MONITORING GLUCOSE CONCENTRATION IN CELL CULTIVATIONS

OptiEnz Sensors
Continuous Monitoring
Real-time Decisions

PRODUCT OVERVIEW

OptiEnz Sensors provides a sensing system for rapid and continuous monitoring of organic chemicals. Fast, accurate, and precise measurements are of high demand in biopharma and industrial biotechnology. Monitoring key analytes during cell cultivation improves process efficiency, increases product yield and quality, and prevents expensive shutdowns. OptiEnz Sensors is now offering a rapid at-line glucose sensor and will soon launch an in-line (in-situ) continuous sensor system.

The OptiEnz at-line sensing system provides an easy-to-use, low-cost product that meets the demands of the industry. The sensor makes measurements in three to four minutes. Simple calibration and assay protocols allow the instrument to be easily operated and eliminate the need for a specialized technician or expensive off-line analysis. No pretreatment other than dilution is required, and measurement accuracy is not impacted by complex media components.

The platform includes an optical transceiver with attached sensor probe, replaceable sensor caps (Figure 1), and PC-based software. Each cap contains an oxygen sensor and up to three sensing elements that can be used for simultaneous measurement of different analytes, extended concentration ranges, or replicate measurements. For example, one sensor cap could be used to simultaneously measure glucose and ethanol, while another sensor cap could be designed to measure low, medium, and high concentrations of glucose. Replaceable sensor caps make the instrument readily customizable and address the unique requirements of each process.

Future product developments include in-line sensing capabilities compatible with traditional and disposable bioreactors, as well as capabilities for monitoring additional analytes.

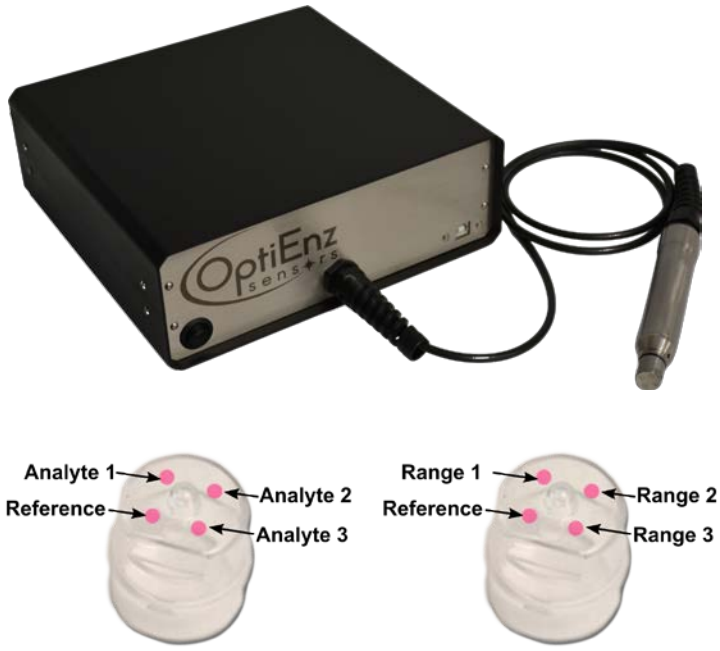

Figure 1: Optical transceiver, sensor probe, and replaceable caps with various sensing element configurations.

Table 1: Product specifications for the at-line glucose sensor.

PRODUCT SPECIFICATIONS	
Measurement Time	3-4 minutes
Sample Volume	0.5 mL
Glucose Range	1-120 g/L
Sample pH Range	2–8
Sample Temp. Range	1–55 °C
Accuracy	10%
Precision	8%
Cap Usage Lifetime	100 samples

METHODS

Calibration

Prior to use, a fast (8-min) calibration was performed at 0, 3, 6, and 9 g/L glucose for a 1–10 g/L detection range. A single calibration is sufficient for 40 sample measurements or four hours of use.

Assay

1) The sensor was inserted into stirred buffer solution and the sample was added directly to the solution (no pretreatment), then allowed to mix for 1 min. Sample addition resulted in a 1:100 (1–10 g/L range) or 1:1000 (10–100 g/L range) dilution in buffered solution.
2) Glucose concentration measurements were recorded for each replicate sensor.
3) The sensor was rinsed with water before inserting into the next sample.

Samples

Using the OptiEnz at-line sensor, glucose concentrations were measured in samples from cultivations of a bacterium (*Pseudomonas putida*) in minimal medium, a yeast (*Dekkera bruxellensis)* in rich medium, and a fungus (*Trichoderma reesei*) in a biomass hydrolysate.[1] For comparison, samples were also evaluated using a YSI 1700 Multiparameter Bioanalytical System and a high performance liquid chromatograph (HPLC) with a pulsed amperometric detector.

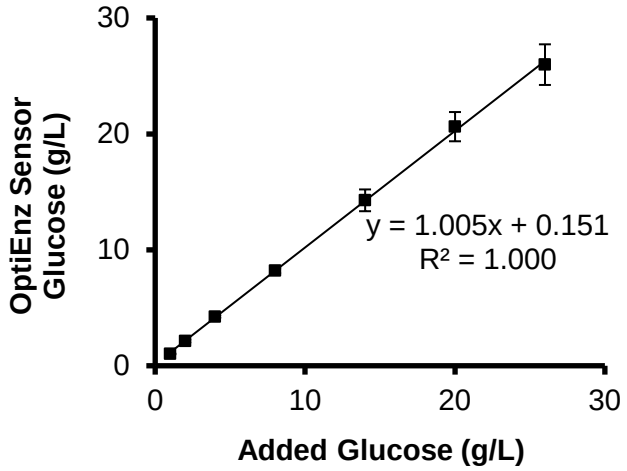

Figure 2: Glucose concentration measurements in buffer samples.

1. Data collected in collaboration with Davinia Salvachua and team at the National Renewable Energy Laboratory, US DOE, Golden, CO.

Table 2: Glucose concentration measurements in buffer samples.

Range (g/L)	Added Glucose (g/L)	OptiEnz Sensor Glucose (g/L)
1-10	1.0	1.02 ± 0.03
	2.0	2.1 ± 0.1
	4.0	4.2 ± 0.1
	8.0	8.2 ± 0.2
10-40	14	14.3 ± 0.9
	20	21 ± 1
	26	26 ± 2

RESULTS

Glucose additions to buffer

Sensors were evaluated across a range of glucose concentrations from 1–26 g/L. Each cap contained triplicate sensors, and reported error values represent the standard deviation of the three sensor measurements (Figure 2 and Table 2). The average measurement accuracy and precision in buffer were 3% and 6%, respectively.

P. putida in minimal medium

Samples of *P. putida* grown on modified minimal medium (M9) were collected throughout six-day cultivations. A steady decline in glucose, from 92 to 0.5 g/L, was measured. The average measurement accuracy (relative to the YSI 1700) and precision were 6.5% and 6%, respectively. A good correlation with the YSI 1700 was obtained across the detection range (R^2 = 0.993 for 1–10 g/L, R^2 = 0.999 for 1–92 g/L). The results are summarized in Figure 3 and Table 3.

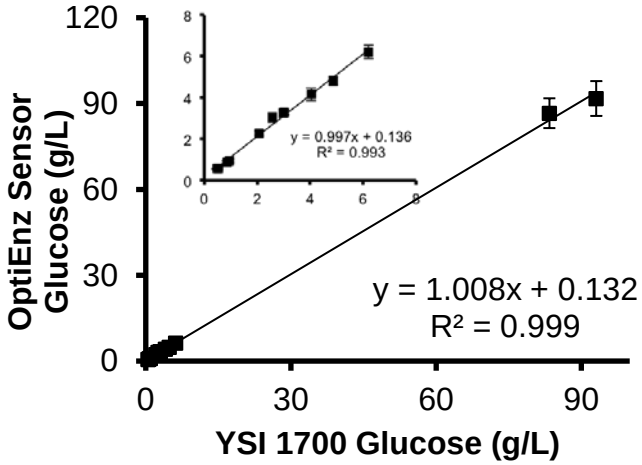

Figure 3: Glucose concentration measurements from *P. putida* cultivation samples.

Table 3: Glucose concentration measurements in *P. putida* cultivation samples.

OptiEnz Sensor Glucose (g/L)	YSI 1700 Glucose (g/L)
0.56 ± 0.01	0.47
0.57 ± 0.05	0.54
0.89 ± 0.06	0.85
0.94 ± 0.08	0.95
2.3 ± 0.1	2.1
3.0 ± 0.2	2.6
3.3 ± 0.2	3.0
3.3 ± 0.1	3.0
4.2 ± 0.3	4.0
4.8 ± 0.2	4.9
6.2 ± 0.3	6.2
87 ± 5	83.4
92 ± 6	93.1

T. reseii in rich medium

Samples of *T. reesei* grown in a rich medium were collected at late growth stage from five bioreactors. Measured glucose concentrations ranged from 1 to 9 g/L. The average measurement accuracy and precision were 4.2% and 4.2%, respectively. A good correlation with the YSI 1700 was obtained across the detection range (R^2 = 0.998). The results are summarized in Figure 4 and Table 4.

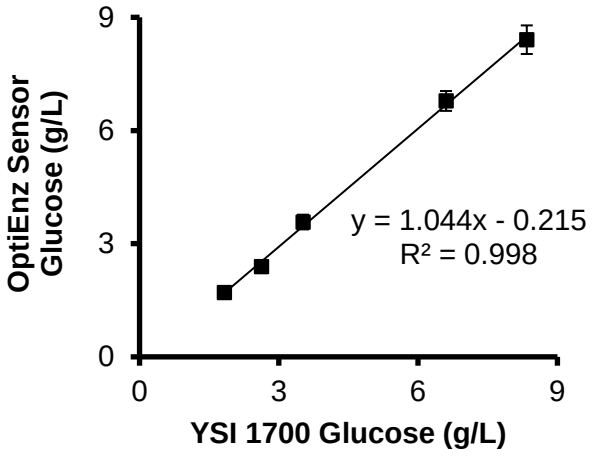

Figure 4: Glucose concentration measurements in *T. reesei* cultivation samples.

Table 4: Glucose concentration measurements in *T. reesei* cultivation samples.

OptiEnz Sensor Glucose (g/L)	YSI 1700 Glucose (g/L)
1.71 ± 0.09	1.8
2.39 ± 0.04	2.6
3.6 ± 0.2	3.5
6.8 ± 0.3	6.6
8.4 ± 0.4	8.3

D. bruxellensis in hydrolysate

Samples of *D. bruxellensis* CBS 11270 grown on biomass hydrolysate supplemented with 15 g/L corn steep liquor and 5 g/L $CaCO_3$ at pH 3.0 were measured for glucose and compared to both the YSI 1700 and HPLC methods. Measured glucose concentrations ranged between 10 and 90 g/L. The average sensor accuracy relative to the YSI 1700 and HPLC measurements was 3.9% and 9.2%, respectively, with an average precision of 4.8%. Good correlations with both the YSI 1700 and HPLC were obtained across the detection range (R^2 = 0.990 and R^2 = 0.998, respectively). The results are summarized in Figure 5 and Table 5.

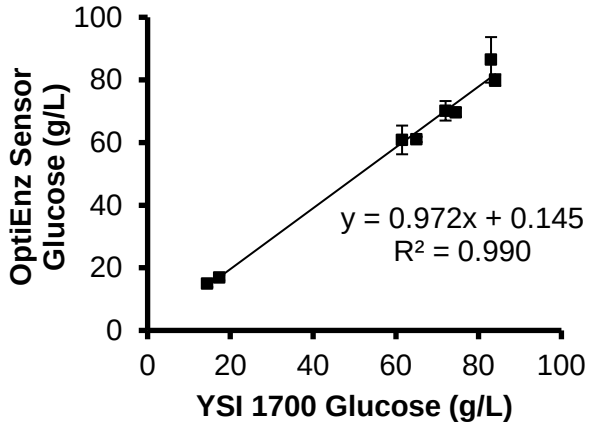

Figure 5A: Glucose concentration measurements in *D. bruxellensis* biomass hydrolysate cultivation samples.

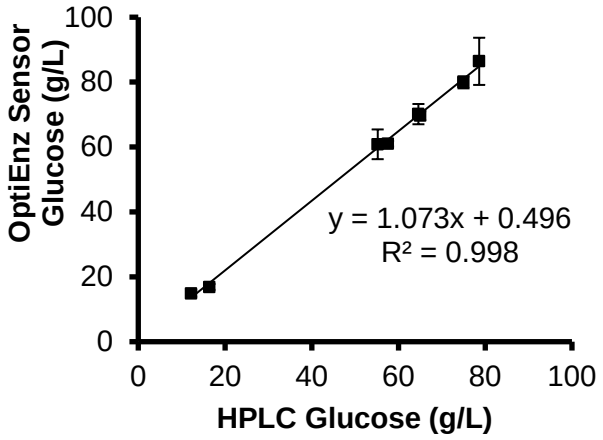

Figure 5B: Glucose concentration measurements in *D. bruxellensis* biomass hydrolysate cultivation samples.

Table 5: Glucose concentration measurements in *D. bruxellensis* biomass hydrolysate cultivation samples.

OptiEnz Glucose (g/L)	YSI 1700 Glucose (g/L)	HPLC Glucose (g/L)
15 ± 1	14.4	12.2
16 ± 1	17.3	16.4
61 ± 1	61.5	55.2
61 ± 5	65.0	57.6
67 ± 3	72.0	64.6
70 ± 1	74.5	65.0
80 ± 2	83.0	75.0
86 ± 7	84.0	78.6

CONCLUSION

The OptiEnz at-line sensing system met or exceeded product specifications when evaluated in bacterial cultivations in minimal medium and fungal cultivations in rich media or biomass hydrolysate. The average OptiEnz measurement accuracy was 7% relative to the YSI 1700 and 9% relative to liquid chromatography (YSI 1700 relative to HLPC accuracy of 11%). Measurement precision was within 6% for triplicate OptiEnz sensor measurements. OptiEnz sensor measurements were linearly correlated with the YSI 1700 and HPLC measurements (R^2 >0.99 across the detection range).

The OptiEnz at-line sensing system provided a rapid, easy-to-use, and low-cost sensor for accurate measurements of glucose in cultivation samples. No filtering or centrifugation steps were required for sample preparation, allowing measurements to be made within three minutes, and providing faster results than either YSI or HPLC.

CONTACT

For further information regarding pricing or product details, please contact OptiEnz Sensors:

Email: info@optienz.com
Phone: 1-855-OPTIENZ
Website: www.optienz.com