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# EVALUATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY MEASUREMENTS OF GLUCOSE AND XYLOSE IN BIOMASS HYDROLYZATE

C. L. Crofcheck, M. D. Montross

**ABSTRACT.** *Measurement of sugars using traditional spectroscopic (UV/Vis) assays or high performance liquid chromatography (HPLC) can be time consuming and expensive. Alternative methods for measuring sugars after enzymatic hydrolysis of biomass would be convenient for screening potential biomass feedstocks and pretreatment methods. Fourier transform infrared spectroscopy (FTIR) has been utilized for measuring composition of various aqueous solutions and is evaluated here as an alternative to UV/Vis and HPLC assays. Solutions of glucose and xylose with concentrations between 0 and 1.5% w/v (total sugar content between 0 and 3.0% w/v) were used to build calibration curves for all three methods. A validation set of 10 samples of varying concentrations of glucose and xylose (between 0 and 1.5% w/v) were used to quantify the performance of the three measurement techniques. The FTIR assay was able to predict the glucose and xylose concentration with a standard error of prediction (SEP) of 0.03% (w/v), lower than the SEP for the HPLC (~0.06%) and UV/Vis (~0.07%) assays. The FTIR assay was also able to accurately measure the sugar concentration of wheat stover (raw and pretreated with sodium hydroxide) after enzyme hydrolysis, although all three techniques produced similar results.*

**Keywords.** *UV/Vis absorbance assay, HPLC, FTIR, Wheat stover.*

Quantification of the individual sugar concentration after the enzymatic hydrolysis of lignocellulosic materials is time consuming and expensive. Analytical techniques using high performance liquid chromatography (HPLC) require extensive sample preparation, long analysis times, and skilled personnel to perform the analysis. *Traditional spectroscopic (UV/Vis) assays* require determination of each compound individually, are labor intensive, and require dilution to the appropriate level to fit within the standard curve.

IR-based spectroscopic techniques provide an opportunity to decrease the time and cost required for sample analyses. Near-infrared (NIR) spectroscopy has been used by numerous researchers to quantify sugars in both dry and wet samples (Giangiacomo and Dull, 1986; Lanza and Li, 1984; Bellon et al., 1993; Hames et al., 2003). Lanza and Li (1984) concluded that it was not possible to determine individual sugars with acceptable accuracy using NIR. Recently, techniques using Fourier transform infrared spectroscopy (FTIR) have been utilized for determining the concentration of sugars in aqueous mixtures (Sivakesava and Irudayaraj,

2000; Rodriguez-Saona et al., 2001), pretreated biomass liquors (Tucker et al., 2000), and during ethanol fermentation (Sivakesava et al., 2001).

Sivakesava and Irudayaraj (2000) utilized FTIR for the determination of glucose, sucrose, and fructose concentrations in aqueous mixtures similar to those found in commercial beverages. The total sugar concentrations varied between 10% and 40% (w/v) with individual sugars between 1.5% and 28% (w/v), where the SEP for glucose was 0.228%. However, FTIR results were not compared to HPLC or UV/Vis assays and the sugar levels were higher than those expected during enzymatic hydrolysis of lignocellulosic materials. Rodriguez-Saona et al. (2001) compared the prediction accuracy of FTIR, HPLC, and standard enzymatic techniques for the determination of glucose, fructose, and sucrose in fruit juice with concentrations between 0 and 8% (w/v). However, they assumed that the HPLC provided the correct reading of the known sugar concentration. PLS models based on transmittance spectral data transformed using the second derivative resulted in a SEP of less than 0.10%.

Sivakesava et al. (2001) monitored an ethanol fermentation using FTIR for the determination of glucose, ethanol, and optical cell density. The SEP for the PLS first-derivative models for glucose was 0.1819% w/v for glucose measurements between 0 to 5% w/v, using HPLC measurements as a reference. Tucker et al. (2000) utilized a FTIR spectrometer for the determination of constituents from liquors produced from dilute acid pretreated softwood and hardwood. Glucose, xylose, mannose, and acetic acid were determined using a PLS model developed for the FTIR and results verified using HPLC. The standard error between the HPLC and FTIR was 0.5% w/v for glucose concentrations between 4.0% and 12.0% w/v, while the xylose standard error was 0.15% w/v for concentrations between 0.4% and 2.0% w/v. While these

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results suggest that FTIR can be used to predict glucose levels which are comparable to HPLC measurements, the glucose levels in the experiment were substantially higher than expected during enzymatic hydrolysis and no comparisons were made to assays.

In this study, an FTIR spectrometer was first calibrated for and then used to measure glucose and xylose concentrations between 0 and 1.5% w/v (total sugar concentration between 0 and 3.0% w/v). The results obtained by calibrated FTIR measurement were compared to those obtained by traditional UV/Vis and HPLC assay methods. In addition, samples of raw and pretreated wheat stover samples were enzymatically hydrolyzed and the glucose and xylose quantified using all three methods.

## MATERIALS AND METHODS

All chemicals and reagents were from Fisher Scientific (Hampton, N.H.) unless otherwise noted. Sugar solutions included glucose, xylose, or a mixture of both. The same sugar stock solutions (same manufacture and lot number) were used for all three techniques.

### MEASUREMENT TECHNIQUES

#### *Uv/Vis Absorbance Assays*

Glucose was measured using the assay described by Russell and Baldwin (1978). A standard curve was constructed with six standards with glucose concentrations between 0 and 0.0025% w/v. Samples were diluted to fall within the range of the standard curve. After the appropriate dilution, 150  $\mu$ L of sample was further diluted with 1 mL of buffer [50-mL triethanolamine buffer (50 mM with pH 7.5)], 40-mg  $MgCl_2 \cdot 6H_2O$ , 20-mg adenosine 5'-triphosphate disodium salt, 20-mg beta-nicotinamide adenine dinucleotide phosphate sodium salt, 5- $\mu$ L hexokinase from *Saccharomyces cerevisiae* (9 U), and 2.5- $\mu$ L glucose-6-phosphate dehydrogenase type XXIII, ammonium sulfate suspension (12.5 U) and incubated for 30 min. The absorbance for each sample was read at 340 nm (Cary 300 Bio Spectrometer, Varian Inc., Walnut Creek, Calif.) and compared to the standard curve to determine the amount of glucose.

Xylose was measured based on the orcinol-sulphuric acid reaction (Brückner, 1955). Dissolving 0.75-g orcinol in 7.5 mL of ethanol made a 10% orcinol solution. Six samples were prepared to produce a standard curve with concentrations between 0 and 0.1% w/v. Samples were diluted to fit within the calibration curve as needed. In a test tube, 25  $\mu$ L of each sample was added to 150  $\mu$ L of the 10% orcinol solution and gently mixed. Three mL of a ferrous chloride solution in hydrochloric acid [0.09 g  $FeCl_3 \cdot 6H_2O$  in 75 mL of concentrated hydrochloric acid (95.5%) and 75 mL of deionized water] were added to each test tube. The test tubes were boiled for 21 min and allowed to cool to room temperature. The samples were read at 600 and 660 nm using the spectrometer and the difference in the readings was used to calculate the xylose concentration based on the standard curve.

#### *FTIR and HPLC Instrumentation*

FTIR spectra were obtained utilizing a Thermo-Nicolet Nexus FT-IR 670 spectrometer (Waltham, Mass.) with a

scanning range of 400 to 4000  $cm^{-1}$ , at spectral resolution of 4 cm, and 128 scans per sample. A pipette was used to transfer the solution to an Attenuated Total Reflectance (ATR) assembly with a ZnSe crystal and a DTGS/KBr detector.

Thirty eight samples were used to develop a FTIR calibration model based on partial least squares. D(+)-glucose monohydrate (Fluka, St. Gallen, Switzerland) and D-xylose were used to produce the calibration samples. Glucose and xylose were weighed using a recently calibrated digital balance (A and D Weighing, HR120, Milpitas, Calif.) with a resolution of 0.0001 g and an accuracy of 0.01% and added to 200 mL of deionized water to create stock solutions for each sugar with a concentration of 2% w/v. The sugar solutions were diluted and commingled to produce glucose and xylose concentrations between 0 and 1.5% w/v at a final volume of 10 mL (total sugar concentration between 0 and 3% w/v). Dilutions were performed using 1000- and 5000- $\mu$ L Eppendorf Research Pro pipettes (Hamburg, Germany) with an accuracy of 0.6%. The sugar concentrations investigated were expected to be typical of enzymatic hydrolysis experiments. The sugar solutions were mixed together in a test tube, equilibrated at room temperature, and centrifuged at 12,000 rpm for 10 min before scanning with the FTIR.

Calibration models were produced with TQAnalyst Professional 6.2.1 software (Waltham, Mass.) using PLS, PLS with the first derivative, principal component analysis (PCA), and Beer's law. The optimum number of latent variables was determined using full cross-validation by leaving one sample out.

The amount of glucose and xylose were also measured using a Varian ProStar HPLC system (Palo Alto, Calif.) with a Varian Model 350 RI detector, a BioRad Aminex7 HPX-87P column (Hercules, Calif.), and a BioRad Carbo-p guard column. The mobile phase was degassed HPLC water, at a flow rate of 0.6 mL/min, and a column temperature of 85°C. All samples for the HPLC were filtered through a 0.2- $\mu$ m syringe filter.

### MEASUREMENT VALIDATION

An initial validation sample set of 10 samples with varying concentrations of glucose and xylose was tested in a manner that would simulate enzyme hydrolysis experiments. Sugars were weighed and dissolved in 50 mL of sodium acetate buffer (6.804 g/L, pH 4.8) with 0.375 g of cellulase (Alltech Inc., Nicholasville, Ky.) in each flask. An eleventh flask with the buffer and cellulase with no sugar added was used as a control and the background spectrum for the FTIR. Sugar concentrations were measured using the FTIR calibration model, UV/Vis, and HPLC.

The glucose and xylose concentrations were measured for wheat nodes, internodes, chaff, and leaves ground (through a 2-mm screen), pretreated or left unpretreated, and then subjected to enzyme hydrolysis in order to verify the measurement capability of the FTIR and compare the performance to the HPLC and UV/Vis assays. Three replicates were run for each component using 3 g ( $\pm 0.1$  g) of wheat material. The samples were pretreated using a procedure similar to Crofcheck and Montross (2004). Samples were soaked in 30 mL ( $\pm 0.1$  mL) of 0.1- or 0.2-N NaOH solution (10% dry weight/volume) for 2 h at room temperature using 50-mL centrifuge tubes. A control sample with 30 mL of deionized water was also included. The

samples were vacuum filtered through a pleated paper filter and washed with 60 mL of deionized water.

The pretreated samples were placed in 125-mL conical flasks with 100 mL of sodium acetate (0.05 M) buffer and autoclaved. The solutions were adjusted to a pH of 4.8 using sodium hydroxide or hydrochloric acid. Sodium azide (0.35g/L, 0.05 M) was added to the flask to prevent the growth of microorganisms. Experiments were conducted with 0.75 g of enzymes from Alltech, Inc. (Nicholasville, Kent.) with a cellulase activity of 10,000 CMCU/g (measured at a pH of 4.8 and a temperature of 50°C) and a xylanase activity of 150,000 XU/g (measured at a pH of 5.3 and a temperature of 50°C). The samples were placed in a shaking incubator (New Brunswick, New Brunswick, N.J.) at a temperature of 50°C for 60 h. Samples (200 µL) were taken at 60 h, placed in a micro-centrifuge tube, boiled for 5 min to inactivate the cellulase (Mandels et al., 1976), and stored at 4°C until analyzed.

## RESULTS AND DISCUSSION

### CALIBRATION DEVELOPMENT

Based on an error propagation estimate (Dally et al., 1984), the estimated maximum error in the sugar concentration of the calibration and validation samples was 0.015% w/v based on the accuracy of the scale and dilution method. The final calibration model used was based on PLS with 8 and 4 latent variables for the glucose and xylose calibration, respectively, between the wavenumbers of 700 and 1530  $\text{cm}^{-1}$ . The root mean squared error (RMSE) of the FTIR calibration was 0.00815 and 0.00375% for the glucose and xylose concentration, respectively. Typical absorbance spectrum for a 1% glucose, 1% xylose, and a 1% glucose and xylose solution are shown in figure 1.

### VALIDATION WITH KNOWN SUGAR CONCENTRATIONS

The 10 validation samples with glucose and xylose concentrations between 0.2% and 1.5% (total sugar concentration between 0.2% and 3.0%) were measured using FTIR, HPLC, and UV/Vis assays. The SEPs for the three methods are included in table 1. The FTIR technique resulted in the lowest SEP with a value of 0.03% for glucose and for xylose, while the SEP of the UV/Vis and HPLC assays were all greater than 0.05%.

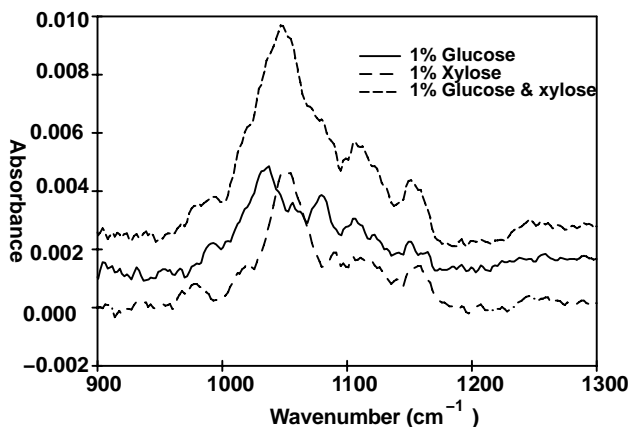


Figure 1. FTIR absorbance spectrum of a 1% glucose, 1% xylose, and 1% glucose and xylose solution.

Table 1. Root mean squared error between the actual and measured amount of glucose and xylose of 10 known samples for the three different measurement techniques.

	Glucose (%)	Xylose (%)
FTIR	0.031	0.030
UV/Vis	0.093	0.066
HPLC	0.075	0.049

A calibration and validation set were tested with the FTIR using centrifuged (12,000 rpm for 10 min) and non-centrifuged samples. The SEP of the glucose and xylose concentration using non-centrifuged samples was 0.052% and 0.062%, respectively. Samples centrifuged had a lower SEP than non-centrifuged samples, suggesting that samples need to be centrifuged prior to analysis with the FTIR to improve accuracy. Therefore, all samples in this study were centrifuged prior to analysis with the FTIR. Based on typical protocols, UV/Vis samples were not filtered or centrifuged, while HPLC samples were filtered using a 0.2-micron syringe filter to prevent damage to the column. FTIR samples were also filtered to see if there was a difference between the filtered and centrifuged results. There was no difference and it was decided to use the less expensive centrifuge method.

The validation data, plotted as actual versus measured with a one-to-one line, for the three methods for glucose and xylose are shown in figures 2 and 3, respectively. In order to compare the predictive power of the three techniques, an analysis was performed to determine if there was a significant difference between the actual versus measured values. Actual versus measured data were fit to a line and the 95% confidence intervals for all three techniques were compared (table 2). For there to be a statistical agreement between the actual and measured values, a slope of one and an intercept of zero should fall within the 95% confidence interval. The confidence intervals for both the slope and the intercept using the FTIR for both glucose and xylose indicated that the

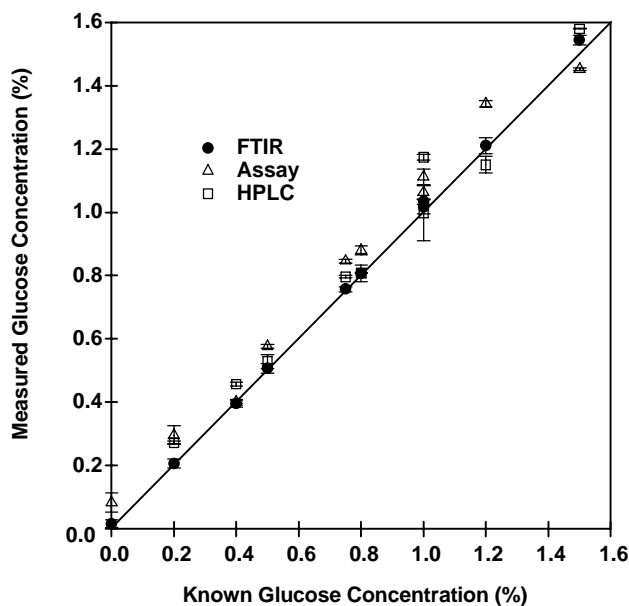


Figure 2. Measured glucose concentrations from the UV/Vis, FTIR, and HPLC assays for 10 independent validation samples of a mixture of glucose, xylose, sodium acetate buffer, and enzymes. The y-axis error bars are based on standard error ( $n = 3$ ), while the known glucose concentration had an estimated error of 0.015%.

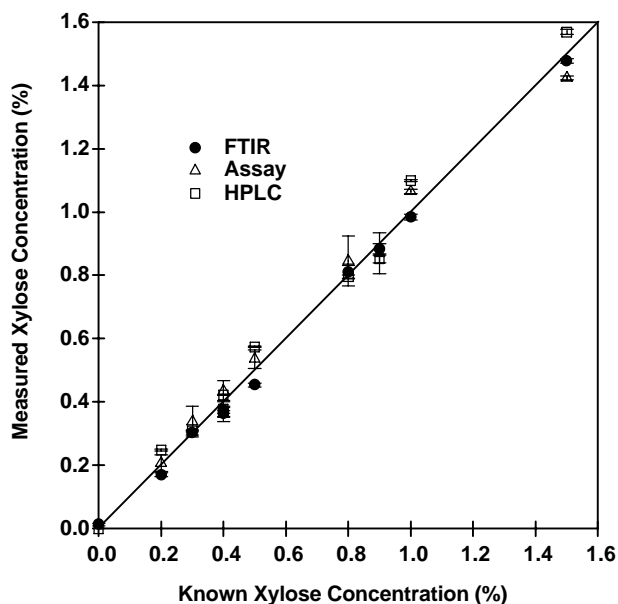


Figure 3. Measured xylose concentrations for the UV/Vis, FTIR, and HPLC assays for 10 independent samples of a mixtures of glucose, xylose, sodium acetate buffer, xylanase, and cellulase. The y-axis error bars are based on standard error ( $n = 3$ ), while the known xylose concentration had an estimated error of 0.015%.

measured and actual values were statistically not different ( $\alpha = 0.05$ ). The confidence intervals using the xylose assay indicated that the measured and actual values were also statistically not different ( $\alpha = 0.05$ ). However, only the FTIR had both regression coefficients within the 95% confidence intervals for glucose and xylose. This suggests that the FTIR measurement was more reliable. Running reference samples with known concentrations of glucose and xylose would be recommended to verify the accuracy of the calibration over time and for different operating conditions.

#### APPLICATION TO ENZYME HYDROLYSIS

Wheat stover fractions were used as a model biomass feedstock to determine the applicability of FTIR for enzyme hydrolysis experiments. The glucose concentrations predicted by the FTIR, HPLC, and UV/Vis assays were very similar (fig. 4). The measured xylose concentration was more variable between the methods (fig. 5). Xylose measured using the UV/Vis was consistently higher than the concentration measured using the FTIR. However, the HPLC consistently measured a lower xylose concentration than the FTIR. The resulting 95% confidence intervals for a linear regression analysis of the data shown in figures 4 and 5 are summarized in table 3. For all comparisons, the slope confidence interval contained one and the intercept interval

contained zero. These results indicate that all three measurement techniques would be appropriate for measuring glucose and xylose concentration during enzymatic hydrolysis. In general, the standard error for the enzyme hydrolyzate measurements were higher than the standard error measurements for the known sugar solutions, hence it was more difficult to find a statistical difference between the three methods. Therefore, the primary factor in deciding which measurement assay to use is determined by available equipment, skill of personnel, and cost per sample.

#### LABOR AND COST ESTIMATION

The costs associated with sample analysis are summarized in table 4. Of the three methods, the equipment cost for the UV/Vis assays are the lowest as almost all labs have access to a UV/Vis, which would eliminate the cost of the spectrometer. FTIR and HPLC systems are not as common and may not be available in all labs. However, for this analysis it is assumed that all labs would have access to the required equipment. The HPLC is assumed to be equipped with an autosampler. It is assumed that an undergraduate student making \$10/h is available to perform all the sample preparation, analysis, and post-processing. All prices were estimated using the 2005 Fisher Scientific catalog (Pittsburgh, Pa.). All samples are assumed to be analyzed in triplicate.

The cost of sample preparation and analysis for the FTIR assay was approximately \$1.95/sample (this approximation includes the cost of supplies, e.g. pipette tips and microcentrifuge tubes, and the cost of labor for three replicates). Sample preparation and analysis for the HPLC assay requires syringes, filters, sample vials, pipette tips, mobile phase solutions, and the appropriate columns and guard columns. In combination with the cost of labor for sample preparation, analysis, and post-processing sugar measurements using the HPLC would cost a minimum of \$6.35/sample (assuming three measurements per sample). This assumed that the HPLC was equipped with an autosampler (without an autosampler the labor cost would be significantly higher). The supplies and labor required to run the UV/Vis assays were based on 45 samples run in triplicate. Considering the reagents and other miscellaneous supplies required during sample preparation and analysis and the eight hours of labor required for preparation, sample dilution, mixing reagents, and running and analyzing the data, the total cost to determine the glucose and xylose concentration using UV/Vis assays was \$2.18/sample (assuming three measurements per sample). However, if the diluted samples did not fall within the standard curve, the samples would need to be rerun resulting in double the analysis cost.

Table 2. The 95% confidence intervals (CI) for the slope and intercept of a line fit to the actual vs. measured values of glucose and xylose for the three different measurement techniques.<sup>[a]</sup>

	Glucose (fig. 2)		Xylose (fig. 3)	
	95% CI		95% CI	
	Slope, $\beta$	Intercept, $\alpha$	Slope, $\beta$	Intercept, $\alpha$
FTIR	<b>0.995</b> > $\beta$ > <b>1.041</b>	<b>-0.019</b> > $\alpha$ > <b>0.021</b>	<b>0.972</b> > $\beta$ > <b>1.016</b>	<b>-0.030</b> > $\alpha$ > <b>0.003</b>
UV/Vis	<b>0.919</b> > $\beta$ > <b>1.020</b>	0.05172 > $\alpha$ > 0.139	<b>0.918</b> > $\beta$ > <b>1.043</b>	<b>-0.036</b> > $\alpha$ > <b>0.055</b>
HPLC	1.004 > $\beta$ > 1.094	<b>-0.017</b> > $\alpha$ > <b>0.056</b>	1.012 > $\beta$ > 1.071	<b>-0.010</b> > $\alpha$ > <b>0.032</b>

<sup>[a]</sup> Confidence intervals, which include a slope of one or an intercept of zero, are shown in bold.

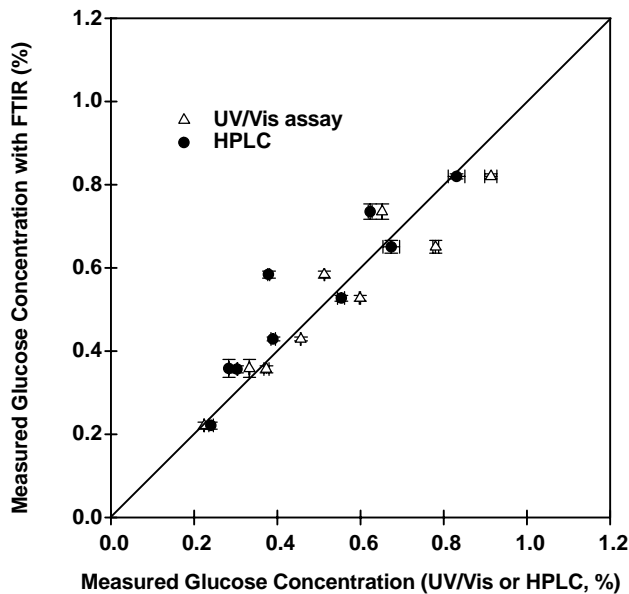


Figure 4. Glucose concentrations measured using FTIR assays compared with concentrations measured using HPLC and UV/Vis assays for wheat stover with three different pretreatment methods followed by enzymatic hydrolysis with cellulase. The y-axis and x-axis error bars are based on standard error (n = 3).

Sample analysis using the FTIR resulted in the lowest cost. Additional costs due to dilution errors with the UV/Vis and HPLC assays were not considered. Both the HPLC and assays could have samples that were not at the proper dilution, such that the samples would need to be reanalyzed with a different sample loop volume or dilution. This would significantly increase the analysis time and sample cost. The FTIR was accurate to a glucose and xylose concentration of 1.5% which would be past the upper limit of concentration expected during enzymatic hydrolysis. An

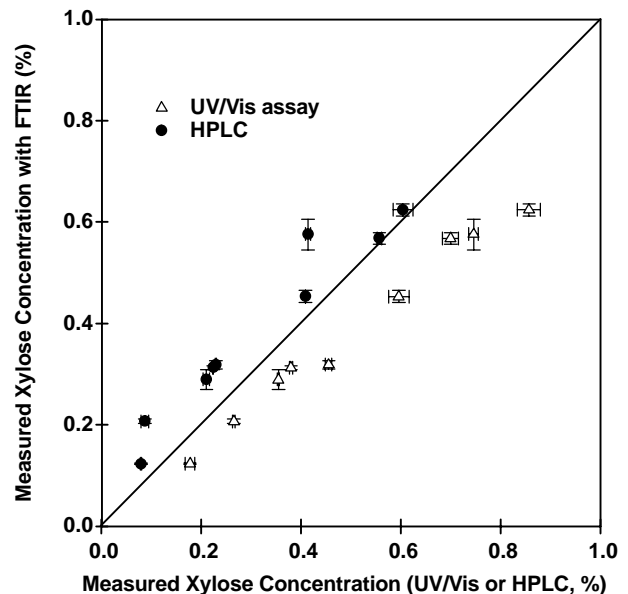


Figure 5. Xylose concentrations measured using FTIR assays compared with concentrations measured using HPLC and UV/Vis assays for wheat stover with three different pretreatment methods followed by enzymatic hydrolysis with cellulase. The y-axis and x-axis error bars are based on standard error (n = 3).

alternative calibration model could be developed for the FTIR that would consider higher sugar concentrations if required. Previous work has indicated that an FTIR is capable of measuring sugar concentrations up to 40% (Sivakesava and Irudayaraj, 2000).

## CONCLUSION

Measurement of sugar concentration during the enzymatic hydrolysis of biomass is a major source of time and error.

Table 3. The 95% confidence intervals (CI) for the slope and intercept of a line fit to the FTIR measurements vs. the HPLC and UV/Vis measurements.<sup>[a]</sup>

	Glucose 95% CI		Xylose 95% CI	
	Slope, $\beta$	Intercept, $\alpha$	Slope, $\beta$	Intercept, $\alpha$
FTIR vs. HPLC	<b>0.609</b> > $\beta$ > <b>1.312</b>	<b>-0.219</b> > $\alpha$ > <b>0.169</b>	<b>0.556</b> > $\beta$ > <b>2.020</b>	<b>-0.459</b> > $\alpha$ > <b>0.118</b>
FTIR vs. UV/Vis	<b>0.745</b> > $\beta$ > <b>1.390</b>	<b>-0.196</b> > $\alpha$ > <b>0.160</b>	<b>0.629</b> > $\beta$ > <b>2.455</b>	<b>-0.4351</b> > $\alpha$ > <b>0.284</b>

<sup>[a]</sup> Confidence intervals, which include a slope of one or an intercept of zero, are shown in bold.

Table 4. Summary of estimated costs per sample when using a FTIR, HPLC, and UV/Vis assays for determination of glucose and xylose after enzymatic hydrolysis.

	FTIR	HPLC	UV/Vis Absorbance (glucose and xylose)
Equipment cost	>\$30,000	>\$40,000 (with autosampler)	>\$5,000 (UV/Vis)
Sample prep.			
Supplies	\$0.15	\$1.78	\$0.30
Labor	\$0.08	\$0.33	\$0.67
Sample analysis			
Supplies	\$0.05	\$3.74	\$0.25
Labor	\$1.67	– (assuming autosampler)	\$1.11
Post-processing			
Labor	– (done during scanning of next sample)	\$0.50	\$0.10
Total cost (not including equipment)	\$1.95	\$6.35	\$2.18

An FTIR assay was used to measure the concentration of known aqueous solutions of glucose and xylose and compared to similar measurements using HPLC and UV/Vis assays. The FTIR had the lowest SEP of the three measurement techniques, between 60% and 300% lower than the UV/Vis and HPLC assays. In addition, the FTIR was able to accurately measure the glucose and xylose released during enzyme hydrolysis of wheat stover fractions that were left alone or pretreated with sodium hydroxide, although, all three techniques produced similar results. If a lab had access to all three pieces of equipment, an FTIR would result in the lowest analysis cost per sample and would require a less knowledgeable operator than UV/Vis or HPLC assays. Analysis costs using the FTIR were at least \$0.23 to \$4.40/sample cheaper than the assays and HPLC, respectively.

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#### REFERENCES

- Bellon, V., J. L. Vigneau, and M. Leclercq. 1993. Feasibility and performances of a new, multiplexed, fast and low-cost fiber optic NIR spectrometer for the online measurement of sugar in fruits. *Appl. Spectrosc.* 47(7): 1079-1083.
- Brückner, J. 1955. Estimation of monosaccharides by the orcinol-sulphuric acid reaction. *Biochem. J.* 60: 200-205.
- Crofcheck, C. L., and M. D. Montross. 2004. Effect of stover fraction on glucose production using enzymatic hydrolysis. *Transactions of the ASAE* 47(3): 841-844.
- Dally, J. W., W. F. Riley, and K. G. McConnell. 1984. *Instrumentation for Engineering Measurements*, 2nd edition. New York: John Wiley and Sons.
- Giangiaco, R., and G. B. Dull. 1986. Near infrared spectrophotometric determination of individual sugars in aqueous mixtures. *J. Food Sci.* 51(3): 679-683.
- Hames, B. R., S. R. Thomas, A. D. Sluiter, C. J. Roth, and D. W. Templeton. 2003. Rapid biomass analysis - New tools for compositional analysis of corn stover feedstocks and process intermediates from ethanol production. *Appl. Biochem. Biotech.* 105: 5-16.
- Lanza, E., and B. W. Li. 1984. Application for near infrared spectroscopy for predicting the sugar content of fruit juices. *J. Food Sci.* 49(4): 995-998.
- Mandels, M., R. Andreotti, and C. Roche. 1976. Measurement of saccharifying cellulose. *Biotechnol. Bioeng.* 6: 21-33.
- Rodriguez-Saona, L. E., F. S. Fry, M. A. McLaughlin, and E. M. Calvey. 2001. Rapid analysis of sugars in fruit juices by FT-NIR spectroscopy. *Carbohydr. Res.* 336(1): 63-74.
- Russell, J. B., and R. L. Baldwin. 1978. Substrate preferences in rumen bacteria - Evidence of catabolite regulatory mechanisms. *Appl. Environ. Microbiol.* 36 (2): 319-329.
- Sivakesava, S., and J. Irudayaraj. 2000. Determination of sugars in aqueous mixtures using mid-infrared spectroscopy. *Applied Engineering in Agriculture* 16(5): 543-550.
- Sivakesava, S., J. Irudayaraj, and A. Demirci. 2001. Monitoring of a bioprocess for ethanol production using FT-MIR and FT-Raman spectroscopy. *J. Ind. Microbiol. Biotechnol.* 26(4): 185-190.
- Tucker, M. P., R. K. Mitri, F. P. Eddy, Q. A. Nguyen, L. M. Gedvilas, and J. D. Webb. 2000. Fourier transform infrared quantification of sugars in pretreated biomass liquors. *Appl. Biochem. Biotech.* 84-86: 39-50.