

MONITORING GLUCOSE CONCENTRATION IN ANAEROBIC CULTIVATIONS



*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.

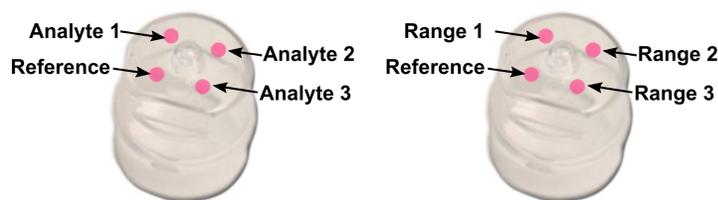


Figure 1: Optical transceiver, sensor probe, and replaceable caps with various sensing element configurations.

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, an 8-min calibration was performed at 0, 3, 6, and 9 g/L. A single calibration is sufficient for 40 sample measurements or four hours of use.

Assay

- 1) The sensor was inserted into a buffer solution and allowed to equilibrate for 1 min. Addition of 0.5 mL of sample from the cultivation provided a 1:100 (1-10 g/L detection range) dilution.
- 2) Glucose concentration measurements were recorded for each of the three replicate sensors.
- 3) The sensor was rinsed with water before inserting into the next sample.

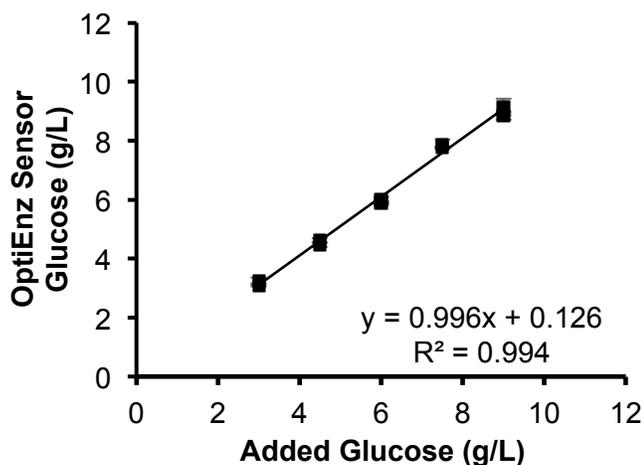


Figure 2: Glucose concentration measurements in buffer samples.

Table 2: Glucose concentration measurements in buffer samples.

Added Glucose (g/L)	OptiEnz Sensor Glucose (g/L)
3.0	3.3 ± 0.1
3.0	3.09 ± 0.04
4.5	4.48 ± 0.09
4.5	4.61 ± 0.08
6.0	5.89 ± 0.05
6.0	6.0 ± 0.1
7.5	7.79 ± 0.02
7.5	7.9 ± 0.2
9.0	8.8 ± 0.1
9.0	9.1 ± 0.3

RESULTS

Sensors were evaluated across a range of glucose concentrations from 3-9 g/L. Each cap contained triplicate sensors, and reported error values represent the standard deviation of the three sensor measurements. The average measurement accuracy and precision in buffer were 3% and 2%, respectively (Figure 2 and Table 2).

Using the OptiEnz at-line sensing system, glucose concentrations were measured in representative samples from bacterial (*Clostridium butyricum*) cultivations and respective cell-free medium. For comparison, samples were also evaluated using high performance liquid chromatography (HPLC) with a refractive index detector.¹ Samples of *C. butyricum* grown in a medium composed of glucose, peptone, yeast extract, and other salts were collected throughout a 15-day continuous anaerobic cultivation. Glucose concentration ranged from 2.2 to 10.2 g/L. The average measurement accuracy and precision in the cultivation samples were 5.5% and 3.5%, respectively (Figure 3 and Table 3). A good correlation with liquid HPLC measurement was observed across the detection range ($R^2 = 0.988$).

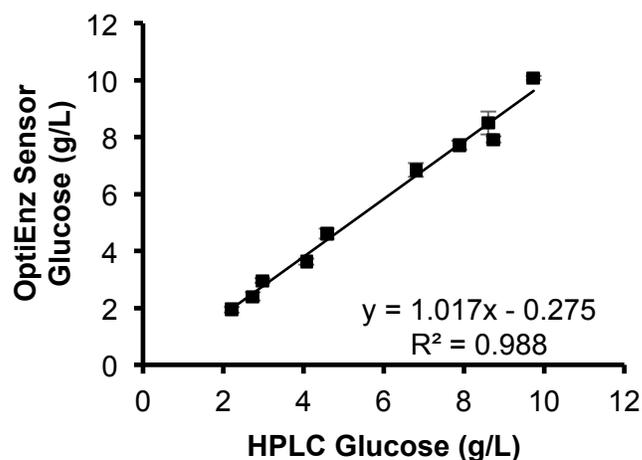


Figure 3: Glucose concentration measurements from *Clostridium butyricum* cultivation.

1. Data collected in collaboration with Xingfeng Huang at Colorado State University, Fort Collins, CO.

Table 3: Glucose concentration measurements in *Clostridium butyricum* samples.

HPLC Glucose (g/L)	OptiEnz Sensor Glucose
2.21	1.9 ± 0.1
2.74	2.4 ± 0.1
2.98	3.0 ± 0.1
4.08	3.6 ± 0.1
4.59	4.6 ± 0.2
6.81*	6.8 ± 0.2
7.89	7.7 ± 0.2
8.61	8.5 ± 0.4
8.74	7.9 ± 0.1
9.74*	10.08 ± 0.07

*Cell-free samples (growth medium only).

CONCLUSION

The OptiEnz at-line sensing system met or exceeded product specifications when evaluated in samples from anaerobic bacterial cultivations. The average OptiEnz measurement accuracy was 5.5% relative to HPLC measurement.

Measurement precision was within 3.5% for triplicate OptiEnz sensor measurements. OptiEnz sensor measurements were linearly correlated with HPLC measurements ($R^2 > 0.98$ across the detection range).

The OptiEnz at-line sensing system provided a rapid, easy-to-use, and low-cost sensor for accurate measurement 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 HPLC.

CONTACT

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