

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

Bob Gleason¹, Nancy Dowe², June Klingensmith¹, William Miller¹
¹ YSI Life Sciences, Yellow Springs, OH, ² National Renewable Energy Laboratory, US Department of Energy, Golden, CO

Abstract

Acid-treated corn stover produces a variety of fermentable sugars that include glucose, xylose, mannose, arabinose, cellobiose, and galactose. HPLC is commonly used to measure glucose and xylose, which are important markers for evaluating the progress and efficiency of ethanol fermentation processes. The HPLC method is accurate and precise; however, it requires significant sample pretreatment and analytical cycle times ≥ 30 minutes. A YSI Biochemistry Analyzer, configured with glucose oxidase and pyranose oxidase immobilized enzyme membranes, was evaluated for its ability to simultaneously measure glucose and xylose during fermentation of pretreated corn stover in a bioethanol production process. In this study filtered corn stover samples were periodically measured for glucose and xylose over a 48-hour period during a lab-scale bioethanol fermentation. Samples were analyzed on both a YSI Biochemistry Analyzer and an HPLC. Comparability of the two analytical methods were evaluated with regards to precision and analysis time. A strong, positive correlation of the two methods was demonstrated. The YSI Biochemistry analyzer performed simultaneous analysis of glucose and xylose in less than 3 minutes, providing a rapid, precise analytical alternative to the HPLC analytical method.

Experimental Plan

Evaluation:

Evaluate comparability of YSI Biochemistry Analyzer and HPLC analytical methods for glucose and xylose monitoring of acid hydrolyzed corn stover fermentation.

Criteria:

- YSI analysis should demonstrate strong, positive correlation with the HPLC method
- YSI analyzer should significantly reduce analysis time.

Fermentation Process

- 48-hour fermentation of acid-hydrolyzed corn stover
- Lab-scale fermentation process for bioethanol production (< 10L)

Process Analytics

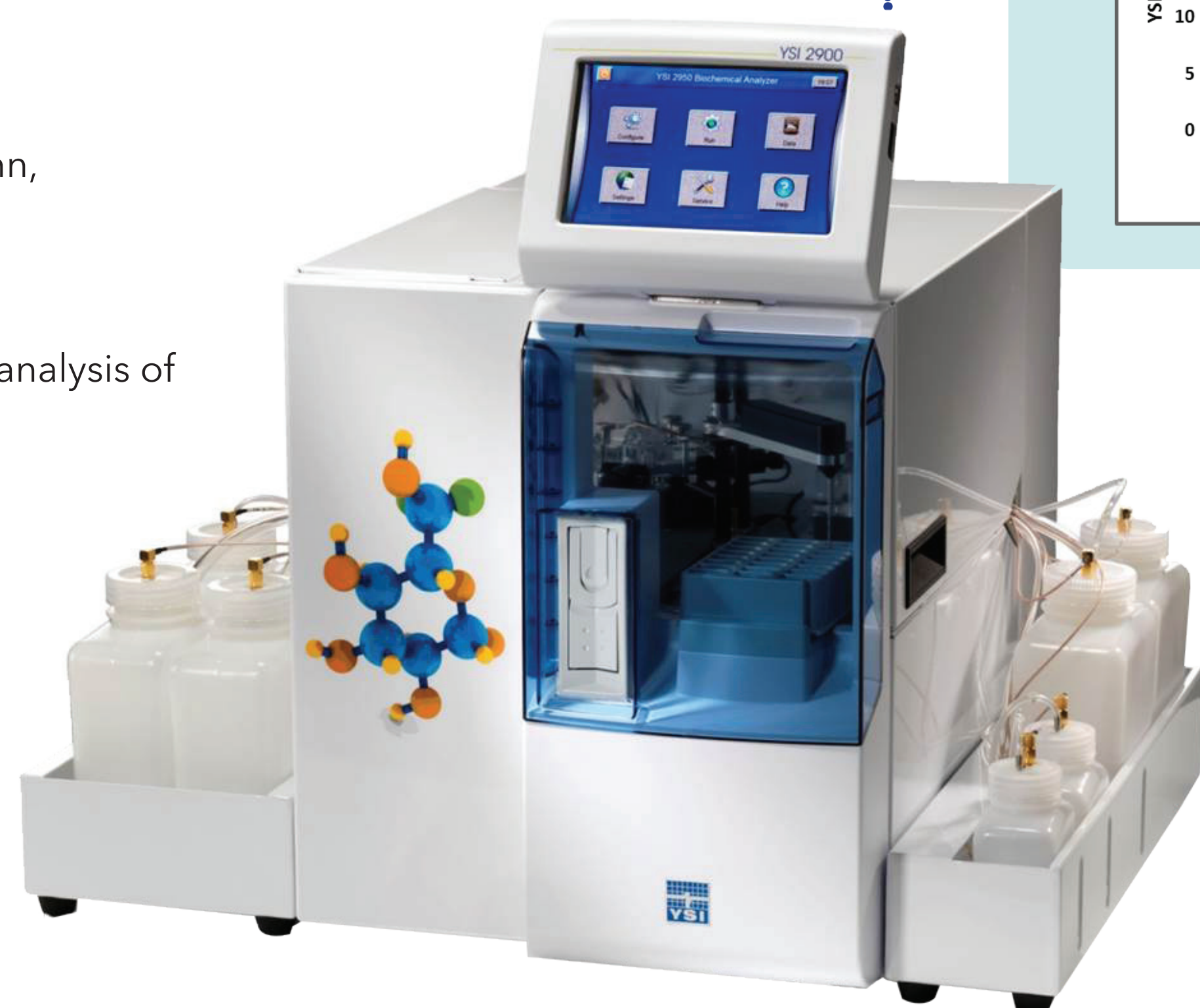
YSI Biochemistry Analyzer:

- Configured with glucose oxidase and pyranose oxidase immobilized membranes.
- Simultaneous glucose and xylose analysis of pre-filtered fermentation samples.
- No sample pH adjustment required.
- 13 μ l sample size

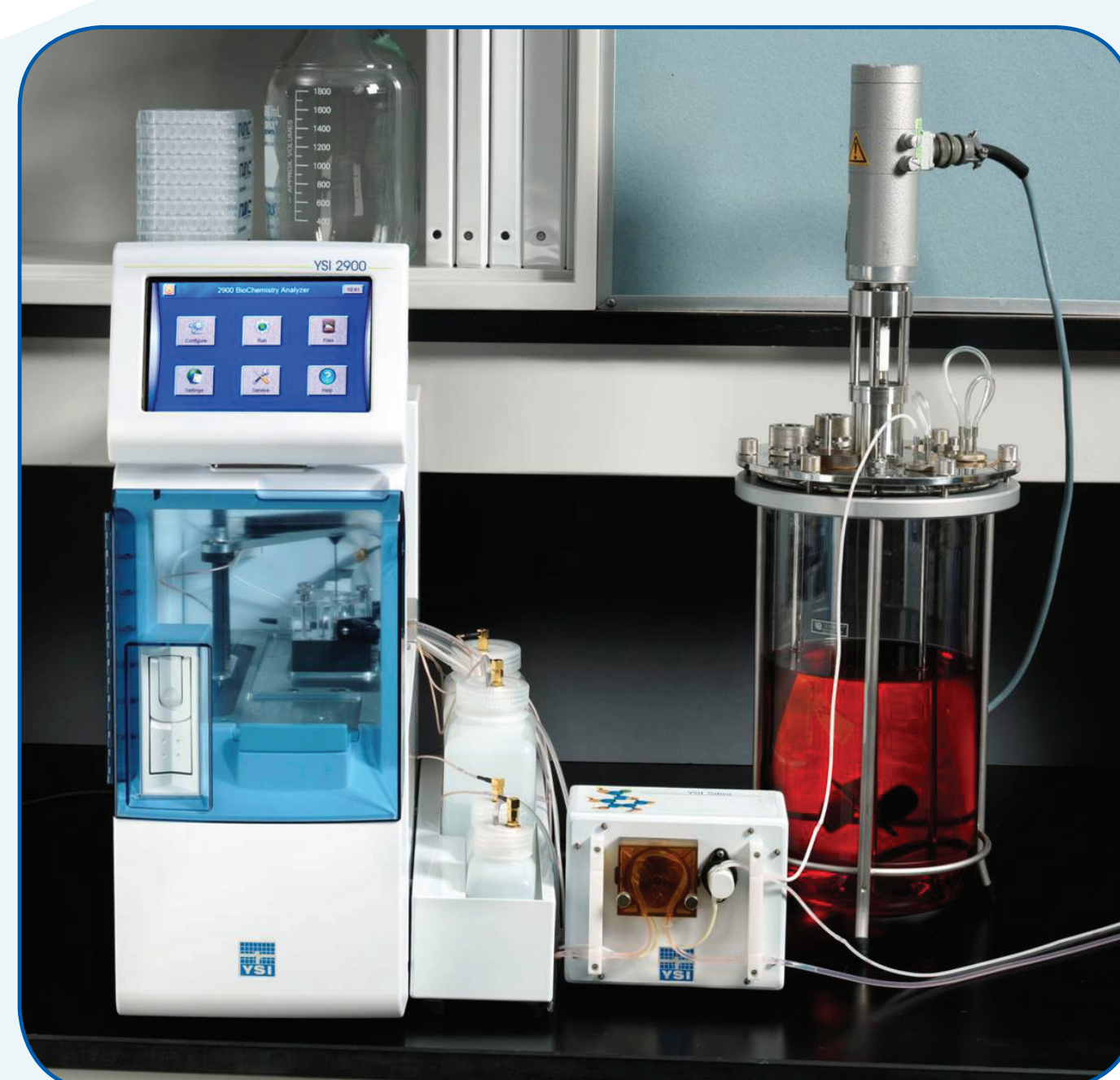
Agilent® 1100 Series HPLC:

- RI detector, Shodex SP0810 column, HPLC-grade H₂O mobile phase
- 0.6 ml/min flow, 85° C
- Simultaneous glucose and xylose analysis of pre-filtered fermentation samples.
- Sample pH adjustment to ~ 7.
- 10 - 50 μ l sample size

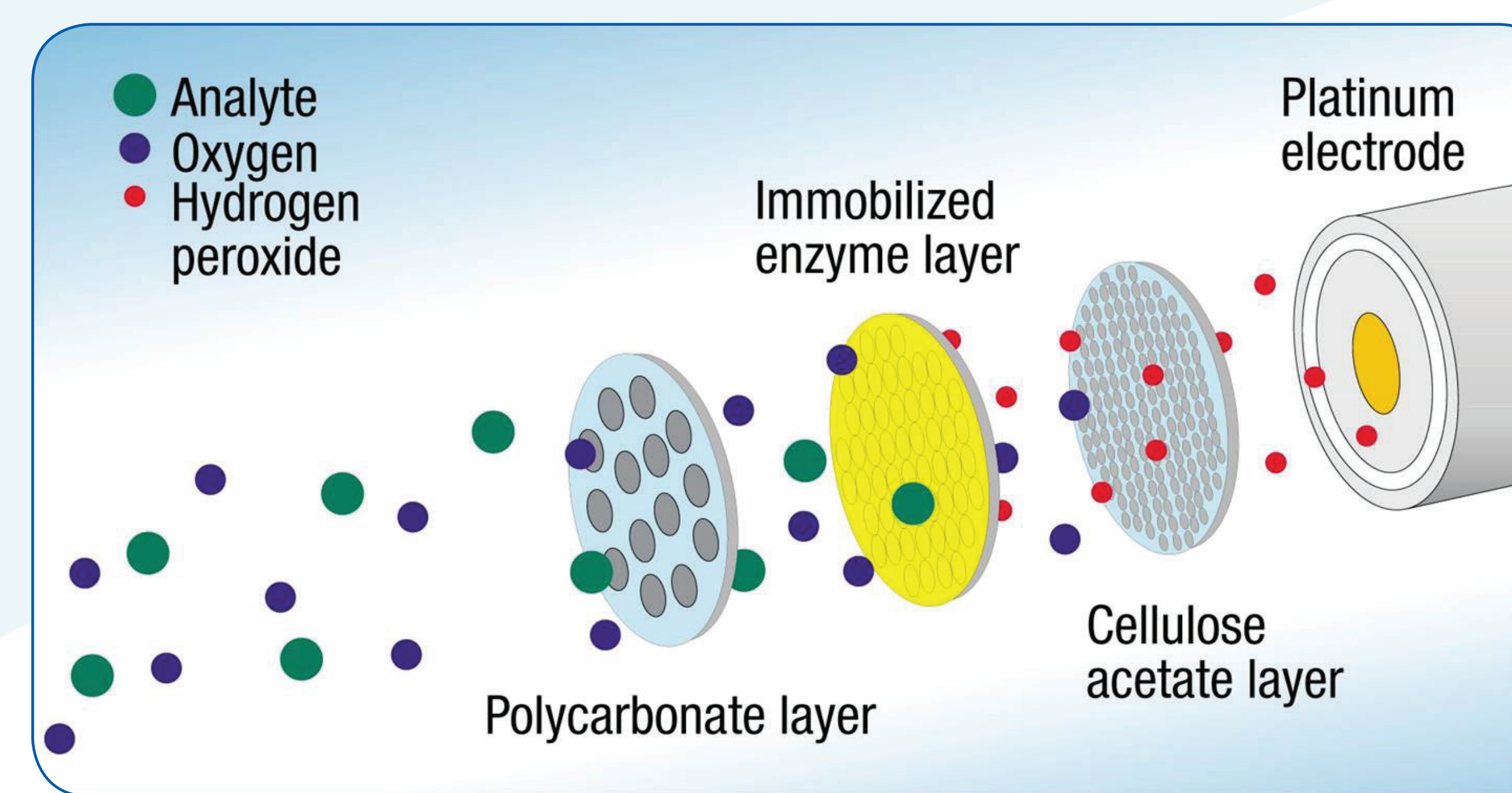
YSI 2950D
Biochemistry
Analyzer



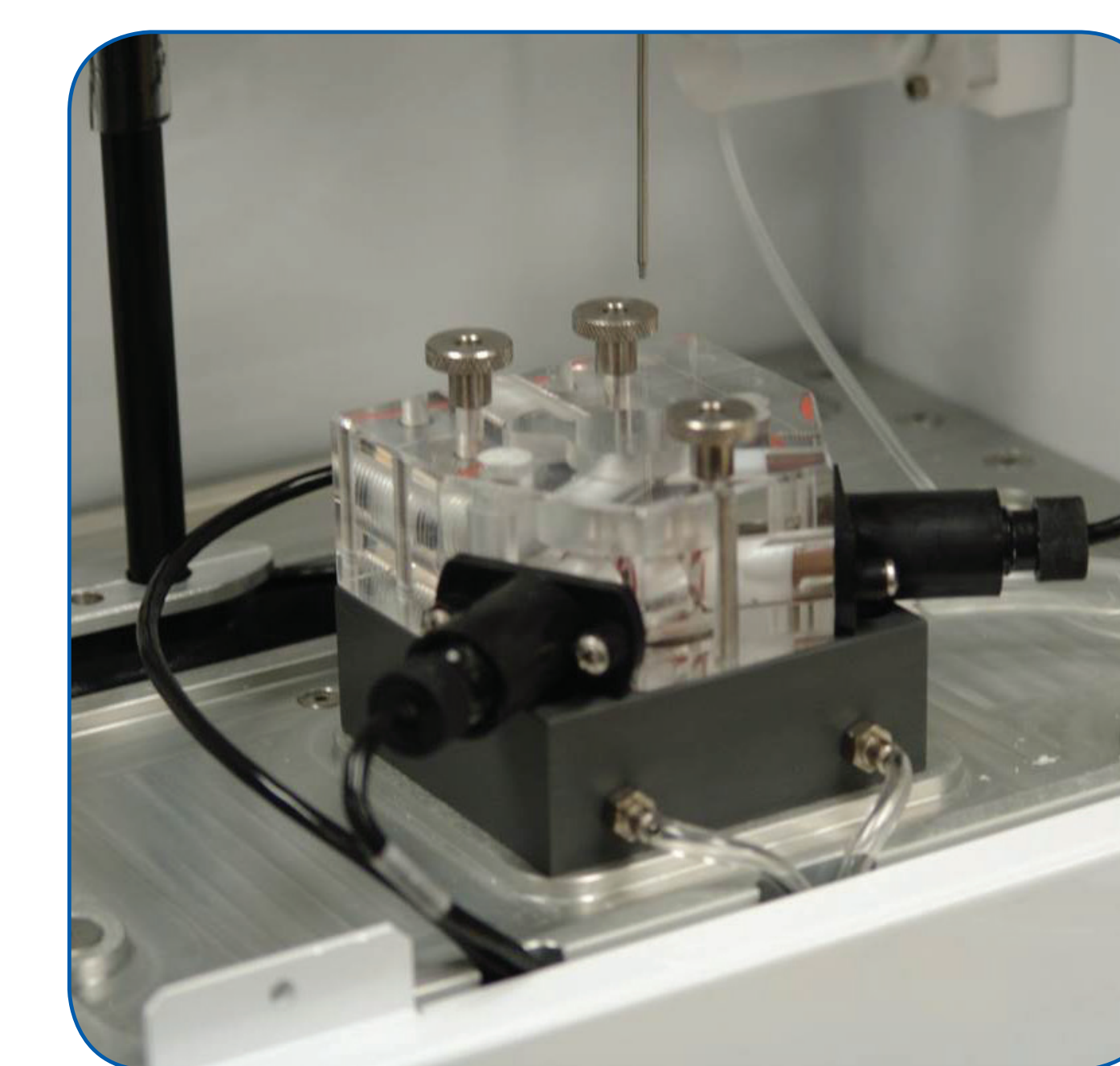
How the Immobilized Enzyme Membrane Technology Works



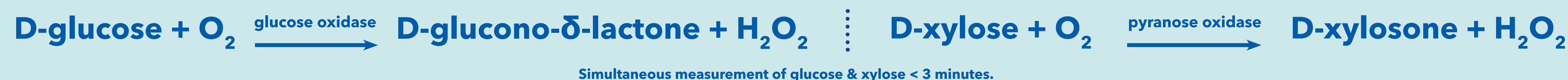
Fermentation samples loaded onto YSI Analyzer or automatically drawn from bioreactor.



Exploded view of membrane and sensor.



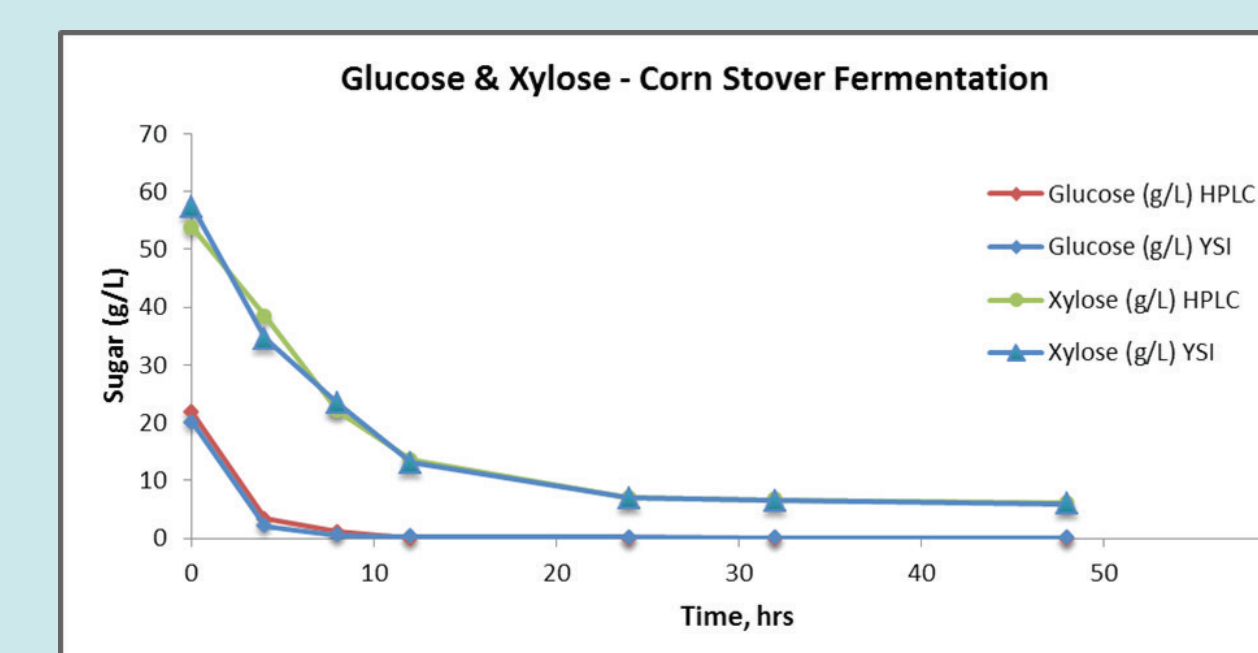
Sample (13 μ l volume) deposited into sample chamber configured with glucose oxidase and pyranose oxidase immobilized enzyme membranes.



Glucose/Xylose Results

Mean (μ) Glucose and Xylose Values for YSI and HPLC samples

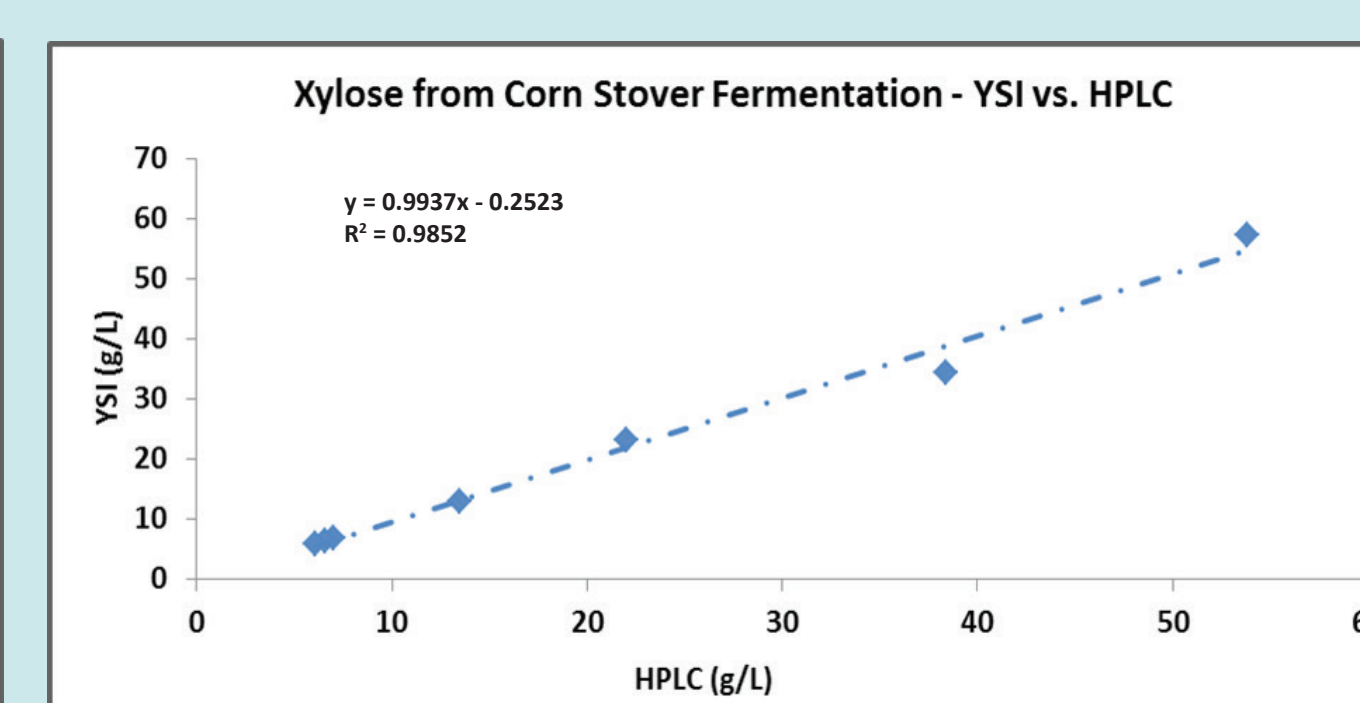
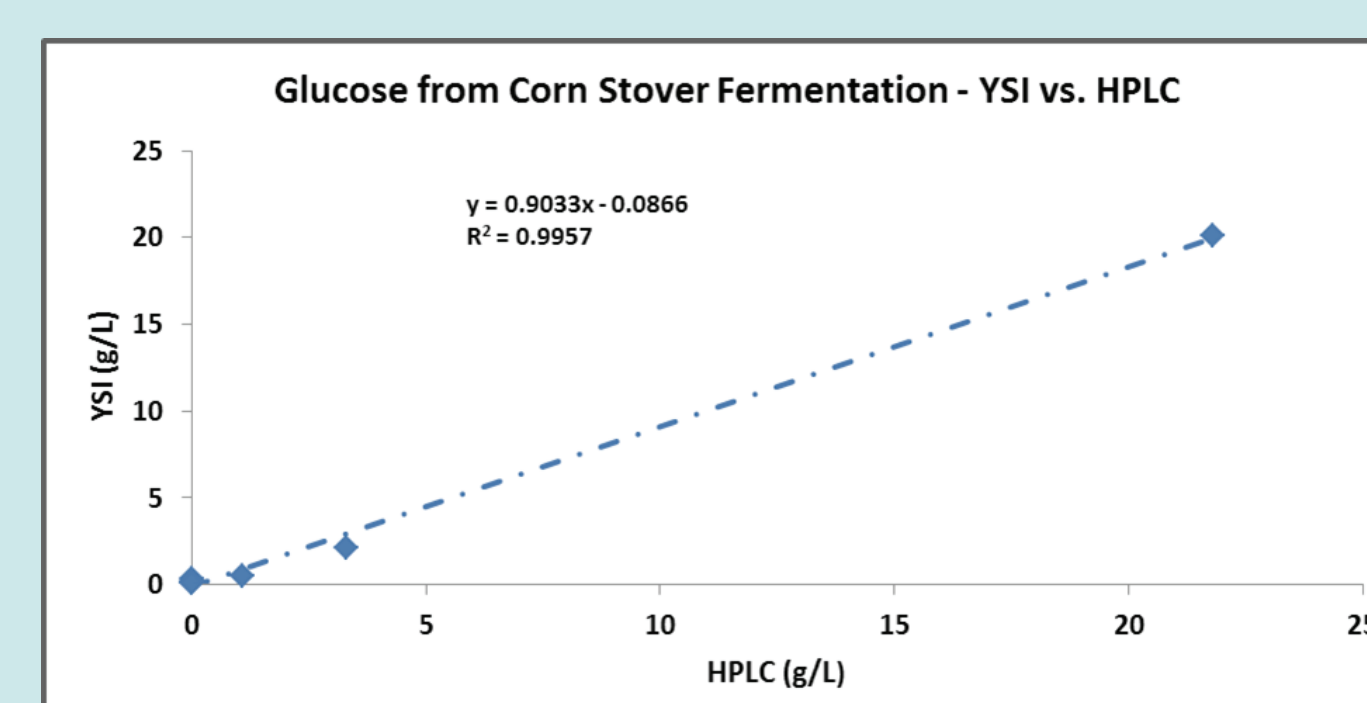
Sample Time (Hr)	Dilution Factor	Number of Samples Analyzed	Glucose (g/L)			Xylose (g/L)		
			HPLC	YSI	bias	HPLC	YSI	bias
0	10x	3	21.8	20.1	-1.7	53.8	57.4	3.6
4	2x	3	3.3	2.1	-1.2	38.4	34.5	-3.9
8	1x	3	1.1	0.5	-0.6	22	23.3	1.3
12	1x	3	0	0.3	0.3	13.5	13.1	-0.4
24	1x	3	0	0.2	0.2	7	7	0
32	1x	3	0	0.1	0.1	6.6	6.5	-0.1
48	1x	3	0	0.1	0.1	6.1	5.9	-0.2



Fermentation Sugar Analysis

- Mean glucose and xylose values for both YSI and HPLC analytical methods demonstrate comparability throughout entire fermentation process.
- No significant interference of arabinose, mannose, galactose and cellobiose on xylose measurement (pyranose oxidase).

Linear Regression Analysis



Analytical Method Comparability



- Strong, positive correlation of YSI and HPLC analytical methods
- YSI analysis time < 3 minutes. HPLC analysis time = 30 minutes.

Conclusions

- YSI immobilized enzyme membrane technology demonstrated comparability to the HPLC method for both D-glucose and D-xylose analysis.
- Simultaneous D-glucose and D-xylose analysis time reduced > 10-fold using the YSI Biochemistry Analyzer.
- YSI Biochemistry Analyzer provides scale-independent process analytical technology for monitoring acid-hydrolyzed corn stover fermentation.
- Rapid, precise analysis achieved with YSI analytical method while reducing sample prep burden.

Acknowledgements - NREL Fermentation Staff



Who's Minding the Planet?™

YSI Life Sciences
1725 Brannum Lane
Yellow Springs, Ohio
45387
USA

800-659-8895
937-767-7241
Fax 937-767-8058
support@ysi.com

Learn more online: ysilifesciences.com