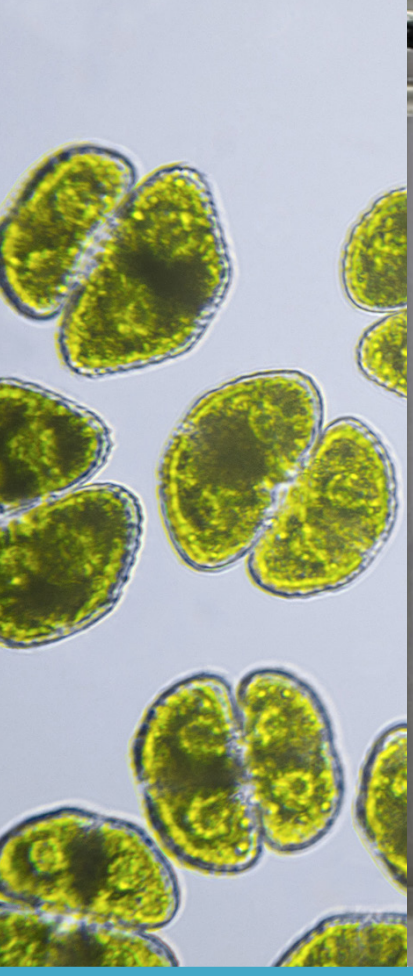

Biofuels Series

RAPID MEASUREMENT OF XYLOSE AND GLUCOSE MONITORING CORN STOVER FERMENTATION IN BIOETHANOL PRODUCTION

Application Note 301LS
YSI Life Sciences

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Rapid Measurement of Xylose and Glucose Monitoring Corn Stover Fermentation in Bioethanol Production

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INTRODUCTION

Simultaneous glucose and xylose concentrations can be measured during fermentation of complex matrices such as pretreated corn stover during the production of bioethanol. A measurement can be made in about one minute with minimal preparation using the YSI 2900 Series. YSI's enzyme electrode technology provides for specific glucose measurements in the range of 0.05 to 9.00 g/L and for xylose measurements in the range of 0.5 to 30.0 g/L (undiluted). Measurements are virtually unaffected by color, turbidity, density, pH, and most chemical substances.

When configured with YSI glucose oxidase and YSI pyranose oxidase membranes, the YSI 2900 Series analyzer measures glucose and xylose with aspiration of just 13 microliters of sample. Most samples require simple filtration and dilution prior to measurement. The sample is automatically flushed from the electrode chamber within 30 seconds after results are displayed and the YSI 2900 Series is ready to measure the next sample. Turn around time per sample is under two minutes.

In the study described glucose and xylose were periodically measured in filtered corn stover fermentation samples over a 48 hour period during lab-scale bioethanol production[1]. Some samples required dilution. Results were compared to HPLC runs of the same samples (see results below). The advantage of a simple, fast, and accurate simultaneous glucose and xylose measurement is to provide near real time data for these two key parameters. Timely information related to the progress of sugar fermentation may help researchers improve ethanol yield.

I. MATERIALS & SETUP

Sample Collection

Collection container (appropriate volume for fermentor sample)
Whatman #2 filter paper, funnel and collection vessel
Nylon syringe filter (0.22 micron); 3 ml luer lock syringe
Collection tubes (1.5 or 2.0 ml microcentrifuge tube)
YSI 2900 Series Analyzer, configured for glucose & xylose measurement

1 YSI greatly appreciates the contributions of Nancy Dowe and her team at the National Renewable Energy Laboratory, US DOE, Golden, CO.

YSI 2900 Series Instrument Setup

See the YSI 2900 Series user's manual for general setup and safety information.

To run these two chemistries simultaneously follow the setup information outlined below.

Under the Configure menu, set/confirm the following parameters.

Sample Size: 13 microliters

Probe A Parameters

Chemistry	Xylose
Unit	g/L
Calibrator	20.0
End Point	45 Sec

Probe B Parameters

Chemistry	Gluose
Unit	g/L
Calibrator	2.50
End Point	45 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	0 Sam
Cal Shift	2%

II. METHOD

A. Calibrate the YSI 2900 Series instrument.

B. Each day, prior to runs, perform the FCN membrane test using YSI 2363 solution.

C. Each day, prior to runs, test the linearity of the system with 0.90% w/v glucose using YSI 1531 (9.00 g/L glucose) linearity standard; followed by running a second linearity test with 30.0 g/L xylose linearity standard. Record only the displayed value for the chemistry you are testing.

continued

D. Collect sample from the fermentation vessel, ensuring a representative sample.

E. Filter the sample using Whatman #2 paper to eliminate the bulk of the particulate matter. Collect the filtrate.

F. If necessary, dilute the sample with reagent water. Record dilution factor. If also running samples for HPLC comparison, check pH using a test strip. Add 1% NH4OH solution to adjust pH to ~7. Record volume of base added[2].

G. Mix samples well and remove at least 2 ml using a clean, disposable 3 mL syringe for each.

H. Secure a 0.22 µ filter cartridge to the luer fitting and dispense the sample into a microcentrifuge tube. This step eliminates small particulates and virtually all cells.

I. Present the sample[3] to the YSI 2900 Series for measurement.

J. Record the glucose and xylose concentrations; adjust with calculations for dilutions, as necessary.

III. CALCULATIONS

Samples that were diluted must be back-calculated by the dilution factor. Cross signal interference between glucose and xylose probes are automatically corrected.

To provide the most reliable results each calibration was run three times to ensure stability before samples were run. Each sample was run in triplicate to verify precision in the measurement. Averages of samples were used to derive the values shown in the tables and graphs.

IV. RESULTS

Seven samples over a 48-hour fermentation of acid-hydrolyzed corn stover where collected and filtered. The samples were collected at 0, 4, 8, 12, 24, 32 and 48 hours to monitor xylose and glucose consumption (main sugars from the acid hydrolyzate). Split samples from each collection time were measured by HPLC[4] configured for a sugar profile, and by the YSI 2900 Series configured for simultaneous xylose and glucose measurement. The results of a representative run are displayed in Table 1.

2 The pH adjustment step is required only for HPLC measurements in this study. The YSI measurement is quite tolerant of samples from pH 4 to 9.

3 An aliquot of this same sample was run by HPLC to obtain a reference value for comparison to YSI glucose and xylose values.

4 Hewlett Packard 1100 with RI detector, Shodex SP0810 column; HPLC grade water mobile phase; 10-50 µL sample; 0.6 mL/min flow; 85°C.

Sample	Glucose			Xylose (g/L)		
Hours	HPLC	YSI	bias	HPLC	YSI	bias
T-0	21.8	20.1	-1.7	53.8	57.4	+3.6
T-4	3.3	2.1	-1.2	38.4	34.5	-3.9
T-8	1.1	0.5	-0.6	22.0	23.3	+1.3
T-12	0	0.3	+0.3	13.5	13.1	-0.4
T-24	0	0.2	+0.2	7.0	7.0	0.0
T-32	0	0.1	+0.1	6.6	6.5	-0.1
T-48	0	0.1	+0.1	6.1	5.9	-0.2

Table 1: Data from Run 072707 at NREL

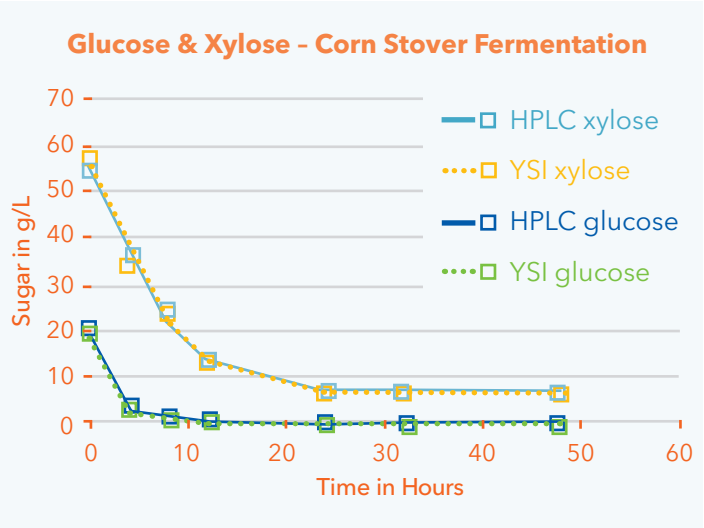

Figure 1: YSI vs. HPLC – Depletion over 48 Hours

Glucose was depleted within 10 hours while xylose depletion did not reach completion. Concentrations of both sugars were relatively stable within 24 hours of the start of fermentation.

Samples T-0 and T-4 were diluted ten-fold and two-fold, respectively, to bring the signals within reportable ranges for xylose. The results from diluted samples were back calculated.

From HPLC data, samples were known to contain several sugars that are potential interferences for the pyranose oxidase membrane. These are Arabinose, mannose, galactose and cellobiose. None were found to have a significant effect on the xylose reading. In the corn stover matrix studied glucose and xylose are by far the dominant sugars from saccharification and YSI values tracked HPLC results very closely.

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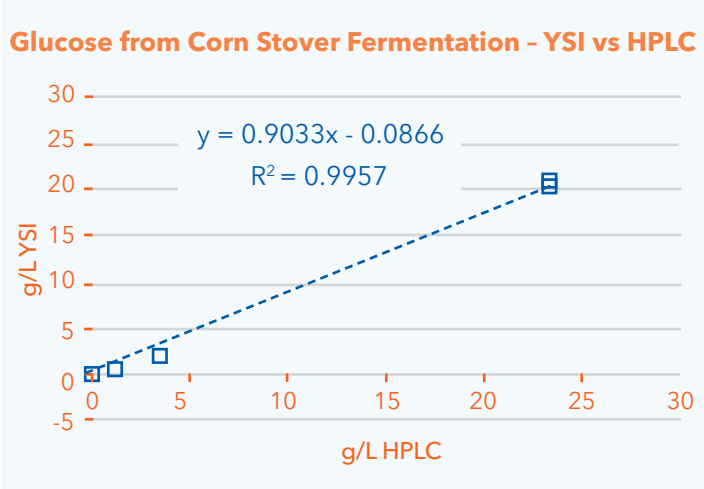

Figure 2: YSI vs. HPLC – Glucose during 48-hour fermentation

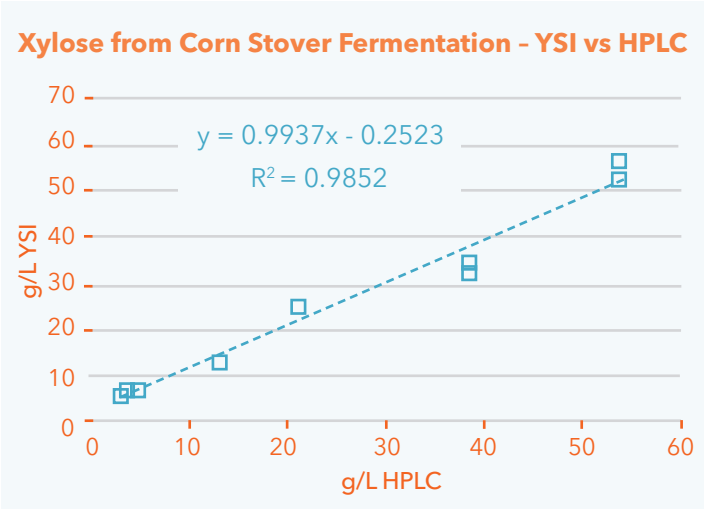

Figure 3: YSI vs. HPLC – Xylose during 48-hour fermentation

Correlation Plots of YSI vs. HPLC for Glucose and Xylose
Correlation data are plotted using values from Table 1 and the results obtained in the repeat run two weeks later at YSI. Therefore each concentration point contains two sets of data.

V. DISCUSSION

Lignocellulosic biomass has been widely studied as a feedstock for fermentation in fuel ethanol production. For example, acid-treated corn stover produces a variety of fermentable sugars that include glucose, xylose, mannose, arabinose, cellobiose, and galactose. Carbohydrate profiles from HPLC analysis showed that glucose and xylose in initial feedstock represent more than half of all carbohydrates in the acid-hydrolyzate; and for that reason can be important markers to evaluate the progress and efficiency of the fermentation process.

HPLC is currently the most common method used to measure glucose and xylose, along with other key metabolites during the production of ethanol. HPLC is quite accurate and precise but is based on separation technology and optical-based measurements that require significant pretreatment of the sample. In addition, time to results after pretreatment may be 30 minutes or more. The YSI 2900 Series configured for xylose and glucose provided results with relative ease of use, speed and accuracy compared with HPLC. Since the YSI is also known to tolerate samples of a broad pH range and show little or no effect to color and turbidity, it provides an excellent complementary method to HPLC when monitoring fermentations using lignocellulosic biomass.

In the preliminary investigation presented above glucose and xylose concentrations proved to correlate well with HPLC results when compared in aliquots of the same samples. Concentrations were compared periodically over the 48-hour fermentation as shown in Figure 1. Although relatively few samples were studied good correlation was observed as shown in Figures 2 & 3.

In conclusion the YSI 2900 Series can be used to monitor glucose and xylose to gain valuable information relevant to maximizing ethanol production efficiency. The YSI technology allows users to collect more data during critical time windows to help better understand process conditions, and in some cases could allow users to adjust process conditions during the fermentation. The technology is applicable to bench-scale, pilot-scale and production scale uses.

continued

ORDERING INFORMATION

YSI Part Numbers:

2900	Biochemistry Analyzer
2365	Glucose Oxidase Membrane Kit
2761	Xylose Membrane Kit
2357	Buffer Kit
2776	Glucose/Lactate Calibrator (2.50 g/L glucose)
1531	Glucose Linearity Test Standard (9.00 g/L)
2767	Xylose Calibrator (20.0 g/L)
2768	Xylose Linearity Test Standard (30.0 g/L)
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation)

RELATED YSI APPLICATION NOTES

Rapid Measurement of Residual Ethanol in Corn Stillage (Beer) during Distillation

Rapid Measurement of Glucose during Fermentation in Bioethanol Production

Dilution of Samples

As with most analytical methods there is a concentration range that provides optimal accuracy and precision. The YSI xylose and glucose methods are specified independently for performance; however these two analytes frequently occur together. Since there is cross-signal interference between the glucose and xylose electrodes compensation equations are applied in mixed solutions of glucose and xylose. In some cases the combined signals exceed the linear range of one or both electrodes. In this situation dilution of sample is recommended to improve accuracy of results.

For further information, please contact:

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