Rapid Measurement of Glucose during Fermentation in Bioethanol Production

I. Introduction

Glucose concentrations in complex matrices such as corn mash in fermentation can be measured in about one minute with minimal preparation using the YSI 2700 SELECT. YSI's unique enzyme electrode technology provides for specific glucose measurements in the range of 0.005 to 0.900% w/v. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of chemical substances.

When configured with YSI 2357 buffer and the YSI 2365 glucose oxidase enzyme membrane, the YSI 2700 analyzer measures glucose after aspiration of just 25 microliters of sample. Some samples may need filtered; and for glucose concentrations that exceed 0.90% w/v (9.00 g/L), dilution of sample may be required. Results are displayed and printed. The sample is automatically flushed from the electrode chamber within 30 seconds after the displayed result and the YSI 2700 is ready to measure the next sample. Turn around time is under two minutes.

In the study described glucose was measured in filtered and diluted corn mash samples obtained from two fermentation stages of a production-scale bioethanol operation; one at 12-hours and the other at 24-hours after yeast addition. To demonstrate reliable performance in the sample matrix, both standard additions and precision studies were performed with these samples. Results are shown below. The advantage of this simple, easy to perform glucose measurement is that operators can perform in-process testing and make process adjustments to maximize ethanol yield.

II. Materials and Setup

Sample Collection

Collection container (250 ml flask)

Whatman #1 filter paper, funnel and 100 ml collection flask

Nylon syringe filter (0.22 micron); 3 ml luer lock syringe

Collection tube (5 ml, 75mm x 100mm - to collect filtrates)

YSI 2700S or 2700D, configured for glucose measurement

YSI 2700 Instrument Setup

See the YSI 2700 user's manual for general setup and safety information.

Chemistry electrode and reagent configuration depends on single or dual channel operation. The information below outlines glucose configuration for a single channel. If you have a 2700D version then assign 'None' to the electrode that you are not using when you set up the electrodes in the menu.

Under Menu choose Setup, and then choose MeasParam to access the electrode assignment configuration. Set or confirm the following parameters.

Sample Size	25 microliters
Cal Method	One Station

Black Probe Parameters

Chemistry	Glucose
Unit	% (w/v)
Calibrator	0.25%
End Point	30 seconds
CalStation#	1

White Probe Parameters

Chemistry	None
Unit	N/A
Calibrator	N/A
End Point	N/A
CalStation#	N/A

Autocal Parameters[1]

Sample Error	ON
Temperature	1°C
Time	60 Min[2]
Sample	0 Sam
Cal Shift	2%
Calibration Station	#1
Sample Station	#2 (or #4 if turntable)

III. Method

A. Calibrate the YSI 2700 by entering Run Mode or, if in Run Mode, by pressing the Calibrate key.

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.

D. When idle for more than 15 minutes during sampling, initiate a calibration to ensure greatest accuracy.

E. Collect about 100 ml of sample from the fermentation tank, ensuring a representative sample.

F. Mix samples well and pour through filter paper to capture particulates. Retain filtrate for additional filtration.

G. Draw about 3 ml of filtrate from F into the syringe, secure a 0.22 micron filter to the luer fitting and then dispense solution into a 5 ml tube.

H. Dilute filtrate as necessary to bring glucose concentration within the YSI analytical method range (0 – 9.00 g/L)[3].

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[1] Enter AutoCal menu from RunMode under Setup Menu.

[2] Calibration is automatically updated hourly. To ensure best accuracy, manually update calibration (press Calibrate key) a few minutes prior to measuring a sample.

[3] In this study a 1:20 dilution was made using reagent water.

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I. Immediately present the sample to Station #2 or #3 of the YSI 2700 for aspiration; and then record the glucose concentration when it is displayed.

J. Record the concentration, adjust with calculations as necessary, and compare to your reference range values.

IV. Calculations

Samples that were compensated for dilution must be back-calculated by the dilution factor.

Example: If you had a solution that you expect to be near 12% w/v glucose with the YSI 2700, you might consider a 20-fold dilution (1 ml sample; 19 ml water) to bring the value into reportable range. You would expect a number near 0.6 % w/v (12% ÷ 20). In this example let us assume the diluted sample now measures 0.62% w/v. Corrected for dilution, the undiluted sample concentration is 12.4% w/v (20 x 0.62%). Since 0.62% w/v is well within the 0 – 0.90% w/v glucose range, record the result.

V. Results

Two samples from a 48-hour yeast fermentation where collected and filtered. The samples were obtained at the 12-hour and 24-hour stages of fermentation where glucose consumption would be well underway. To demonstrate method performance, instrument precision (n=10) and standard additions (spike and recovery of known glucose addition) were performed. In the spikes 5 mg and 10 mg of glucose in water were delivered to each of two filtrate aliquots of 10 ml. The spiked concentrations were compared to the original filtrates to determine percent recovery.

YSI 2700 Precision for Unspiked Filtrate Samples

Samples 12-HR and 24-HR were selected for precision studies. Ten (10) replicates of each sample were performed. Results are shown in the table below.

Sample	Replicates	Mean % w/v	STD % w/v	CV (%)
12-HR	10	14.04	0.198	1.41%
24-HR	10	4.52	0.025	0.55%

The standard deviation (STD) was determined for each replicate series. YSI precision results fall within the YSI-specified limits of 2% CV or 0.002% w/v, whichever the greater limit.

Percent Recovery of Spiked Samples

A glucose spike standard of 100 g/L (100 mg/ml) was prepared in reagent water using high purity D-Glucose. The solution was mixed for a minimum of 4 hours at room temperature to allow for mutarotational equilibrium. Spikes of 5 mg and 10 mg glucose were delivered by pipet (50 uL & 100 uL) into 10 ml volumetric flasks. Sample filtrate was added to the 10 ml mark to produce the spiked solutions. Original solutions (before spike) and spiked solutions were measured using the YSI 2700. Results are displayed in the table below and percent recoveries calculated.

All diluted samples were back-calculated by the 20-fold dilution factor and results are expressed in concentration units of glucose % w/v. These units would be equivalent to g/100 ml.

Sample	Spike, µl	Unspiked*	Spiked*	Calculated	Recovery
12-HR	50	14.09	14.98	15.02	99.7%
12-HR	100	13.79	15.68	15.66	100.2%
24-HR	50	4.50	5.45	5.48	99.4%
24-HR	100	4.48	6.47	6.43	100.5%

*Glucose values in % w/v units

VI. Discussion

Glucose is the primary simple sugar that results from the enzymatic hydrolysis of starch and therefore the main carbon source for yeast in the production of ethanol in fermentation. In typical corn bioethanol production, the consumption of glucose over the course of 24 to 48 hour yeast fermentation is a common method of monitoring ethanol production efficiency. Although ethanol measurement is also important, glucose concentration profiles, and sometimes glucose consumption rates, are often more easily measured and are of great importance early in the fermentation process when adjustments to process can be made. In addition, a set glucose concentration at the end of fermentation is often the signal to move the 'beer' to distillation.

HPLC is the analytical method of choice to measure not only glucose, but also dextrins, maltose, glycerol, acetic acid, lactic acid, and ethanol. All have relevant meaning to measuring efficiency of the bioethanol production process. However, the YSI 2700 has proven to be a valuable tool in monitoring glucose during fermentation. The enzyme-electrode based system not only provides rapid, accurate glucose measurements (sometimes 30 minutes before an HPLC result) but also is simple to use and easy to maintain. Results are available earlier than HPLC and allow operators to make adjustments when they count.

In the data presented in this study performance of the method indicates precision and accuracy that is comparable to HPLC. Precision of samples studied proved to be better than 2%. Percent recoveries were < +/- 1% showing that glucose measured by the YSI 2700 was not affected by metabolic by-products or the ethanol concentrations present during fermentation. Once filtered (and diluted as necessary), the sample was presented to the instrument and glucose results were displayed and printed in one minute. Total turn-around time was less than two minutes. The YSI 2700 automatically updates calibration on a user-defined schedule, or may be initiated at the user's command.

In conclusion the YSI 2700 used in glucose monitoring provides valuable information relevant to maximizing ethanol production. The measurements allow the operators to make timely decisions thus improving plant efficiency and reducing costs.

VII. Ordering Information

YSI Part Numbers:

2700S (or 2700D) Biochemistry Analyzer
2365 Glucose Oxidase Membrane Kit
2357 Buffer Kit
2776 Glucose/Lactate Calibrator (2.50 g/L glucose)
1531 Glucose Linearity Test Standard (9.00 g/L)
2363 Potassium Ferrocyanide Test Solution
2392 NaCl Solution (for membrane installation)