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Fourier Transform Infrared Quantification of Sugars in Pretreated Biomass Liquors

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Abstract

The process of converting renewable lignocellulosic biomass to ethanol requires a number of steps, and pretreatment is one of the most important. Pretreatment usually involves a hydrolysis of the easily hydrolyzed hemicellulosic component of biomass using some form of thermal/chemical/mechanical action that results in a product that can be further hydrolyzed by cellulase enzymes (the cellulosic portion). The sugars produced can then be fermented to ethanol by fermentative microorganisms. If the pretreatment step is not severe enough, the resultant residue is not as easily hydrolyzed by the cellulase enzyme. More severe pretreatment conditions result in the production of degradation products that are toxic to the fermentative microorganism. In this article, we report the quantitative analysis of glucose, mannose, xylose, and acetic acid using Fourier transform infrared (FTIR) spectroscopy on liquors from dilute-acid-pretreated softwood and hardwood slurries. Comparison of FTIR and high-performance liquid chromatography quantitative analyses of these liquors are reported. Recent developments in infrared probe technology has enabled the rapid quantification of these sugars by FTIR spectroscopy in the batch reactor during optimization of the pretreatment conditions, or interfaced to the computer controlling a continuous reactor for on-line monitoring and control.

Index Entries: Fourier transform infrared; biomass; hardwood; softwood; pretreatment; acid hydrolysis.

Introduction

The process of converting renewable lignocellulosic biomass to ethanol requires a number of steps. One of the early process steps involves the pretreatment of the raw biomass, which partially hydrolyzes the

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hemicellulosic portion of the feedstock. This important step can be accomplished in a number of ways, one of which is using dilute-acid catalysis at moderately high temperatures (~140–230°C). The extent of the pretreatment reaction step must be controlled and optimized for each feedstock in order to maximize the production of xylose from hardwoods and herbaceous crops, or glucose, mannose, and xylose from softwood feedstocks. This pretreatment step may also increase the susceptibility of the cellulosic portion to enzymatic hydrolysis by cellulases, if employed in the process, thus increasing overall fermentation yield to ethanol. If a less severe pretreatment occurs, the removal of hemicellulosic sugars from the solids may be incomplete and the percentage of oligomeric sugars in solution may increase. Posthydrolysis of the oligomeric sugars may increase the capital cost of the biomass-to-ethanol plant, because additional vessels will be needed to complete the hydrolysis to fermentable monomeric sugar *(1)*. Thus, the reactor should be monitored and controlled to maximize the concentration of soluble xylose, or glucose and mannose produced, and minimize the degradation of the hemicellulosic sugars to furfural and 5-hydroxymethyl-2-furfural (HMF). Monitoring acetic acid production may also be useful as an indicator of the extent of hydrolysis. Furfural and HMF reduce overall yield and are known to be toxic to fermentative microorganisms *(2,3)*. Less severe pretreatment conditions affect the degree of cellulose digestion by cellulase enzymes, and harsher conditions convert sizable amounts of the soluble sugars to toxic by-products, thus lowering the overall yield of a biomass-to-ethanol plant.

Dilute-acid pretreatment is usually a short process step involving only a few minutes at high temperature within the reactor. Monitoring and control of this reactor must be rapid and accurate in order for the feedback to have any effect on the pretreatment. Currently, high-performance liquid chromatography (HPLC) is used to analyze samples from the pretreatment reactor. This time-consuming test may take an hour or so. Although this method is quite accurate, it is too slow to enable control of the short dilute-acid pretreatment step. As a result, several kilograms to several tons of toxic products (depending on reactor size and feed rate) may be pumped to the fermentation train. This could poison the fermentation train and cause downtime and operating losses owing to disposal costs. However, if an on-line method for monitoring and control of the pretreatment reactor is available, ethanol production can be increased by maximizing the amount of hemicellulosic sugars available for fermentation and reducing losses caused by poisoning of the fermentation train with toxic furfural and HMF from too harsh a pretreatment.

The utilization of midinfrared (mid-IR) spectroscopy, in particular Fourier transform infrared (FTIR) spectroscopy, for the qualitative and quantitative analysis of compounds has increased in importance over the last two decades as more commercial instruments have become available. FTIR analysis is a rapid and nondestructive technique for the qualitative and quantitative identification of compounds in solids and liquids (and

some gases) in the mid-IR region *(4)*. FTIR spectroscopy may help identify other compounds in the pretreated slurries that may be toxic to the fermentation process because comprehensive spectral libraries are available to help identify these unknown compounds.

FTIR analysis of dissolved compounds in aqueous solutions has been severely restricted in the past because of the high IR background absorbance of water. Extremely short path length cells (not practical for analyzing high-solids slurries) were needed to obtain transmission spectra *(5)*. However, in the attenuated total reflection (ATR) mode *(6–9)*, the high background absorbance owing to water can be partially controlled by using single or multiple reflections within the ATR crystal to control the total depth of penetration of the evanescent radiation into the solution. This allows the attenuation of the incident radiation by the dissolved species to give the mid-IR spectra without the high background absorbance owing to water completely obscuring the spectra. Recent developments in ATR cells and probes have extended the application of mid-IR (especially FTIR) spectroscopy to the qualitative and quantitative analysis of solutes dissolved in aqueous solutions in harsh environments associated with a chemical reactor *(10)*. Probes are currently manufactured that enable FTIR monitoring of chemical reactions up to 250°C and pressures to 1500 psi.

FTIR spectroscopy of solid wood samples has been employed in the past with considerable success using diffuse reflectance IR Fourier transform spectroscopic methods (DRIFTS) to study whole wood, pretreated wood, wood surfaces, pulp, and paper substrates *(11–15)*. FTIR spectroscopy in the transmission mode has been used to study wood solids *(16,17)* and combined with IR microspectroscopy to study a wide range of pulps *(18)*. DRIFTS spectroscopy has been used successfully to characterize lignins in pulped wood *(19,20)*. However, the use of FTIR-ATR spectroscopy to study solids has been severely limited in the past because of the soft ATR crystals available. In addition, the dilute acid found in fermentation broths and pretreatment liquors attacks some of the ATR crystals (i.e., ZeSe), which quickly degrades their optical throughput. These drawbacks have recently been overcome because ATR cells are now available that utilize crystals of considerable hardness and chemical inertness to allow mid- and near IR analysis of solids pressed tightly against the crystal surface, permitting a more uniform penetration by the evanescent radiation *(7)*. Diamond-composite probes with Hastelloy sheaths are available for insertion directly into reaction vessels or fermentors via the standard Ingold port *(10)*. The diamond-composite sensor is highly chemically inert, exhibits high hardness and mechanical strength, and is optically transparent in the visible and most of the mid-IR region. Because a diamond surface has the lowest coefficient of friction of all the available crystal materials, few materials stick to this surface, allowing extended measurements in flow-related applications *(10)*. The ability of lignin and other materials in the reactor to coat the optical surface of the diamond-composite probe should be reduced because of this low coefficient of friction.

In this study, we used a diamond-composite insertion probe to follow the neutralization reaction that occurs in a slurry sample of pretreated yellow poplar sawdust when lime is added to a stirred mixture. The process of overliming of pretreated slurries occurs when the pH of the slurry is increased past pH 7.0 to 10.0, held there for a brief period of time and then decreased to pH 6.0 by the addition of acid. This process of adding excess lime has been shown, in the past, to decrease the toxicity of pretreated wood slurries to fermentative microorganisms *(2,21,22)*. The use of FTIR and a diamond-composite insertion probe monitoring the overliming process demonstrates the utility of the FTIR-ATR technique to follow chemical reactions in a pretreated hardwood slurry composed of 16% insoluble solids and 19% total solids. In addition, a diamond-composite cell that matched the specifications of the insertion probe was fitted into the sample compartment of two FTIR spectrometers to obtain mid-IR spectra of pretreated softwood slurries, liquors, and washed solids. The partial least squares (PLS) option in the commercially available software package TQ Analyst™ (Nicolet, Madison, WI) was used to regress the spectra into methods capable of predicting sugar concentrations in unknown samples of pretreated softwood liquors. A series of batch pretreatment experiments in a 4-L steam-explosion reactor at National Renewable Energy Laboratory (NREL) Digester (Golden, CO) was used to simulate variations in a continuous process, because operation of the Sunds CD-300 Laboratory Hydrolyzer (Sunds DeFibrator, Norcross, GA) in the NREL-DOE Process Development Unit (PDU) to obtain samples of pretreated material from a continuous reactor is resource intensive *(23)*. Application of this technique to this series of batch pretreatment experiments demonstrates the possibility of using FTIR-ATR to solve the problem of rapidly determining (in a couple of minutes) the soluble and insoluble components in slurries from a pretreatment reactor.

Materials and Methods

Feedstock Preparation and Pretreatment

Yellow poplar sawdust (approx 50–55% solids) was obtained from SawMiller (Haydenville, OH) and shipped to NREL in plastic-lined cardboard totes for storage at –20°C. The totes were thawed for several days prior to dilute-acid (0.32% w/w H_2SO_4) pretreatment at 200°C and 4.6-min residence time in a Sunds CD-300 Laboratory Hydrolyzer (Sunds DeFibrator) in the pretreatment section of the NREL-DOE PDU as described elsewhere *(24)*. The FTIR-ATR technique was applied to a sample of this slurry using a diamond-composite insertion probe to follow the neutralization reaction that occurs when lime is added to a stirred mixture of this slurry. FTIR spectra from monitoring the process of overliming was followed by adjusting the pH of the slurry past pH 7.0 to pH 10.0, holding at pH 10.0 for a brief period, then decreasing the pH to 6.0 by the addition of acid.

A synthetic mixed solids waste (MSW) feedstock was prepared from construction lumber (35% based on oven-dried basis), prunings from Cali-

fornia almond trees (20%), wheat straw (20%), office wastepaper (12.5%), and newsprint (12.5%). The MSW was pretreated with dilute sulfuric acid (0.4% w/w) in a 4-L steam explosion reactor (NREL Digester) at 210°C for 3 min as described elsewhere *(25)*. The NREL Digester (4-L steam explosion reactor) was custom designed by NREL and fabricated by an American Society of Mechanical Engineers–certified shop for installation at NREL. The steam-jacketed 4-L reactor is closed by two rapidly opening ball valves for feeding acid-impregnated feedstocks and discharging pretreated material. Direct steam injection into the reactor is used for rapid heat up to pretreatment temperature. The NREL Digester discharges directly into a flash tank for rapid quenching of the pretreatment reaction *(24)*.

The Pacific Wood Fuels Company, Redding, CA, harvested a feedstock consisting of softwood forest thinnings from a site near Quincy, CA. The trees selected for thinning were specifically chosen to represent statistically the area and consisted of approx 70% white fir (*Abies concolor*) and 30% Ponderosa pine (*Pinus ponderosa*). The freshly harvested whole trees (including bark, limbs, and needles) were immediately chipped on-site and shipped to NREL. The 8-t load of chips was milled using a Mitts and Merrill rotary knife mill (Model 10 × 12, Reduction Technology, Leeds, AL) equipped with a 0.5-in. (12.7-mm) rejection screen followed by mixing on large tarpaulins using a front-end loader. Pretreatment of this feedstock for first- and second-stage experiments was performed using the 4-L steam explosion reactor under various conditions ranging from 180 to 215°C, 0.35 to 2.5% (w/w) sulfuric acid, and 120 s to 9 min residence times as described elsewhere *(1)*.

High-Performance Liquid Chromatography

Sugar concentrations in the pretreated slurry liquors were determined using a Hewlett-Packard 1090 LC equipped with an HP 1097A refractive index detector, and Bio-Rad Aminex HPX-87X or HPX-87P (Bio-Rad, Hercules, CA) ion-moderated partition chromatography columns. The procedures for analyzing chemical composition of the washed pretreated solids (lignin and individual carbohydrates) and hydrolyzate liquor (monomeric and oligomeric sugars, organic acids, furfural, and HMF) were reported previously *(24)*.

IR Spectroscopy

IR spectra monitoring the process of overliming a pretreated yellow poplar slurry were obtained using an ASI Applied Systems ReactIR 1000™ spectrometer and a six-reflection diamond-composite Hastelloy C-276 insertion BioProbe™ (ASI Applied Systems, Millersville, MD). The spectrometer was equipped with an optional liquid nitrogen–cooled mercury-cadmium-telluride detector. A 1-m articulated light conduit coupled the BioProbe to the spectrometer and allowed flexible positioning of the probe to accommodate the hot (60°C) water bath and sonicator probe. A 500-mL sample of whole slurry from a pilot-scale production run with

the NREL Sunds hydrolyzer using yellow poplar sawdust as feedstock *(24)* was overlimed by slowly raising the pH of the warm (60°C) slurry to 10.0 over the course of 20 min by adding lime. However, the pH continued to rise slowly to 11.0, at which time a small amount of sulfuric acid was used to lower the pH to 9.5. After approx 8 min (with no further change in pH), the pH was readjusted to 10.0 for the remainder of the 1-h incubation period. The pH was then lowered to 6.0 with sulfuric acid for overnight incubation. Sixty-four scans at 8-cm^{-1} resolution were averaged and plotted every 2 min for the first hour of the overliming process, then every 20 min thereafter for the 18-h duration of the experiment. The whole slurry was maintained at 60°C and continuously stirred.

Spectra for the liquor samples were obtained using a six-reflection diamond-composite ASI DurasamplIR™ (ASI SensIR Technologies, Danbury, CT) cell in a Nicolet Avatar 360® (Nicolet, Madison, WI) spectrometer by averaging 512 scans at 2-cm^{-1} resolution. The liquor samples were obtained by centrifuging a well-mixed sample of the slurry for 5 min at 12,000*g* and using the supernatant for the measurement, or pressing a sample of the slurry through a 0.2-μ filter with a syringe and using the filtrate. Washed solids were obtained by resuspending the centrifuged solids a minimum of four times in 10 vol of deionized water followed by centrifugation. Spectra for the washed solid samples were obtained by averaging 512 scans at 8-cm^{-1} resolution using a Nicolet Impact® spectrometer and the same ASI DurasamplIR cell as used for liquids.

PLS Method

A calibration method for slurry liquors was determined using the PLS regression analysis option in TQ Analyst™ (version 1.2 [Nicolet Instrument Corp., Madison, WI]) on 23 liquor sample spectra. HPLC data for each sample were entered into the software package spreadsheet prior to calibrating the FTIR method. The software automatically calculates the number of factors to use by calculating the predicted residual error sum of squares prior to calibrating the method. The mid-IR regions selected for analysis of the four sugars was 1500 cm^{-1} to 830 cm^{-1}, 1683 to 804 cm^{-1} for acetic acid and 1562–1503 cm^{-1} for HMF. Results from the PLS regression analysis were then used to plot the FTIR-predicted concentration values against the HPLC-determined concentration values for each sugar, acetic acid, HMF, and furfural. A similar process was used to arrive at a PLS method for predicting glucose and lignin composition in 18 washed, pretreated softwood solid residues.

Results and Discussion

Figure 1 presents the ability of the FTIR-ATR technique to monitor the overliming process. The total insoluble solids of this slurry was determined to be 16%, with the total solids determined to be 19%, requiring constant stirring of the reaction mixture. The carbonyl peak owing to protonated acetic acid is found at 1718 cm^{-1}. This band is split and shifted to 1555 and

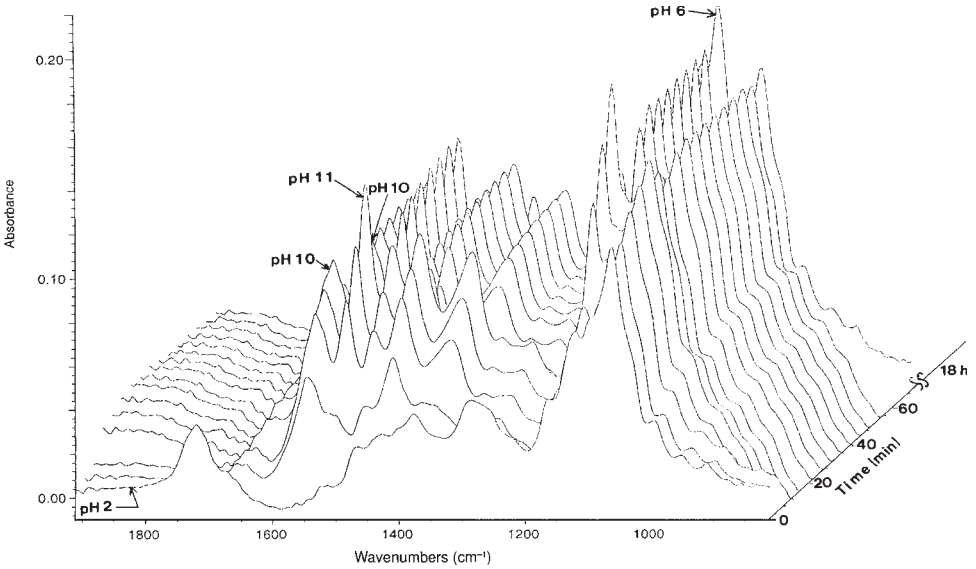

Fig. 1. Titration of slurry of pretreated yellow poplar sawdust consisting of 16% insoluble solids and 19% total solids using lime to adjust the pH. The whole slurry was incubated at 60°C with constant mixing to keep the solids in suspension, and the overliming was monitored using the FTIR-ATR technique.

1418 cm^{-1} as a result of the formation of the acetate anion by increasing the pH with the addition of lime. The broad absorption band owing to sulfate ion at 1100 cm^{-1} decreases as calcium is added and insoluble gypsum is formed. The spectra were taken and plotted every 2 min; however, every third spectra is shown for clarity.

Figure 2 shows the PLS regression analysis for glucose from a calibration set of 23 solution spectra of samples from pretreated softwood forest thinnings. The HPLC compositional values were measured for each sample and plotted vs the FTIR regression value predicted using the model developed from the calibration set. Although a limited number of samples were available, the fit was reasonable (r^2 = 0.9604).

Table 1 lists the correlation coefficient (r), r^2, and standard error of estimate (SEE) for four sugars, acetic acid, and HMF determined by PLS regression analysis for the FTIR-ATR calibration set of 23 solution spectra of samples from pretreated softwood forest thinnings. Acetic acid (r^2 = 0.9917) and HMF (r^2 = 0.9884) were found to give relatively good fits using this sample set.

Method validation requires that an analytical method have precision and accuracy. An FTIR spectrometer is expected to have a frequency error of approx 0.01 cm^{-1} *(4)*. The intensity error using the Nicolet Avatar 360 FTIR-ATR (ASI DuraSamplIR) combination was determined to have a standard deviation of 0.002 absorbance units for eight replica spectra obtained from one pretreatment liquor for a major absorbance band at 1053 cm^{-1}. In each replicate spectra, a baseline was drawn from 1500 to 822 cm^{-1}, and

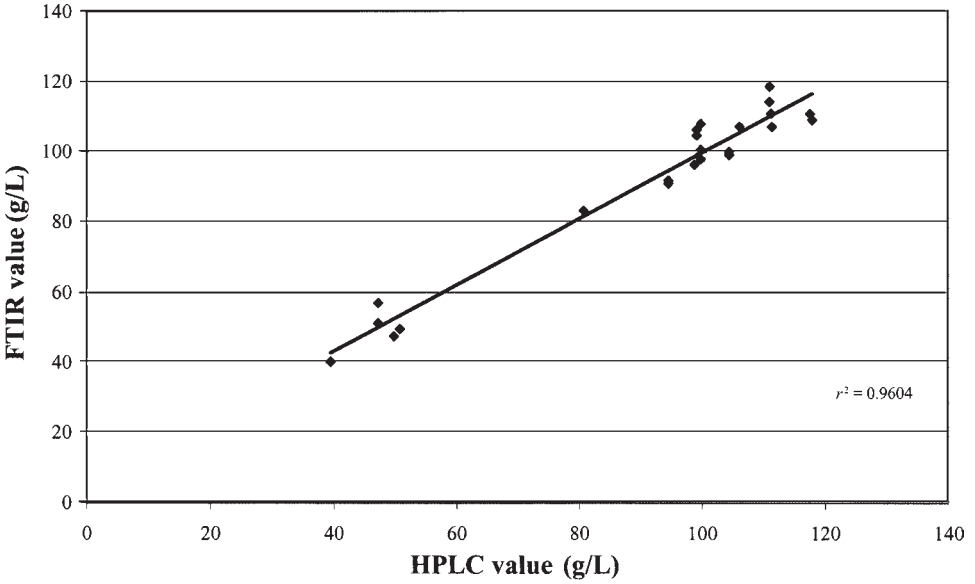

Fig. 2. PLS regression method developed for glucose using FTIR-ATR spectra and HPLC-measured values from liquors for a calibration set of 23 first- and second-stage pretreated softwood slurries.

Table 1
Correlation Coefficients (r), r^2, and SEE
for FTIR-ATR Calibration Set Regression Analysis of 23 Solution Spectra
of Liquor Samples from Pretreated Softwood Slurries

Soluble component	r	r^2	SEE (g/L)
Glucose	0.9800	0.9604	5.0
Mannose	0.9863	0.9728	2.8
Galactose	0.9926	0.9853	0.6
Xylose	0.9866	0.9734	1.5
Acetic acid	0.9958	0.9917	0.4
HMF	0.9942	0.9884	0.3

the height of the major absorption band at 1053 cm^{-1} was measured. The root means square (RMS) of the noise of this FTIR-ATR combination was determined to be 0.00054 absorbance units in the region between 2600 and 2400 cm^{-1} using a standard software tool in the Omnic® software package (Nicolet). The six-reflection diamond-composite cell used in this study has an apparent lower limit of detection of 1–2 g/L. As an example, the lower detection limit of our method for glucose was calculated as 0.6 g/L using a lower limit of the signal-to-noise ratio (S/N) of 3:1 *(26)*. However, using an S/N ratio of 10:1 as the lower quantitation limit *(26)* yields a value of approx 2 g/L for glucose with this six-reflection ATR cell and FTIR spec-

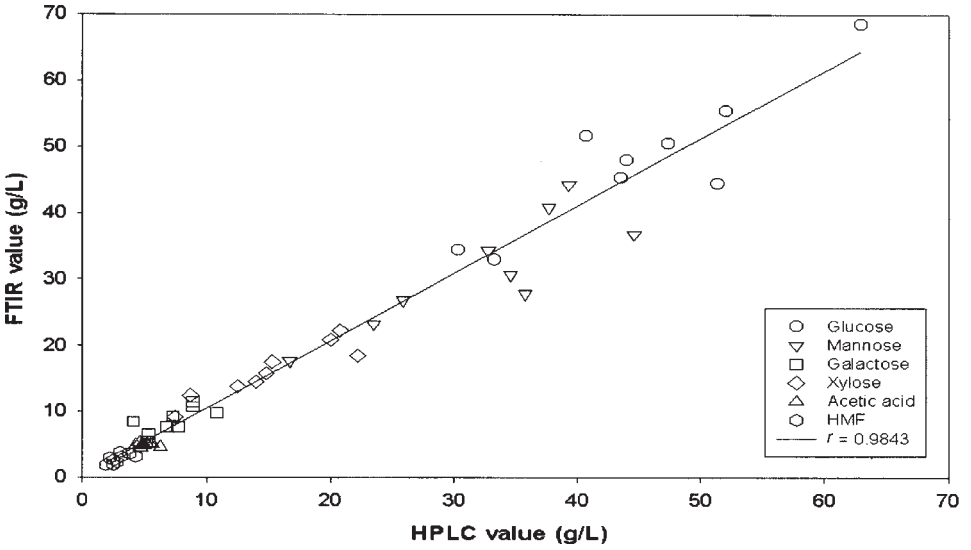

Fig. 3. PLS regression analysis for predicting the composition of nine first-stage pretreated softwood liquors and liquor obtained from pretreated MSW. FTIR predicted composition vs HPLC-measured composition.

trometer combination. This limits the accuracy of component concentration prediction for solutions and pretreated liquors with low concentrations of dissolved species. However, 9-, 12-, and 23-reflection models of these cells are commercially available and should extend the limits of detection of solutes to lower levels, unless the background IR absorption by water increases sufficiently to interfere with the analysis.

Figure 3 shows the prediction of the sugar composition of nine softwood pretreatment samples using the PLS method we have described vs the values measured by HPLC. The nine samples were obtained from first-stage pretreatment experiments of whole-tree forest thinnings over the temperature range from 180 to 190°C, 2.5% H_2SO_4, and 2- to 9-min residence times. These nine samples were treated as unknowns and were not included in the calibration set. In addition, the prediction of composition using the softwood PLS regression method for the liquor from MSW gave values comparable to the values measured by HPLC, perhaps because the MSW contained construction lumbers, office paper, and newsprint, all of which are derived from softwood sources. However, when this PLS method, which was developed with softwood samples, is applied to spectra obtained from hardwood liquors of yellow poplar sawdust pretreated in the Sunds Hydrolyzer, prediction of the composition of the pretreated liquor completely failed (data not shown). Apparently, the matrix within the liquor from pretreated hardwood samples differs enough from that of the softwood calibration samples that the prediction accuracy decreases substantially. The prediction accuracy of the method for softwood is expected to improve when a larger database of calibration samples becomes avail-

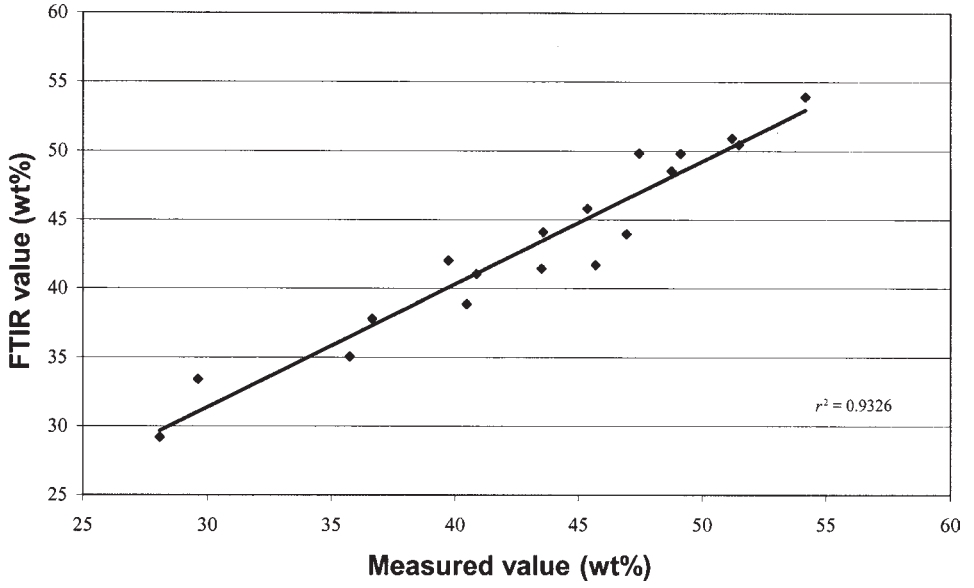

Fig. 4. PLS method developed for glucose in solids using washed solids from 18 first- and second-stage pretreated softwood slurries.

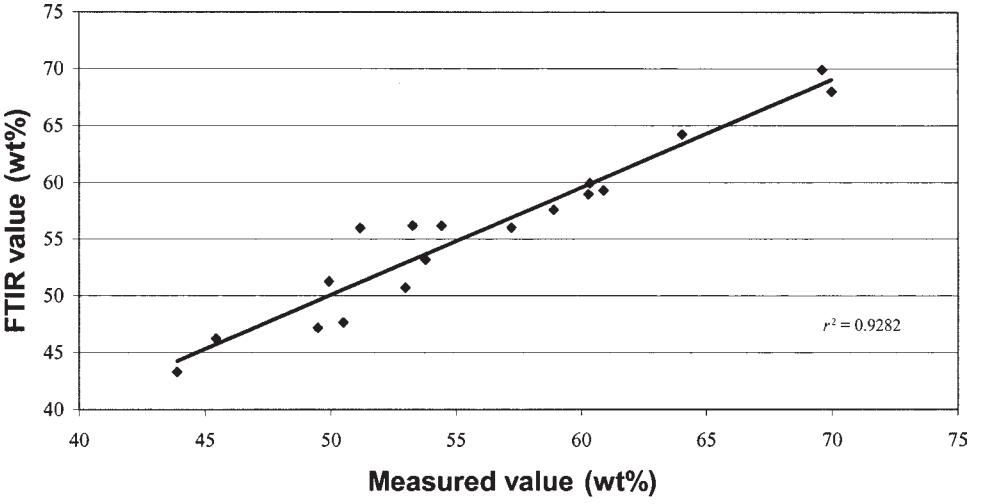

Fig. 5. PLS method developed for lignin in solids using washed solids from 18 first- and second-stage pretreated softwood slurries.

able. However, the resources required to enlarge this database will be substantial.

The same ASI DurasamplIR composite-diamond cell was used to obtain spectra for 18 washed residues of pretreated softwood samples. The results of the PLS regression analysis following entering of the compositional data for the solids is shown in Fig. 4 for residual glucose and Fig. 5 for residual lignin. A number of samples were excluded from the

analysis because the glucan determinations were unusually low (one was 3.77%) and the lignin contents were unusually high (with the same sample the Klason lignin determination was found to be 96%). In addition, the mass balance closures around the harshly pretreated samples gave low glucan recoveries and >110% lignin recoveries; thus, we excluded them from the analysis. Considerable deviation in the PLS method developed for pretreated softwood solid residues results if data for samples from harsh pretreatment conditions are used in the analysis. We excluded solid samples in which mass balance closure for lignin was determined to be >110%.

Reports in the literature indicate that cellulose can be modified to products giving low glucose values when determined by the Klason lignin determination *(27)*. We are currently working on methods to circumvent this problem. Accurate glucan and lignin values are needed in the calibration sets in order for PLS models to predict these components in washed solids. With accurate calibration sets for solids, the FTIR-ATR technique can then be applied to predicting the composition of solids, as well as to liquors and slurries from pretreated biomass.

Conclusion

The FTIR-ATR technique allows mid-IR spectra to be rapidly obtained on slurries, washed solids, and liquors from pretreated biomass. The pretreated softwood slurries used in this study were obtained from a very complex feedstock consisting of chipped whole trees, including limbs, bark, and needles. As this preliminary study indicates, the diamond-composite Hastelloy insertion probe is capable of allowing quantitative FTIR-ATR measurements to be taken inside pretreatment reactors. This analysis can be rapid enough to potentially allow monitoring and control of the reactor to maximize product yield. The accuracy of predicting the composition of pretreated liquors should improve as larger databases of FTIR spectra and compositional values are accumulated. However, methods developed using PLS for one feedstock must be redeveloped when changing to other feedstocks or markedly altering reactor conditions. Once that new method has been validated, the FTIR-ATR technique can be used to monitor and control the reactor. Options are available for some of the current FTIR spectrometers that allow for 4- to 20-mA output control with decisions based on values from the PLS method. Diamond-composite probes are available with 9, 12, and 23 reflections to allow lower levels of detection of dissolved species.

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