the biomass. All monomeric sugars (glucose, xylose and arabinose) were analyzed using HPLC. The system consists of a Shimadzu LC-2010 (Milford, MA) equipped with a Waters 410 refractive index detector. An Aminex HPX-87P column (Bio-Rad, Sunnyvale, CA, USA) with a de-ashing guard cartridge (Bio-Rad) was used for monomeric sugars concentration analysis in hydrolysate. Degassed HPLC grade water was used as the mobile phase at 0.6 ml/min at a column temperature of 85°C. An Aminex HPX-87H column (Bio-Rad, Sunnyvale, CA, USA) was used to quantify the sugar concentrations in the acid hydrolysis samples for compositional and oligomers analysis. 5 mM sulfuric acid (H2SO4) was used as the mobile phase at 0.6 ml/min at a column temperature of 50°C. The HPLC sample injection volume was 10 µl. Standard curves were generated using different concentrations of mixed sugars.[3]

3.0 RESULTS AND DISCUSSION

The comparison on compositions between URS and AFEX pretreated rice straw were made based on the native-whole unextracted rice straw, extractives-free rice straw and corrected native-whole rice straw. Table 3.1 presents the compositions of URS and AFEX pretreated rice straw, AC1RS and AC2RS from

the native-whole unextracted material, extractives-free material, and corrected native-whole material, respectively. These composition results were expressed as the percentage of the oven-dried, native-whole unextracted rice straw.

3.1 Native-Whole Unextracted URS and AFEX Pretreated Rice Straw

In general, the compositions of the structural components of the native-whole unextracted URS were made up of structural carbohydrates with 61.6%, Klason lignin with 26.2%, and acetyl group with 1.7%. The carbohydrates were composed of glucan and xylan with 35.7% and 21.3% respectively as the major components while arabinan was only 4.5%. The non-structural components of the native-whole unextracted URS accounted for about 16.8% of the rice straw, mainly ash and nitrogen (Table 3.1).

The structural components of the native-whole unextracted AC1RS and AC2RS yielded about 58.4% to 58.6% for structural carbohydrates, 22.4% to 22.9% for Klason lignin, and 1.3% to 1.4% for acetyl group. The carbohydrates of these AC1RS and AC2RS constituted approximately by 34.0% for glucan, 21.0% for xylan, and 4.0% for arabinan. The non-structural components of native-whole unextracted AC1RS and AC2RS, ash and nitrogen were about 19.9% to 20.6% (Table 3.1). A statistical paired t-test on the mean composition of the components between the native-whole unextracted URS and AFEX pretreated rice straw, AC1RS and AC2RS, indicated that the differences in compositions of carbohydrates components (glucan, xylan and arabinan), acetyl group, and ash were statistically insignificant (t-stat<tcritical and p>0.05). This was due to a “dry to dry” AFEX process, which prevented the loss of holocellulosic components during pretreatment of rice straw (Mosier *et al*. 2005; Teymouri *et al*. 2005). R. Kumar *et al*. (2009) reported that the yields of the components left after pretreatment, particularly the carbohydrates, were 100% for each glucan, xylan, and arabinan, and current results in this study were consistent with their results.[14]

However, the compositions of Klason lignin and nitrogen between the native-whole unextracted URS and AFEX pretreated rice straw (AC1RS and AC2RS) showed significant differences (t-stat>tcritical and p<0.05). The reduction of Klason lignin in AC1RS and AC2RS was potentially due to the lignin degradation during the AFEX pretreatment, which solubilized and redeposited on biomass surface. R. Kumar *et al*. characterized changes in physical features of corn stover and poplar before and after AFEX pretreatment.[14] They reported that some lignin droplets appear to be present on the surface of AFEX pretreated solids at a 3000 magnification, suggesting that some lignin melted during AFEX pretreatment and agglomerated on the surface. These results are consistent with reports by Chundawat[5] that carbon rich components, which were lignin, were found on the biomass surface after AFEX pretreatment. During the two-stage acid hydrolysis used in compositional analysis, this newly redeposited and melted lignin on the biomass surface would be released the in the form of acid soluble lignin.[23] Therefore, this would reduce the amount of Klason lignin as observed in AC1RS and AC2RS.

Table 3.1 Composition of native-whole, extractive-free, and corrected native-whole of URS, AC1RS and AC2RS

Components	Composition of rice straw (expressed as the percentage of the oven-dried, native-whole unextracted rice straw)								
	Native-whole unextracted material			Extractive-free material			Corrected native-whole material		
	URS	AC1RS	AC2RS	URS	AC1RS	AC2RS	URS	AC1RS	AC2RS
Structural components:									
1. Struc. Carbo	61.6±1.7	58.4±0.7	58.6±0.9	55.2±0.6	51.6±0.2	49.7±0.3	57.8±0.6	57.2±0.3	57.8±0.4
Glucan	35.7±1.3	34.0±0.7	34.0±0.9	32.7±0.6	31.8±0.2	32.1±0.3	34.4±0.6	33.8±0.2	34.6±0.4
Xylan	21.3±0.3	20.5±0.1	20.0±0.2	19.0±0.2	16.8±0.1	15.0±0.1	19.7±0.2	19.8±0.2	19.5±0.1
Arabinan	4.5±0.2	3.9±0.0	4.3±0.6	3.5±0.1	2.9±0.0	2.6±0.1	3.7±0.1	3.6±0.0	3.7±0.1
2. Lignin	26.2±0.4	22.4±0.4	22.9±0.3	21.0±0.5	16.6±0.2	16.9±0.2	19.8±0.8	15.4±0.8	15.8±1.0
3. Acetyl group	1.7±0.1	1.3±0.1	1.4±0.2	1.6±0.0	<0.1	<0.1	1.6±0.1	1.4±0.1	1.7±0.0
Non-structural components:									
1. Ash	16.3±0.2	16.9±0.1	16.4±0.2	14.1±0.2	13.5±0.1	13.4±0.2	14.1±0.2	13.5±0.1	13.4±0.2
2. N_2 - native	0.5±0.2	0.5±0.2	0.5±0.2	0.2±0.2	0.2±0.2	0.2±0.2	0.5±0.2	0.5±0.2	0.5±0.2
3. N_2 – AFEX	0.0	2.5±0.4	3.7±0.4	0.0	1.9±0.3	1.9±0.3	NR	2.5±0.4	3.7±0.4
4. Extractives	NR	NR	NR	14.0±1.8	25.3±0.6	30.2±2.3	6.7±1.8	11.8±1.4	12.8±1.0

All values are means of duplicate ± standard deviation

In contrast, the increase of nitrogen in native-whole unextracted AC1RS and AC2RS was solely due to the addition of ammonia in AFEX pretreatment itself. Apparently, from Table 3.1, AFEX C2 condition yielded more nitrogen in AC2RS, 3.7%, when compared to AFEX C1 condition with 2.5% nitrogen content in AC1RS. This finding is interesting as the ratio of ammonia to solid under AFEX C2 condition (1:1) was half than the ratio under AFEX C1 condition (2:1). This may indicate that with AFEX C2 condition, where higher reaction temperature (140oC) was applied, more ammonia was able to penetrate the cellulose that resulted in the formation of ammonia-cellulose complexes. This led to the incorporation of ammonia into the cellulose crystal lattice, causing lattice transformation and crystal plane widening[13], knowing as swelling effect.[15,17] Most of the ester linkages were broken during this process, resulting in solubilization of lignin residues and re-deposition on the surface when ammonia was removed from the reactor. Previous work on AFEX pretreatment of several biomass including rice straw also indicated similar trend of compositional changes in the components discussed above.[2,30]

3.2 Extractives-Free and Corrected Native-Whole of AFEX Pretreated Rice Straw

The extractives in plants influence the compositional analyses of structural carbohydrates, acid insoluble lignin, and ash, particularly in herbaceous biomass, which consist high amount of extractives.[23,25,26] The pretreated rice straw must be analyzed for the carbohydrates content in order to determine the efficiency of the AFEX pretreatment and estimate the theoretical amount of glucan available in order to calculate the glucan conversion in the subsequent downstream process. Therefore, as with URS, the extractives-free material of AC1RS and AC2RS were used in determining and correcting the composition of the components in the rice straw after the pretreatment.

Apparently, the compositions of the extractives-free AC1RS and AC2RS revealed the actual effect of the AFEX pretreatment on the rice straw (Table 3.1). These compositions of AC1RS and AC2RS were statistically different when compared to the composition of native-whole unextracted AC1RS and AC2RS, primarily in the structural carbohydrates, Klason lignin and acetyl group (t-stat>tcritical and $p<0.05$). The structural carbohydrates of AC1RS and AC2RS decreased significantly from 58.4% and 58.3% in the native-whole pretreated rice straw to 51.6% and 49.7% in the extractives-free rice straw respectively, primarily due to the hemicellulose solubilization in the extractives. Hemicelluloses in extractives-free AC1RS and AC2RS were less by 19.1% and 27.5%, respectively with respect to hemicellulose in native-whole unextracted AC1RS and AC2RS. The Klason lignin of AC1RS and AC2RS varied from 22.4% and 22.9% in the native pretreated rice straw to 16.6% and 16.9% in the extractives-free rice straw, approximately 26% reduction for each. The acetyl group of AC1RS and AC2RS indicated that more than 90% of the acetyl group in the native-whole of AC1RS and AC2RS were easily extracted into the extractives, leaving less than 0.1% in the extractives-free AC1RS and AC2RS.

Basically, hemicelluloses of rice straw are characterized experimentally and composed mainly of α–L-(1-3)-arabino-(4-O-methyl-α-(1-2)-D-glucurono)-β-(1-4)-D-xylan and/or arabino-glucuronoxylan, AGX.[27] The xylan backbone, consists of β-(1-4)-D-xylopyranosyl units, is typically substituted by monomeric 4-O-methyl-α-D-glucopyranosyluronic acid residue (4-O-MeGlcA) and α–L-arabinofuranosyl unit at C2 and/or C3 main chain. A significant portion of the xylose in cereal straw cell walls is acetylated, mainly on C2 and C3 and the acetyl groups account for 1% - 2%.[21,29] Lignin exists in plant tissue as a dependent polymer, and is always associated with cellulose, hemicelluloses and other polymers as lignin-carbohydrates complexes (LCCs) through covalent bonds. In herbaceous plants such as rice straw, LCCs contain ferulic bridges, which are attached to lignin and carbohydrates (AGX) via ether and ester bonds respectively. Alkali cleaves the ester bond components of such bridges, liberating the ferulic acid (FA) residue and lignin from carbohydrates yielding small amount of FA (1% to 4%).[4,27,28] Experimental analysis on isolated LCCs from rice straw reveals that it contains 64% carbohydrates, 3% uronic acid, 33% lignin, 4% acetyl group, 4% trans-p-coumaric acid and 1% trans-ferulic acid.[1]

The decrease in hemicelluloses, Klason lignin, and ash after the solvent extractions of AC1RS and AC2RS was complemented by the increase in extractives from extractions of AC1RS and AC1RS. The total extractives extracted out from the rice straw, including the water soluble products, soluble lignin, soluble proteins, soluble salts and minerals, and others, significantly increased with the increase in pretreatment severity, from 14.0% in URS, to 25.3% in AC1RS, and 30.2% in AC2RS, implying the presence of additional amount of solubilized components from AFEX pretreated rice straw. Hence, this observation showed that during AFEX pretreatment, ammonia was able to chemically cleave these ester linkages of AGX via ammonolysis and the structure of lignin and AGX in LCCs.[3,4] These hemicelluloses and lignin residues were easily extracted and solubilized in subsequent solvent extractions.

Table 3.2 characterizes the composition of the total extractives from URS, AC1RS and AC2RS rice straw samples based on extractives in water and ethanol extractions. The LCCs cleavage was evidenced by the increase in soluble oligomeric sugars found in water extractions of AFEX pretreated samples. In comparison to URS water extraction, AC1RS and AC2RS water extraction yielded 4.1 and 6.2-fold increase of oligomeric xylose, 4.1 and 7.3-fold increase of oligomeric arabinose as well as 16 and 19-fold increase of acetyl that solubilized the water extraction (Table 3.2). This increase in acetyl solubility is likely due to the dissolution of O-acetyl group on xylan-pyranose backbone side chain in this alkaline treatment.

In general, xylan in cell wall of graminaceous plants, like rice straw, contains 1% - 2% of O-acetyl group.[12,20] Hemicellulose components, xylose, arabinose and acetyl, dissolved and solubilized more during water extraction of AC2RS compared to AC1RS showing more disruptions of LCC ester linkages occurred under the more severe AFEX C2 pretreatment conditions. Higher severity pretreatments can be higher in any/all of the pretreatment factors such as temperature, residence time, alkali/acid concentration, and moisture content, causes more release of hemicelluloses and lignin degradation products during pretreatments.[3,6,8,11]

Table 3.2 Composition of extractives from URS, AC1RS and AC2RS

All values are means of triplicate ± standard deviation

Type of extractives	Composition in %		
	URS	ACIRS	AC2RS
Total extractives	14.03±1.84	25.33±0.55	30.24±2.3
Identified water extractives	2.97±0.05	7.15±0.04	10.69±0.08
Glucose oligo	*0.68±0.06*	*0.80±0.07*	*0.88±0.19*
Xylose oligo	*0.72±0.05*	*2.95±0.11*	*4.49±0.10*
Arabinose oligo	*0.15±0.01*	*0.70±0.02*	*1.10±0.02*
Acetyl	*0.09±0.02*	*1.43±0.09*	*1.70±0.03*
Sucrose	*0.10±0.01*	*0.86±0.02*	*1.85±0.21*
Glucose	*0.54±0.16*	*0.29±0.01*	*0.55±0.06*
Fructose	*0.68±0.0*	*0.12±0.06*	*0.11±0.01*
Extractable crude protein (native)	2.08±0.29	2.08±0.29	2.08±0.29
Extractable nitrogen (AFEX)	-	0.62±0.38	1.74±0.22
Ethanol extractives	2.35±0.08	2.41±0.73	2.72±0.35
Extractable ash	2.26±0.38	3.42±0.08	2.98±0.36
Other extractives	6.62±1.80	12.81±1.14	13.01±0.83
Corrected extractives	6.72±1.85	11.80±1.36	12.76±0.97

Other possible extractives such as gums, resins, pitch, waxes, sterols, flavinoids, tannins, terpenes, quinones, non-structural sugars, chlorophyll and other minor building blocks [9] were not quantified in this study. However, it could be seen that the fraction of other possible extractives identified as other extractives was higher in both AC1RS and AC2RS extractions when compared to URS extraction. The other extractives in both AC1RS and AC2RS extractions approximately yielded 1.9-fold

increase compared to other extractives in URS. It could be seen that both ethanol extractives and extractable ash were not significantly different from these extractions of URS, AC1RS and AC2RS (t-stat<tcritical and p>0.05).

The corrected native-whole compositions of URS, AC1RS, and AC2RS were reproduced after subtraction of the identified extractives from the reported extractives, and correcting their components for any loss, degradation or double-counting, particularly the carbohydrates, lignin and extractives.[9] The corrected native-whole compositions of the carbohydrates, mainly glucan, xylan, and arabinan, in URS, AC1RS and AC2RS were similar, and the differences between URS and AFEX pretreated rice straw were statistically insignificant (t-stat<tcritical and p>0.05) (Table 3.1). This indicated good total sugar recovery of the pretreated rice straw after the pretreatment as reported in previous work.[16,17,24] The lowest value of Klason lignin and extractives indicated that some false materials were successfully removed from them. Therefore, these corrected native-whole compositions were taken as the actual compositions for both URS and AFEX pretreated rice straw.

■4.0 CONCLUSIONS

Overall, the compositional analysis performed on the URS and AFEX pretreated rice straw (AC1RS and AC2RS substrates), either the native-whole or the corrected compositions, conclusively indicated that the differences in the structural carbohydrates components (glucan, xylan, arabinan, and acetyl) were statistically insignificant (t-stat<tcritical and p>0.05) showing good total sugar recovery of the pretreated rice straw after the pretreatment. This was due to the "dry to dry" AFEX pretreatment process, which prevented the loss of holocellulosic components during pretreatment of rice straw. However, the compositions of Klason lignin and nitrogen between URS and AFEX pretreated rice straw (AC1RS and AC2RS) showed significant differences (t-stat>tcritical and p<0.05). The decrease of Klason lignin in AC1RS and AC2RS was potentially due to the lignin degradation during the AFEX pretreatment, which solubilized and redeposited on biomass surface. In contrast, the increase of nitrogen in AC1RS and AC2RS was solely due to the addition and incorporation of ammonia from AFEX pretreatment itself into the rice straw.

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References

[1] Azuma, J. & Koshimjima, T. 1988. Lignin-carbohydrate Complexes from Various Sources. *Methods in Enzymology* 161: 12–18.
[2] Balan, V., da Costa Sousa, L., Chundawat, S. P., Vismeh, R., Jones, A. D. & Dale, B. E. 2008. Mushroom Spent Straw: A Potential Substrate for an Ethanol-based Refinery. *Journal of Industrial Microbiology and Biotechnology* 35: 293–301.
[3] Balan, V., Sousa, Lda. C., Chundawat, S. P., Marshall, D., Sharma, L. N., Chambliss, C. K. & Dale, B. E. 2009. Enzymatic Digestibility and Pretreatment Degradation Products of AFEX-treated Hardwoods (Populus nigra). *Biotechnology Progress.* 25(2): 365–375.
[4] Buranov, A. U. & Mazza, G. 2008. Lignin in Straw of Herbaceous Crops. *Industrial Crops and Products* 28: 237–259.
[5] Chundawat, S.P., Venkatesh, B. & Dale, B.E. 2007. Effect of Particle Size Based Separation of Milled Corn Stover on AFEX Pretreatment and Enzymatic Digestibility. *Biotechnology and Bioengineering*. 96(2): 219–231.
[6] Chundawat, S. P., Vismeh, R., Sharma, L. N., Humpula, J. F., da Costa Sousa, L., Chambliss, C. K., Jones, A. D., Balan, V. & Dale, B. E. 2010. Multifaceted Characterization of Cell Wall Decomposition Products Formed During Ammonia Fiber Expansion (AFEX) and Dilute Acid Based Pretreatments. *Bioresource Technology.* 101(21): 8429–8438.
[7] Demirbas, A. 2009. *Biofuels*. Securing the Planet's Future Energy Needs. London: Springer London.
[8] Fengel, D. & Wegener, G. 1984. *Wood: Chemistry, Ultrastructure, Reactions*. De Gruyter, Berlin.
[9] Hames, B. R. 2009. Biomass Compositional Analysis for Energy Applications. In: *Biofuels: Methods And Protocols*, Mielenz, J. R. (Ed). Humana Press, Thousand Oaks, California, USA. 145–167.
[10] Harun, S., Yu, X., Balan, V., Dale, B., Takriff, M. S., Hassan, O. and Jahim, J. 2011. Statistical Optimization of AFEX Process Conditions for Fermentable Sugar Production from Rice Straw. *Proceedings of 18th Regional Symposium on Chemical Engineering Vietnam.* 2011: 40.
[11] Hendriks, A. T. W. M. & Zeeman, G. 2009. Pretreatments to Enhance the Digestibility of Lignocelluloses Biomass. *Bioresource Technology.* 100: 10–18.
[12] Jeffries, T.W. 1994. Biodegradation of Lignin And Hemicelluloses. In: Biochemistry of Microbial Degradation, Ratledge, C. (Ed.). Kluwer Academic Publishers, Netherlands. 233–277.
[13] Krässig, H. A. 1993. Effect of Structure and Morphology on Accessibility and Reactivity. In: *Cellulose: Structure, accessibility, and reactivity*, Polymer Monographs (11). Gordon and Breach Science Publishers, Singapore. 215–258.
[14] Kumar, R., Mago, G., Balan, V & Wyman, C. E. 2009. Physical and Chemical Characterizations of Corn Stover and Poplar Solids Resulting From Leading Pretreatment Technologies. *Bioresource Technology.* 100: 3948–3962.
[15] Lin, K. W., Ladisch, M. R., Schaefer, D., Noller, C. H., Lechtenberg, V. & Tsao, G. T. 1981. Review on Effect of Pretreatment on Digestibility of Cellulosic Materials. *AIChE Symposium Series.* 77: 102–106.
[16] Moniruzzaman, M., Dale, B. E., Hespell, R. B. & Bothast, R. J. 1997. Enzymatic Hydrolysis of High-moisture Corn Fiber Pretreated by AFEX and Recovery and Recycling of the Enzyme Complex. *Applied Biochemistry and Biotechnology*. 67: 113–126.
[17] Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M. & Ladisch, M. 2005. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresource Technology.* 96: 673–686.
[18] National Agricultural Innovation Project. 2010. Novel Biotechnological Processes for Production of High Value Products from Rice Straw and Bagasse. New Delhi, India: National Agricultural Innovation Project. (online) http://www.naip.icar.org.in/ES/ESC4Rice.pdf (30 June 2012).
[19] Palonen, H. 2004. Role of Lignin in the Enzymatic Hydrolysis of Lignoncellulose. PhD Thesis, Espoo 2004, VTT Publication 520, VTT Biotechnology, VTT Technical Research Center of Finland.
[20] Ren, J.-L., Sun, R.-C. & Peng, F. 2008. Carboxymethylation of Hemicellulose Isolated from Sugarcane Bagasse. *Polymer Degradation and Stability.* 93: 786–793
[21] Ren, J.-L. & Sun, R. C. 2010. Hemicellulose. In: Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels, Sun RC (Ed). Elsevier, UK. 73–119.
[22] Sluiter, J. B., Ruiz, R. O., Scarlata, C. J., Sluiter, A. D. & Templeton, D. W. 2010. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. *Journal of Agricultural and Food Chemistry.* 58: 9043–9053.
[23] Tandjo, D. 1996. Influence of Extractives on the Chemical Analysis of Switchgrass. Master of Science Thesis Oregon State University, Oregon, USA.
[24] Teymouri, F., Laureano-Peréz, L., Alizadeh, H. & Dale, B. E. 2005. Optimization of the Ammonia Fiber Explosion (AFEX) Treatment Parameters for Enzymatic Hydrolysis of Corn Stover. *Bioresource Technology.* 96: 2014–2018.
[25] Thammasouk, K. 1996. The Role of Solvent Extraction in the Chemical Characterization of Rorn Stover Feedstock. Master of Science Thesis Oregon State University, Oregon, USA.
[26] Thammasouk, K., Tandjo, D. & Penner, M. H. 1997. Influence of Extractives on the Analysis of Herbaceous Biomass. *Journal of Agricultural and Food Chemistry.* 45(2): 437–443.
[27] Sun, R. C., Tomkinson, J., Ma, P.L. & Liang, S. F. 2000. Comparative Study of Hemicelluloses from Rice Straw by Alkali and Hydrogen Peroxide Treatments. *Carbohydrate Polymers.* 42: 111–122.

[28] Sun, Y. & Cheng, J. 2002. Hydrolysis of Lignocellulosic Material for Ethanol Production: A Review. *Bioresource Technology*. 83: 1–11.
[29] Xiao, B., Sun, X. F. & Sun, R. 2001. Chemical, Structural, And Thermal Characterizations of Alkali-soluble Lignins and Hemicelluloses, and Cellulose from Maize Stems, Rye Straw, and Rice Straw. *Polymer Degradation Stability*. 74: 307–319.
[30] Zhong, C., Lau, M. W., Balan, V., Dale, B. E. & Yuan, Y. J. 2009. Optimization of Enzymatic Hydrolysis and Ethanol Fermentation from AFEX-treated Rice Straw. *Applied Microbiology Biotechnology*. 84(4).