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Photoacoustic Spectroscopy in the Assessment of the Quantitative Composition of the Biomass – Barley Straw

Kingsley L. Iroba and Lope G. Tabil

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1. Introduction

Agricultural biomass residue is a naturally occurring polymeric compound comprised of cellulose, hemicellulose, lignin, and extractives [1]. Biomass has the potential to be used as raw material for the biofuel industry. Cellulose occurs as crystalline fibers with random amorphous portions. The crystallinity of cellulose fibers is a key obstacle for effective biomass enzymatic hydrolysis [2]. Hemicellulose functions as a matrix for the cellulose and acts as a linkage between the crystalline cellulose and amorphous lignin [1-2]. Lignin is a polyphenolic compound that acts as a cementing material for biomass [1]. Lignin binds the hemicellulose and cellulose within the matrix. It provides rigidity and mechanical strength, holding it together [3]. The complex structure of lignocellulosic biomass presents resistance and 'recalcitrance' to biological and chemical degradation during enzymatic hydrolysis/saccharification and the subsequent fermentation process. This leads to a very low conversion rate, which makes the process less economically feasible [4-7]. Pretreatment strategies are employed to overcome the aforementioned challenges by deconstructing/disrupting the lignocellulosic matrix [8], while making cellulose and hemicellulose accessible for the production of bioethanol.

Radio frequency (RF)-alkaline and steam explosion pretreatment of biomass barley straw have shown that pretreatment enhanced the modification of the natural binding characteristics of lignocellulosic biomass [8-9]. Subsequently, this improved the physical properties of the produced pellets from the pretreated biomass [10]. RF heating has been considered as an energy efficient heating method that is an alternative to conventional methods (such as hot air). In RF heating, there is a volumetric heat generation inside the product, which is a result

of the interaction between the RF waves and the ions/molecules of the product [11]. Therefore, heat flows from inside the product to the outside, unlike conventional heating methods in which heat is transferred from the heating medium to the product via conduction or convection [8]. RF heating has the following advantages: 1) uniform electric field strength inside the application chambers, therefore preventing uncontrolled heating, overheating, local hot spots, and product degradation; 2) large penetration depth (10-30 m); and 3) higher energy efficiency [11-13]. RF has been successfully applied to leather drying [13], quantification of hydroxycinnamic acids and lignin in perennial forage and energy grasses [14], thermal therapy [15], and in other research fields such as food processing (blanching, tempering, pasteurisation, sterilisation) and medicine [11]. Recently, Izadifar et al. (2009) [12] demonstrated that RF can be used for the extraction of podophyllotoxin from rhizomes of *Podophyllum peltatum*.

Steam explosion is operated by introducing the feedstock into the reactor and heating under steam pressure (2000-5000 kPa; 200-260°C) for a few minutes [16]. Steam explosion induces chemical effects because "water itself acts as an acid at high temperatures" [17]. The sudden thermal expansion involved in the termination of the reaction causes the particulate structure of the biomass to open up [17]. Lam et al. 2011 [18] investigated the steam explosion of Douglas Fir (*Pseudotsuga menziesii*) at a reaction temperature of 200-220°C and a retention time of 5-10 min. Excoffier et al. (1991) [19] and Ferro et al. [20] (2004) have applied steam explosion on lignocellulosic biomass. These authors reported that steam explosion is effective in the pretreatment of biomass.

The traditional methods of determining the chemical compositions of biomass involve gravimetric and analytical procedures. These procedures are time-consuming, laborious and expensive to perform, with low sample throughput and often results in a corresponding degradation of natural polymers [1, 21-22]. In contrast, procedures involving infrared (IR) spectroscopy are useful tools in rapidly extracting information about the structure of biomass constituents and the associated chemical changes resulting from various biomass treatments [1]. Infrared spectroscopy offers researchers an alternative method that is easier, robust and rapid. Fourier transform infrared (FTIR) spectroscopy has been successfully applied in a variety of species in wood surface characterization, for estimating the carbohydrate and lignin components [1, 22]. The majority of the carbon based molecules in plants and animals alike are highly active in the IR. FTIR is associated with superior spectral resolution and provides information on the fundamental molecular vibrations [22]. This permits better discrimination of structural and compositional differences, and often better structural interpretation [22]. Furthermore, FTIR analysis requires only small amounts of biomass plant material, which helps when screening samples available in limited quantities [22]. Applications of FTIR have also been found in biomedical and medicine research such as cancer and bone [23-24].

Mid-infrared spectroscopy and near infrared reflectance spectroscopy (NIRS) are the two types of IR spectroscopy that have found application for the measurement of chemical composition in lignocellulosic biomass. NIRS has been used in the prediction of the chemical composition in bulk plant samples. While mid-IR spectroscopy in contrast to NIRS predicts the fundamental molecular

vibrations, as such it gives a better information and insight into the molecular bonds present in the sample [25]. FTIR has been successfully applied for the comparison of Fourier transform infrared (FTIR) spectra of individual cells acquired using synchrotron and conventional sources [23]. The authors reported that the main beneficiaries of this rapid development (FTIR) on the instrumental front are the applications-based sciences, such as biomedical and cell-biological fields, for which results have been produced that are unavailable by standard methodologies. Stewart et al. (1995) [26] used FTIR spectroscopy to investigate the changes in composition and structure of oak wood and barley straw that has been subjected to chemical and biochemical treatments. The samples were also analyzed gravimetrically for residual neutral sugar composition, lignin and uronic acid contents. The spectroscopic techniques provided complementary information to the traditional approach. The FTIR fingerprint of wheat endosperm arabinoxylan (AX) has been investigated using a set of polysaccharides exhibiting variation of their degree of substitution and xylo-oligosaccharides comprising xylose units mono-or disubstituted by arabinose residues [27]. Yu (2005) [28] applied synchrotron FTIR microspectroscopy for the investigation of the molecular chemistry of various feed tissues. These images revealed spatial intensity and distribution of chemical functional groups in various feeds tissues within cellular dimensions. The researcher reported that such information can be used for plant breeding program for selecting superior variety of plant for targeted feed purposes and for prediction of feed quality and nutritive value. This proved that synchrotron FTIR microspectroscopy can be used for biological structure study.

Changes in plant cell wall composition following pretreatment largely influence the end use performance. The ability to easily monitor these compositional changes is, therefore, important. Hence, the objective of this investigation is to study and rapidly estimate the relative chemical composition of lignin, cellulosic and hemicellulosic polymers in pretreated and non-treated lignocellulosic barley straw using FTIR-PAS. This work focuses on the effects of radio frequency-alkaline and steam explosion pretreatments on the cell compositions of barley straw.

2. Material and methods

2.1. Material procurement and preparation

Barley straw of the “Xena” variety was grown in Maymont, SK (56.667°N, 107.794°W) and obtained from RAW Ag Ventures Limited (Maymont, SK) in October 2009. To increase the surface area of the biomass, the straw was ground using a hammer mill (Model No. GM13688, Glen Mills Inc., Maywood, NJ) with screen size of 1.6 mm. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation and to provide flow of the biomass in and out of the hammer mill. The initial moisture content of the straw was 8.09% (wet basis). The moisture content was measured based on ASABE standard method, ASAE S358.2 (2008). As a comparison between pretreatment methods, the 1.6 mm biomass grind was subjected to two different pretreatment methods: radio frequency (RF)-alkaline pretreatment using a RF machine (1.5 kW & 27.12 MHz

laboratory dryer, Strayfield, Theale, Reading) in a blown glass reactor (volume 4.25 liters) stationed in University of Saskatchewan, Saskatoon, SK, and steam explosion (SE) pretreatment located at the Clean Energy Research Center, University of British Columbia, Department of Chemical and Biological Engineering, Vancouver, BC. The material and operating variables considered in both methods of pretreatment are shown in Table 1 (a-b). Each pretreatment was performed in two replicates. For more details on the RF-alkaline and SE pretreatment, see [8-9].

Variables	Levels
Hammer screen size	1.6 mm
NaOH solution concentration	1% w/v
Biomass: NaOH solution ratio	1:4 - 110 g biomass and 440 g NaOH solution 1:5 - 110 g biomass and 550 g NaOH solution 1:6 - 100 g biomass and 600 g NaOH solution 1:7 - 100 g biomass and 700 g NaOH solution 1:8 - 90 g biomass and 720 g NaOH solution
Soaking time	1 h
Temperature	70, 80, and 90°C
Residence time	20 minutes
(a)	
Variables	Levels
Temperature (°C)	140, 160, and 180
Moisture Content (% mass fraction of water)	8, 30, and 50
Retention Time (min.)	5 and 10
(b)	

Table 1. a) RF-alkaline material and operating variables using blown glass reactor; (b) SE material and operating variables with corresponding levels

2.2. Chemical composition analysis of lignocellulosic biomass

The chemical composition analysis of the RF-alkaline and SE pretreated biomass grind was performed using the National Renewable Energy Laboratory standard (NREL) [30] at a laboratory facility at the Agriculture and Agri-Food Canada, Saskatoon, SK. Each sample was replicated twice. The NREL standard uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The first step uses 72% H_2SO_4 , while the second step uses 4% H_2SO_4 . The lignin fractionates into acid insoluble and acid soluble material. The acid insoluble lignin is the residue (remaining solids) from the hydrolysis suspension. Acid-soluble lignin moieties were quantified using the Waters Acquity Ultra Performance Liquid Chromatography–MS system (Acquity 2004-2010, Waters Corp., Milford, MA), which has the capability of separating and quantifying the various lignin components. The complex

carbohydrates are hydrolyzed into monomeric forms (xylose, arabinose, mannose, glucose, and galactose) and subsequently quantified using UPLC-MS. The percentage hemicellulose was obtained by adding up the percentage xylose, arabinose, mannose, and galactose, while the percentage glucose was assigned to percentage cellulose. Further details on the material preparation, physical characteristics of the biomass grind, radio frequency alkaline technique, steam explosion process, and the chemical composition analysis using the NREL standard can be obtained in the research studies of [8-9]. The pretreated and non-treated samples were further ground to screen size of 0.354 mm using a precision grinder (Falling Number, Model No. 111739, Huddinge, Sweden).

2.3. Preparation of reference materials of known concentration

In order to develop a predictive model that will be rapidly used for the quantitative prediction of the chemical composition contained in the RF-alkaline and SE pretreated biomass, pure cellulose (microcrystalline powder), hemicellulose (xylan from birch wood), and lignin (hydrolytic) powders (Sigma-Aldrich Canada Ltd., St. Louis, MO) were mixed in different proportions (Table 2). These were used as reference spectra to determine the relationship between the respective quantity in the mixture and the representative sample FTIR spectra. The FTIR wavenumber-dependent instrumental effects were corrected by using carbon black reference spectrum.

Reference Mixtures	Cellulose (%)	Hemicellulose (%)	Lignin (%)
C1H0L0	100	0	0
C0H1L0	0	100	0
C0H0L1	0	0	100
C7H2L0	75	25	0
C2H7L0	25	75	0
C0H2L7	0	25	75
C0H7L2	0	75	25
C7H0L2	75	0	25
C2H0L7	25	0	75
C5H2L2	50	25	25
C2H5L2	25	50	25
C2H2L5	25	25	50
C3H3L3	33	33	33
Total	13	13	13

C, H, and L represent Cellulose, Hemicellulose, and Lignin, respectively

Table 2. Reference materials: Pure cellulose, hemicellulose, and lignin mixtures used to obtain the reference spectra (adapted from [21])

2.4. Fourier Transformed Infrared Photoacoustic Spectroscopy (FTIR-PAS)

Intensity of spectra generally increases as the particle size decreases [30]. To avoid moisture interference, the biomass samples were further dried using the forced-air convection dryer [31] (Shaw et al. 2007) set at 40°C for 48 h. Photoacoustic intensities are lower for samples with high moisture content. This might be due to lower efficiency of heat transfer between the moist cellulose surface and the carrier gas [32]. The Infrared data/spectra of the reference materials and biomass samples were collected using Mid-IR beamline (01B1-1) with energy range of 4000-400 cm^{-1} , at the Canadian Light Source Inc. (CLS, University of Saskatchewan, Saskatoon, SK). The beamline has a MTEC Model 300 photoacoustic cell (MTEC Photoacoustic Inc., Ames, IA) for FTIR-PAS of bulk samples. The sample cup was filled with reference biomass sample (52-75 mg, depending on the pretreatment type and combination) and purged with helium gas to remove water vapor and CO_2 . Helium gas is also needed in the medium because of its sound propagating properties. The collected FTIR spectra of the reference materials and biomass samples were recorded using Globar source (silicon carbide rod). When the radiation is incident on the sample, the energy of the Infrared beam is being absorbed by the sample layer. The photoacoustic signal is generated by thermal expansion of the gas caused by heat associated with the thermal wave emanating from the sample. The photoacoustic signal is carried by a carrier gas (Helium) to a microphone which is transferred to the FTIR electronics (detector) for processing; this ultimately produces the needed spectrum [33-34]. The spectrum for each reference material and biomass samples were recorded separately averaging 64 interferograms (number of scans) collected from wavenumbers of 2000-400 cm^{-1} at a resolution of 4 cm^{-1} . The higher the number of scans the better the signal (lesser noise). Stuart (1997) [36] reported that the signal-to-noise ratio (SNR) is proportional to the square root of the number of scans, n ($\text{SNR} \propto n^{0.5}$). Therefore, the higher the number of scans, the higher is the SNR. Resolution of 4 cm^{-1} was used to be able to discriminate between too close overlapping peaks, help increase the SNR and subsequently obtain higher resolution. The OPUS 6.5 (Bruker Optics Inc. Billerica, MA) software was used for the collection of the FTIR-PAS spectra. Three replicates were performed for each reference and biomass samples.

2.5. Determination of concentration

Beer-Lambert discovered that the amount of light transmitted by a solid sample was dependent on the thickness of that sample [35]. The Beer-Lambert law which can be applied to all electromagnetic radiation, states that the absorbance of a material is directly proportional to the thickness and concentration of the sample as shown: $A = \epsilon CL$. A =absorbance of the material, C =concentration, L =pathlength of the sample, and ϵ =constant of proportionality, which is referred to as the molar absorptivity [35]. Infrared spectra, particularly in the spectra of solid samples are often associated with the presence of asymmetric bands. As such, peak height cannot be used for the quantitative analysis of the spectra, because the baseline will vary from sample to sample. Instead, peak-area measurements should be used [35].

2.6. Quantitative and qualitative analysis of the FTIR-PAS spectra

The two quantities of greatest interest in virtually any type of spectroscopy are, of course, band positions (wavenumbers) and intensities, the former generally conveying qualitative information, the latter quantitative [30]. Therefore, these two variables were used for the FTIR data analysis.

2.6.1. Spectrum manipulation

There are techniques that assist in both qualitative and quantitative interpretation of spectra. OriginPro software (Data analysis and graphing Version 8.6, OriginLab Corporation Northampton, MA) was used for the spectrum manipulation, quantitative, and qualitative analysis of the FTIR-PAS Spectra:

2.6.2. Baseline correction, subtraction, and rescaling

The Baseline Mode is a tool for choosing a baseline mode and creates the baseline. A user-defined baseline treatment was applied in this analysis. A common flat baseline of 0.012, joining the points of lowest absorbance (via fitting Pro) on the peak was selected and applies across all the reference materials and biomass sample spectra. Thereafter, baseline subtraction was performed from the input data, such that the absorbance difference between the selected baseline and the top of the band is then used. This helps to improve the accuracy of the peak finding. Rescaling of the baseline to zero was subsequently performed, so that all the spectra will have a common origin of zero.

2.6.3. Smoothing

Smoothing is a signal processing technique typically used to remove or diminish noise from signals/spectrum. After a spectrum is smoothed, it becomes similar to the result of an experiment obtained at a lower resolution [35, OriginLab Corporation manual]. The features are blended into each other and the noise level decreased. A smoothing function is basically a convolution between the spectrum and a vector whose points are determined by the degree of smoothing one wish to apply [35]. There are multiple smoothing methods that work differently depending on the nature of the signal and the noise contained in the signal. Each method offers a different performance. In this present analysis, Savitzky-Golay was used. The Savitzky-Golay filter method performs a local polynomial (order of 2) regression around each point, and creates a new, smoothed value for each data point. This method is superior to other methods (such as adjacent averaging) because it tends to preserve features of the data, such as peak height and width, which can be "washed out" by adjacent averaging (OriginLab Corporation, Northampton, MA, 2012). To increase the smoothness of the result, one can increase the "window size," used in each local regression (OriginLab Corporation, Northampton, MA, 2012). As such, the window size in this analysis was increased from one to two. But for very large window size, adjacent averaging may depart from the input signal too much, whereas

Savitzky-Golay can still preserve the overall profile (OriginLab Corporation, Northampton, MA, 2012).

2.6.4. Peak finding settings

Manual peak editing was performed so as to effectively select the required respective peaks. The second (2nd) derivative was used to search for all the hidden peaks and heavily overlapped bands included in the spectrum data. Differentiation is used to resolve and locate peaks in an envelope. Sharp bands are enhanced at the expense of broad ones, and this may allow easier selection of a peak, even when there is a broad band beneath it [35].

2.6.5. Characteristic peak assignment

The characteristic wavenumbers for pure cellulose, hemicellulose, and lignin listed in Table 3, were used for the peak assignment. Five characteristic peaks were identified for the pure cellulose, six characteristic peaks for pure hemicellulose, and six characteristic peaks for pure lignin (Figure 1a-c). The number of the peaks identified for the respective reference materials depends on the mixture of the reference materials. All seventeen peaks were identified for the treated and non-treated biomass samples.

2.6.6. Peak integration

To obtain quantitative values from the area under the manipulated spectrum/peaks, the area under the respective peaks were integrated and output into excel file.

2.6.7. Normalization of photoacoustic infrared spectra

Prior to the spectrum manipulation, the FTIR-PAS biomass sample spectra were corrected for wavenumber-dependent instrumental effects by dividing the reference carbon black ("background") spectrum intensity. This strategy implicitly assumes that the stability of the instrumentation used is adequate to ensure reliable results, even though the sample and reference spectra were collected at different times [30]. Carbon black is featureless, in the sense that it does not show any major characteristics peak [30]. Photoacoustic (PA) cell intensities varied with sample packing in the PA cell [36]. Stuart (1997) [35], also reported that absorbance varies linearly with the sample thickness. Therefore, the effect of bulk density of the reference materials and biomass samples was corrected by dividing the integrated areas with respective mass of the reference materials and biomass samples contained in the PA sample cup.

The model was further standardized by normalizing the corrected integrated area data from 0 to 1. This was performed by dividing individual reference materials and biomass samples corrected integrated area data by corresponding maximum corrected integrated area data value. The aforementioned steps were performed for the three major components of lignocellulosic biomass (cellulose, hemicellulose, and lignin). Therefore, this normalization approach ensures that the predictive model is adaptable for quantitative analysis of FTIR-PAS spectra obtained for any lignocellulosic biomass.

3. Regression analysis

This portion of the analysis is divided into two parts: the training and verification analysis:

3.1. Training analysis

The normalized data of cellulose, hemicellulose, and lignin components in the reference materials and the combined biomass samples (i.e. combination of data from RF and SE analysis) were correlated to their percentage compositions. The RF and SE normalized data were combined so as to develop a general model that can be applied on a barley straw sample, irrespective of the pretreatment method applied on the biomass. The percentage compositions of the reference materials (Table 2) and the percentage compositions (Tables 4 & 6-8) of the combined biomass samples obtained from the NREL Standard were used as the dependent variable, while two out of the three replicates from the combined normalized data (obtained from the PA spectra) were randomly selected and used as the independent variables. Five independent variables were used for the cellulose (because it has five characteristic wavenumbers), while six independent variables were used for both hemicellulose and lignin (they both have six characteristic wavenumbers each). Multiple linear regression analysis was conducted at 5% significance level using IBM SPSS Statistics (Superior Performing Statistical Software, version 20 for Windows, 2012; IBM, Armonk, New York, NY). Regression models (equations 1-3) for cellulose, hemicellulose, and lignin were developed based on the generated regression coefficients. The developed models have the capability to predict quantitatively, the percentage compositions of cellulose, hemicellulose, and lignin in any lignocellulosic biomass.

3.2. Verification analysis (Validation)

Subsequently, the normalized data (based on the respective characteristic peaks) from the third replicate of biomass sample was substituted into the developed predictive models to estimate/predict the percentage compositions of lignocellulosic components present in the non-treated, RF-alkaline and SE pretreated biomass samples (Tables 4 & 6-8).

4. Results and discussion

4.1. Reference material spectra

Figure 1 (a-c) shows the normalized spectral results obtained from a typical reference cellulose (100%), hemicellulose (100%), and lignin (100%), respectively, after smoothing and baseline corrections. Generally, FTIR-PAS techniques permit to obtain spectra which present specific characteristic key bands of individual components. In principle, such band position allows the discrimination of different species and provides important information about the chemical compositions of the material.

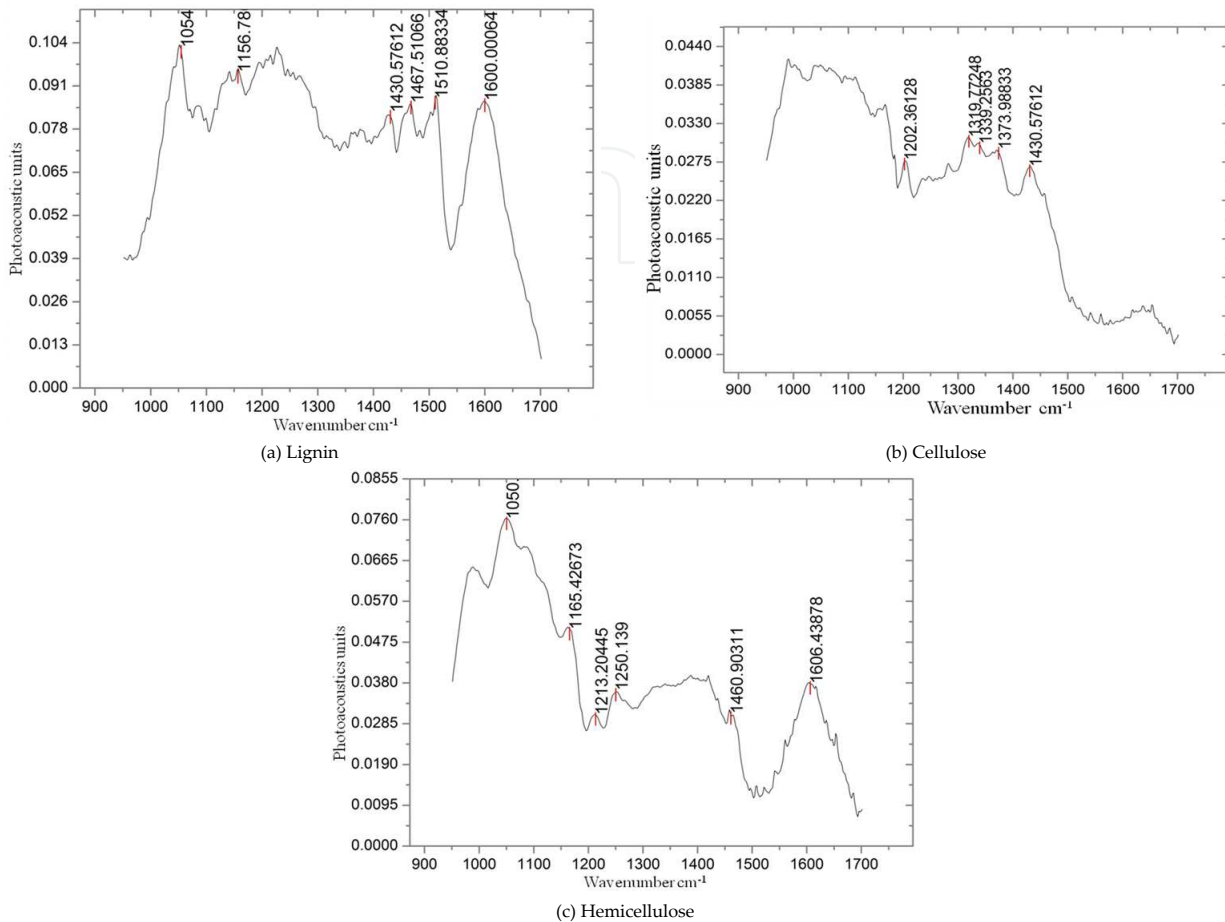


Figure 1. FTIR-PAS spectra of a) pure lignin (hydrolytic), b) cellulose (microcrystalline powder), and c) hemicellulose (xylan from birch wood) powders showing prominent peaks/band positions at the various characteristics wavenumbers.

4.2. Characteristic peak positions

The various prominent (characteristic) peak (band) positions and the corresponding peak assignments for cellulose, hemicellulose, and lignin are presented in Table 3. These are distinct peaks at different wavenumbers associated with these three biomass components. Lignin spectrum has six prominent peaks at wavenumbers of 1599 (X_1), 1511 (X_2), 1467 (X_3), 1429 (X_4), 1157 (X_5), and 1054 cm⁻¹ (X_6) (Figure 1a). Cellulose spectrum has five distinct peaks at wavenumbers of 1431 (X_1), 1373 (X_2), 1338 (X_3), 1319 (X_4), and 1203 cm⁻¹ (X_5) (Figure 1b). Hemicellulose spectrum has six characteristic peaks at wavenumbers of 1606 (X_1), 1461 (X_2), 1251 (X_3), 1213 (X_4), 1166 (X_5), and 1050 cm⁻¹ (X_6) (Figure 1c).

Wavenumbers (cm ⁻¹)	Cellulose 100%	Hemicellulose 100%	Lignin 100%	Peak Assignment
1650-1600	-	1606	-	1604-acetylated hemicellulosic polysaccharides [37].
1600-1550	-	-	1599	1595-aromatic ring stretch, strongly associated with aromatic C-O stretching mode [26]; 1595- phenylpropanoid polymer [37]; 1600-quadrant ring stretching (aromatic compound of lignin) [38-39]; 1600-aromatic skeletal vibration [1].
1550-1500	-	-	1511	1515-semicircle ring stretching (aromatic compound of lignin) [40-41]; 1510-lignin aromatic compound [28]; 1510-very strong aromatic ring stretch, aromatic C-O stretch [26, 40]; 1513-aromatic C=C stretch [41]; 1510-semi-circle stretch of para-substitute benzene rings [42]; 1550-protein [42]; 1502-aromatic skeletal vibration-softwood lignin [1]; 1505-aromatic skeletal vibration of benzene ring in lignin [1]
1500-1450	-	1461	1467	1462-C-H lignin deformation (methyl and methylene) [1].
1450-1400	1431	-	1429	1425- C-H in-plane lignin deformation with aromatic ring stretching [1]; 1430-CH ₂ in-plane bending vibrations [43]; 1430- C-H cellulose deformation (asymmetric) [1]; 1433-aromatic C=C stretch [41].
1400-1350	1373	-	-	1370-weak C-O stretching [39, 44]; 1372-C-H cellulose deformation (asymmetric) [1]; 1380-C-H symmetric and asymmetric deformation [41]; 1382-C-O stretch [45]; 1378-cellulose (microcrystalline) [37].
1350-1300	1319, 1338	-	-	1335-weak C-O stretching [39, 44]; 1336-C-H ring in-plane bending vibrations [43]; 1336- O-H in-plane cellulose deformation [1]; 1318-CH ₂ cellulose wagging [1].
1300-1250	-	1251	-	1258- acetylated glucomannan [37]; 1258-arabinoxylans (hemicellulose) [45].
1250-1200	1203	1213	-	1246 cellulosic material [39]; 1246-weak C-O stretching [44]; 1258- acetylated glucomannan [37]; 1250-acetylated hemicelluloses [43]; 1201- O-H cellulose deformation [1]
1200-1150	-	1166	1157	1160 glycosidic linkage [27]; 1162-C-O-C ring vibrational stretching [43]; 1165-arabinoxylans (hemicellulose) [45];
1100-1050	-	-	1054	1086- C-O of secondary alcohols [1].
1050-1000	-	1050	-	1044-arabinoxylans (hemicellulose) [45]; 1035-C-O, C = C and C-C-O vibrational stretching [43]; 1045-C-OH bending [44, 27]; 1025-non-structural CHO [39].

Table 3. Characteristics peaks/bands position and assignment of pure cellulose, hemicellulose, and lignin (adapted from [21])

4.3. Radio frequency-alkaline treatment on lignin

It was observed that NaOH concentration is a major factor in the pretreatment process. The swelling initiated by NaOH creates pores in the biomass matrix [46-48], which helps enhance the reactivity of the biomass matrix to any externally added material such as enzyme [8]. A similar finding was reported by [49]. The lignin values obtained from the traditional wet chemistry chemical composition analysis and through FTIR-PAS as shown in Table 4 depicts that in most cases, there is a decrease in the lignin in the pretreated samples as compared to the non-treated samples. This may be due to the unloosened lignified matrix in the non-treated sample which is tightly bound to the other constituents, unlike the RF alkaline pretreated samples with broken bonds which has been structurally separated, and disrupted. This disaggregation may be attributed to the interaction between the biomass and NaOH solution in the presence of the RF heating which is believed to be responsible for this solid loss [8]. It also seems that the disruption and deconstruction of the lignified matrix is associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF radiation and the ions and molecules from the NaOH solution and the biomass [8]. Lignin removal is an important part of the pretreatment process, because lignin can effectively inhibit/prevent the cellulase enzymes from hydrolysing the cellulose. This lignin reduction resulting from the alkaline pretreatment had also been reported by [50-51].

Lignin estimated by FTIR-PAS was higher than those obtained with NREL procedures. The laborious and time consuming traditional method that uses 72% H₂SO₄ seems to create a more stringent condition that may lead to altering and further degrading the native cell wall compositions (such as lignin and complex carbohydrates), structure, and possibly generating artifacts. FTIR-PAS is a rapid, direct, non-invasive, and non-destructive chemical analytical technique. This rapid method can detect molecular chemical characteristics of biological materials at high spatial resolutions without altering the inherent biomass structure such as the tissue [40, 44].

The difference in the lignin values from the traditional approach and FTIR-PAS may also be attributed to the spectrum manipulations and the assumption that the reference lignin sample is 100% pure. The FTIR-PAS qualitative and quantitative analytical chemical information can be connected to the structural information within cellular dimension [42].

Equation 1 shows the predictive model for lignin generated from the training and verification analysis of the combined values from RF and SE using the methods described in section 6.3.8.

Lignin predictive model:

$$\%L = -33.92X_1 + 52.61X_2 + 32.16X_3 + 208.14X_4 + 98.46X_5 - 56.59X_6 + 17.33 + \varepsilon \quad (1)$$

%L=%lignin (dry matter basis), X_i=regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 1a), with the regressors representing the respective wavelengths as 1599 (X₁), 1511 (X₂), 1467 (X₃), 1429 (X₄), 1157 (X₅), and 1054

Temperature (°C)	Biomass: NaOH solution ratio	Lignin (%)		
		Wet chemistry	FTIR-PAS	Standard error
Non-treated	-	20.12	22.62	1.25
70	1:4	19.56	15.80	1.88
80	1:4	19.22	15.78	1.72
90	1:4	20.66	20.40	0.13
70	1:5	17.81	16.77	0.52
80	1:5	18.22	20.63	1.21
80 ^W	1:5	15.62	18.15	1.27
90	1:5	17.64	19.38	0.87
90 ^W	1:5	15.37	15.90	0.27
24	1:6	21.57	23.65	1.04
70	1:6	17.91	15.98	0.97
90	1:6	18.38	21.63	1.62
24	1:7	17.99	22.34	2.17
70	1:7	17.82	17.55	0.13
80	1:7	18.10	25.22	3.56
80 ^W	1:7	12.39	20.57	4.09
80 ^{DW}	1:7	19.90	27.74	3.92
80 ^{TW}	1:7	20.38	26.49	3.06
90	1:7	18.05	20.19	1.07
90 ^W	1:7	10.98	18.78	3.90
70	1:8	18.53	22.20	1.83
80	1:8	18.86	21.60	1.37
90	1:8	19.27	17.45	0.91

Standard error=standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; W=washed after pretreatment; DW=pretreated with distilled water; TW=pretreated with tap water.

Table 4. Lignin composition of RF-alkaline pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

cm^{-1} (X_6). 17.33=intercept, and ϵ =error term/stochastic variable which describes the noise (errors that could emanate from the equipment, environment, or the experimenter).

Table 5 shows the R^2 and mean square error values of the various biomass components from the regression analysis. However, the R^2 values are too low; this might be due to the associated stochastic variables and the spectrum manipulations.

Biomass composite	R ² value	Mean square error
% Lignin	0.68	9.10
% Cellulose	0.34	13.86
% Hemicellulose	0.31	15.86

Table 5. R² and mean square error values from the regression analysis

Washing the pretreated samples reduces the lignin. This may be due to loss of solid lignin during the washing process [8]. This investigation shows that the concentration of NaOH solution and the ratio of biomass to the NaOH solution are the dominant contributing factors, while temperature plays a lesser role. The heat provided by the RF is needed to assist the alkaline solution in the deconstruction and disaggregation of lignocellulosic biomass matrix. It was also observed from this investigation that biomass can be alkaline pretreated even at room temperature if the required ratio of biomass and NaOH solution is applied.

4.4. Radio frequency-alkaline treatment on cellulose and hemicellulose

The difference in the cellulose and hemicellulose values from the traditional approach and FTIR-PAS may be attributed to the spectrum manipulations and the assumption that the reference cellulose and hemicellulose samples are 100% pure. Equations 2-3 show the cellulose and hemicellulose predictive models generated from the training and verification analysis of the combined values from RF and SE using the methods described in section 3.

Temperature (°C)	Biomass: NaOH solution ratio	Cellulose (%)			Hemicellulose (%)		
		Standard error	Wet chemistry	FTIR-PAS	Standard error		
Wet chemistry	FTIR-PAS						
Non-treated	-	42.51	50.37	3.93	29.98	23.82	3.08
70	1:4	22.25	28.65	3.20	23	18.10	2.45
80	1:4	22.37	27.69	2.66	22.14	28.47	3.16
90	1:4	26.93	26.09	0.42	26.24	20.73	2.75
70	1:5	24.21	4.98	9.61	21.63	30.37	4.37
80	1:5	21.07	29.42	4.18	21.38	24.56	1.59
80 ^w	1:5	27.69	26.55	0.57	21.6	15.81	2.90
90	1:5	24.65	37.03	6.19	21.05	8.44	6.31
90 ^w	1:5	33.44	34.50	0.53	26.08	22.58	1.75
24	1:6	30.93	14.95	7.99	29.12	21.00	4.06
70	1:6	28.25	29.02	0.39	23.18	22.88	0.15
90	1:6	30.37	20.85	4.76	22.36	39.31	8.47

Temperature (°C)	Biomass: NaOH solution ratio	Cellulose (%)			Hemicellulose (%)		
24	1:7	25.78	27.08	0.65	23.4	26.21	1.40
70	1:7	27.39	11.74	7.82	24.27	28.81	2.27
80	1:7	26.08	17.83	4.12	26.75	10.70	8.02
80 ^W	1:7	38.20	19.86	9.17	27.72	8.48	9.62
80 ^{DW}	1:7	21.88	2.06	9.91	19.12	5.76	6.68
80 ^{TW}	1:7	24.98	6.09	9.44	21.04	9.97	5.54
90	1:7	21.33	23.74	1.20	22.27	9.55	6.36
90 ^W	1:7	33.31	12.98	10.17	20.69	9.71	5.49
70	1:8	26.73	27.91	0.59	26.76	22.69	2.03
80	1:8	18.44	42.08	11.82	18.32	31.06	6.37
90	1:8	22.68	21.66	0.51	19	25.64	3.32

Standard error=standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; W=washed after pretreatment; DW=pretreated with distilled water; TW=pretreated with tap water.

Table 6. Cellulose and hemicellulose compositions of RF-alkaline pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Cellulose predictive model:

$$\%C = 28.63X_1 + 48.60X_2 + 35.83X_3 - 51.71X_4 - 29.24X_5 + 37.16 + \varepsilon \quad (2)$$

Where %C=%cellulose value, X_i =regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 1b), with the regressors representing the respective wavelengths as 1431 (X_1), 1373 (X_2), 1338 (X_3), 1319 (X_4), and 1203 cm^{-1} (X_5), 37.16=intercept, and ε =error term/stochastic variable.

Hemicellulose predictive model:

$$\%H = -14.25X_1 - 90.42X_2 + 34.14X_3 - 39.18X_4 + 71.17X_5 + 122.90X_6 + 30.42 + \varepsilon \quad (3)$$

%H=%hemicellulose wet chemistry value, X_i =regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 1c), with the regressors representing the respective wavelengths as 1606 (X_1), 1461 (X_2), 1251 (X_3), 1213 (X_4), 1166 (X_5), and 1050 cm^{-1} (X_6), 30.42=intercept, and ε =error term/stochastic variable.

Ramesh and Singh (1993) [2] reported that barley straw theoretically contains about 40% cellulose, 20% hemicellulose, and 15% lignin. Marsden and Gray (1985) [52] also reported that barley straw theoretically contains about 44% cellulose, 27% hemicellulose, and 7% lignin.

These values are comparable with the values obtained from the non-treated sample in this investigation. It should be noted that the variance in the chemical composition between the reported theoretical values and the values obtained from this investigation may be attributed to differences in locations where the crop was grown, weather conditions, the barley variety grown, and different methods of analysis.

4.5. Steam explosion treatment on lignin

The efficacy of FTIR-PAS techniques for studying changes in plant cell wall composition following steam explosion pretreatment has been evaluated. Table 7 shows that the SE pretreated samples have higher lignin content as compared to the non-treated. This may be due to the carbonization of the sample resulting from the direct contact of biomass with the walls of the reactor during the steam explosion pretreatment.

Temperature (°C)	Moisture content (%) [*]	Retention time (min)	Lignin (%)		
			Wet chemistry	FTIR-PAS	Standard error
Non-Treated	-	-	20.12	22.62	1.25
140	8	5	23.79	26.25	1.23
160	8	5	22.72	31.46	4.37
180	8	5	40.58	21.41	9.58
140	30	5	22.05	28.23	3.09
160	30	5	21.69	24.53	1.42
180	30	5	33.01	25.99	3.51
140	50	5	21.18	25.41	2.11
160	50	5	23.31	33.21	4.95
180	50	5	25.04	32.60	3.78
140	8	10	21.56	31.63	5.04
160	8	10	21.66	25.13	1.73
180	8	10	32.75	33.45	0.35
140	30	10	21.25	29.37	4.06
160	30	10	20.9	27.47	3.28
180	30	10	37.31	29.21	4.05
140	50	10	21	23.54	1.27
180	50	10	31.82	31.16	0.33

Standard error=standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; ^{*}= % mass fraction of water.

Table 7. Lignin composition of SE pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Hemicellulose degrades easily and some volatile organic compounds vaporize as volatile components, while cellulose behaves as a fixed carbon (solid combustible residue). This may account for the increase in the lignin content. Lam et al. (2011) [18] investigated the steam explosion of Douglas fir (*Pseudotsuga menziesii*) at a reaction temperature of 200-220°C and a retention time of 5-10 min. These researchers reported that there was increase in lignin content from 30 to 43% attributed to the thermal degradation of hemicellulose during the steam explosion treatment. Chen and Kuo (2011) [53] reported that cellulose and lignin are both locked in biomass from the mild carbonization process. This indicates that the degraded cellulose may appear as residue resulting to the increase in the lignin content.

The interaction between moisture content and temperature and also between retention time and moisture content had a statistically significant effect ($P < 0.01$) on the lignin. The interaction among the three variables (moisture content, temperature, and retention time) also had a significant effect on the lignin. The difference in the lignin values obtained using the FTIR-PAS and the traditional approach may be attributed to the spectrum manipulations and the assumption that the reference lignin sample is 100% pure.

4.6. Steam explosion treatment on cellulose and hemicellulose

From Table 8, in comparison with the non-treated biomass, it is evident that retention time, moisture content, and temperature had a significant effect on the cellulose and hemicellulose content. The decrease in the sugars content increased at higher retention time and temperature. Wang et al. (2009) [54] also reported that the retention time and temperature are the process parameters required for the optimization of steam explosion process. In this present study, less degradation of the simple sugars was observed at higher moisture content. High feedstock moisture content acts as acid catalyst to hydrolyze biomass during steam explosion. However, the direct contact of biomass with the walls of the reactor will limit and affect the extent of the hydrolysis. Therefore, a combination of carbonization and acid catalyzed hydrolysis occurred which ultimately led to the degradation of the simple sugars and increase in the lignin. The obtained results from the wet chemistry demonstrated that the hemicellulose contained in the biomass was highly degraded (79% to 89%) compared to cellulose (58% to 77%) as reported in chapter 4. The high degradation of hemicellulose was due to its amorphous nature, which degrades easily and evaporates as volatile components during the carbonization process. Presumably, the crystallinity of cellulose was responsible for the less degradation of this component. These degradations can be explained by considering the monomers of hemicellulose and cellulose which consist primarily of sugars. Degradation of cellulose and hemicellulose during steam explosion/thermal pretreatment of biomass has been reported by [16, 19-20, 55-57]. It was reported that hemicellulose is very reactive and was nearly completely removed at 200°C, while both cellulose and lignin can be dissolved partially at higher temperatures. Shaw (2008) [57] performed steam explosion on poplar and wheat straw at 200-205°C, steam pressure of 1.66-1.73 MPa for 4-4.5 min. This author reported a decrease in cellulose and hemicellulose content with an increase in the lignin content after the steam explosion treatment of both biomass samples. Chen and Kuo (2011) [53], Yang et al. (2007) [59], and Khezami et al. (2007) [60] studied the effects of thermal process on biomass. These

researchers showed that thermal pretreatment removes moisture and light volatiles from biomass. Bergman et al. (2005) [61] and Lipinsky et al. (2002) [62] reported that during the torrefaction process, biomass was partly decomposed giving off various condensable and non-condensable gases, with a carbon-rich solid as a final product. Mohammad and Karimi (2008) [7] reported a corresponding decrease in total sugar recovery with increasing temperature during steam explosion.

Furthermore, the difference between the cellulose and hemicellulose values estimated using the FTIR-PAS and the measured values using the traditional approach might also be associated with the spectrum manipulations and the assumption that the reference cellulose and hemicellulose samples are 100% pure. The main advantage of this correlation is that based on collection of FTIR-PAS spectra, it provides a rapid, easy, economical, non-destructive, and non-laborious estimation of the chemical composition of biomass. This may be of particular interest in the contexts where more sophisticated and expensive equipments for experimental measurement of biomass chemical compositions are not always available. However, care must be taken in selecting the steam explosion pretreatment conditions in order to prevent excessive degradation of the chemical properties of the complex carbohydrates, since the yields of hemicelluloses and cellulose were dependent on the pretreatment conditions of the steam explosion.

Temp. (°C)	M.C. (%)	R.T. (min.)	Cellulose (%)			Hemicellulose (%)		
			Wet chemistry	FTIR-PAS	Standard error	Wet chemistry	FTIR-PAS	Standard error
Non-treated	-	-	42.51	50.37	3.93	29.98	23.82	3.08
140	8	5	11.19	20.53	4.67	3.42	12.39	4.48
160	8	5	11.17	17.93	3.38	3.29	13.10	4.90
180	8	5	9.58	18.54	4.48	3.74	6.60	1.43
140	30	5	14.82	19.87	2.53	3.8	17.79	7.00
160	30	5	14.63	17.53	1.45	4.13	12.54	4.21
180	30	5	12.07	17.30	2.62	3.88	11.44	3.78
140	50	5	15.81	22.06	3.12	6.58	15.95	4.69
160	50	5	14.51	19.74	2.62	5.11	15.18	5.04
180	50	5	15.77	20.24	2.23	5.23	14.02	4.40
140	8	10	10.02	16.91	3.45	3.9	16.01	6.05
160	8	10	9.11	19.49	5.19	4.47	9.44	2.49
180	8	10	8.74	16.08	3.67	3.23	12.03	4.40
140	30	10	15.38	15.66	0.14	5.03	14.91	4.94
160	30	10	13.57	16.95	1.69	6.59	6.50	0.05
180	30	10	12.7	16.24	1.77	4.54	12.47	3.96

Temp. (°C)	M.C. (%)	R.T. (min.)	Cellulose (%)			Hemicellulose (%)		
			Wet chemistry	FTIR-PAS	Standard	Wet chemistry	FTIR-PAS	Standard
			error			error		
140	50	10	16.02	17.51	0.74	6.33	14.01	3.84
180	50	10	12.35	15.95	1.80	4.69	10.70	3.01

Standard error=standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; Temp.=temperature; M.C.=moisture content; R.T.=retention time.

Table 8. Cellulose and hemicellulose compositions of SE pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

5. Conclusion

Lignocellulosic biomass has been identified as a potential feedstock for the biofuel industry. Quantitation of lignocellulosic biomass components (lignin, cellulose, and hemicellulose) is often performed using the traditional acid hydrolysis followed by gravimetric determination. This approach is complicated and time consuming. FTIR-PAS was used in light of the need for rapid analysis of biomass materials and wood-based materials at large. The samples were initially pretreated using RF-alkaline and steam explosion techniques and analyzed gravimetrically using the traditional approach to elucidate compositional information. Thereafter, the effect of the pretreatment conditions on barley straw grind was further analyzed based on their FTIR-PAS spectra. In order to develop a predictive model that will be swiftly used for the quantitative prediction of the chemical composition of the biomass, reference materials: pure cellulose (microcrystalline powder), hemicellulose (xylan from birch wood), and lignin (hydrolytic) powders were mixed in different proportions with known concentrations. The reference materials were used to generate standard spectra to determine the relationship between the respective quantity of components in the mixture and the FTIR spectra of representative biomass sample. The FTIR wavenumber-dependent instrumental effects were corrected by using carbon black reference spectrum. Multiple linear regression models for cellulose, hemicellulose, and lignin were developed based on the generated regression parameters, with coefficient of determination 0.68, 0.34, and 0.31, respectively, and mean square error of 9.10, 13.86, and 15.86, respectively. This study reflects that pretreatment can also lead to the degradation of the energy potentials. However, non-treated biomass resulted in the least conversion yield during the enzymatic hydrolysis [63]. The FTIR-PAS technique has advantage because it is a quick, easier, and non-destructive method. The structure of the biomass is maintained when spectra are measured directly from the bulk of grind biomass surface. Consequently, this study has led to the following conclusions: i) Lignin matrix structurally disrupted and released during pretreatment process; ii) Pretreatment enhances the accessibility and digestibility of the cellulose and hemicellulose; iii) This increased the conversion rate and assisted in reducing the costs and amount of enzymes required for the next stage of process (enzymatic hydrolysis) by 64% and 33% for RF and SE pretreatment,

respectively. This implies that the PA infrared spectra can be used for biofuel feedstock identification and analysis of the chemical composition of biomass before it is processed. This innovative approach could be easily adopted by the biofuel industry and extended to any form of lignocellulosic biomass feedstock.

Author details

Kingsley L. Iroba* and Lope G. Tabil

*Address all correspondence to: kli931@mail.usask.ca

Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, Canada

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