In Situ Real-Time Monitoring of a Fermentation Reaction Using a Fiber-Optic FT-IR Probe

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The fermentation of sucrose was monitored in real time using a midinfrared fiber-optic probe. A partial least squares treatment of the resulting spectra is presented.

With the development and commercialization of a wide range of biotechnology processes, the need for simple, robust, and adaptable methods of monitoring the progress of fermentation reactions has been widely recognized. Preferably, the chosen monitoring methods will be capable of integration into process control systems. Thus, real-time methods with little or no sample removal or preparation are needed.

In a 1986 study of the off-line use of Fourier transform–infrared (FT-IR) spectroscopy to monitor substrate and product concentrations in a fermentation reactor, Alberti and co-workers (1) demonstrated the feasibility of using FT-IR to monitor the reactions in a batch *Saccharomyces cerevisiae* fermentation using a glucose substrate. By using attenuated total reflectance (ATR) in a Barnes Circle cell configuration, they obtained quantitative results in a cloudy media that would be intractable using transmission methods. They also showed that it is possible to use partial least squares (PLS) curve fitting for multivariate analysis to quantitate substrate and product sugars and alcohols. In 1989, Sadeghi-Jorabchi and co-workers (2) discussed the application of FT-IR using ATR techniques to monitor fermentation technology and fat processing. They showed that FT-IR could be used to measure the concentration of sucrose, glucose, and fructose during fermentation, and confirmed the observed trends by enzymatic analysis. Fairbrother and co-workers (3) used FT-IR for on-line analysis of lactose and lactic acid in their 1991 paper on whey fermentation. Like Alberti and co-workers, they used a Barnes Circle cell, and they acknowledged that a remote-sensing device such as a fiber-optic probe would allow for true in situ monitoring of fermentation reactions.

A problem with the work cited above is that samples have to be removed from the batch reactor and pumped into or through the Circle cell used in each case. This means modifying the reactor and potentially perturbing the reaction. In the case of cloudy media such as fermentation broths, it also involves the possibility of fouling or blockage in the sampling apparatus. True in situ reaction monitoring, without the need to modify the reactor, requires a sampling device that can be placed inside a reactor with minimal or no changes to the reactor geometry. Such a capability is provided by the fiber-optic probe with an ATR crystal attached, as was used in our study.

MATERIALS AND METHODS

Chemicals. We obtained sucrose, fructose, glucose, and ethanol from Aldrich Chemical Co. (Milwaukee, WI) and used commercial, household-grade *S. cerevisiae*.

Fermentation. We prepared a 20% (w/v) solution of sucrose in water and maintained it at room temperature (approximately 20 °C) with continuous stirring at 200 rpm. After acquisition of initial spectra from the sucrose solution, *S. cerevisiae* (0.5 g) was added, and the solution was stirred at room temperature for 36 h.

Analytical method. We used a Remspec (Sturbridge, MA) fiber-optic ATR probe and detector module coupled with a Vector 22 FT-IR spectrometer (Bruker, Billerica, MA) to obtain spectra during the fermentation. The zinc selenide ATR crystal at the end of the probe was immersed in the fermentation medium. Three spectra were collected from the starting sucrose solution before the addition of the yeast, and then spectra were collected at intervals of 1 min for a total of 33.33 h as the fermentation progressed. We collected each spectrum at the rate of 3.5 scans/s and a resolution of 4 cm^{-1} using 100 scans for each spec-

Table I. Actual and predicted values of components in solutions included in the PLS1 training set.

Fructose			Glucose			Ethanol			Sucrose		
Actual	Predicted	Error	Actual	Predicted	Error	Actual	Predicted	Error	Actual	Predicted	Error
1.00	0.90	0.10	1.00	1.07	0.07	1.00	1.01	0.01	1.00	1.07	0.07
2.00	2.01	0.01	2.00	2.06	0.06	2.00	2.17	0.17	4.00	3.92	0.08
2.50	2.49	0.01	2.50	2.49	0.01	2.50	2.50	0.00	7.50	7.44	0.06
5.00	4.98	0.02	5.00	4.97	0.03	5.00	5.17	0.17	13.00	12.86	0.14
7.50	7.48	0.02	7.50	7.45	0.05	7.50	7.59	0.09	16.50	16.51	0.01
10.00	9.95	0.05	10.00	9.82	0.18	10.00	9.90	0.10	20.00	20.11	0.11
1.00	0.94	0.06	1.00	0.86	0.14	1.00	1.02	0.02	20.00	19.74	0.26
1.00	1.05	0.05	1.00	0.98	0.02	10.00	9.92	0.08	1.00	0.94	0.06
1.00	0.90	0.10	10.00	10.23	0.23	1.00	0.92	0.08	1.00	1.06	0.06
10.00	10.03	0.03	1.00	1.01	0.01	1.00	0.93	0.07	1.00	0.91	0.09
1.00	1.13	0.13	1.00	1.01	0.01	1.00	1.09	0.09	16.50	15.95	0.55
1.00	1.03	0.03	1.00	1.00	0.00	7.50	7.48	0.02	1.00	0.97	0.03
1.00	1.06	0.06	7.50	7.45	0.05	1.00	1.11	0.11	1.00	1.09	0.09
7.50	7.52	0.02	1.00	0.96	0.04	1.00	0.87	0.13	1.00	1.04	0.04
1.00	1.04	0.04	1.00	1.06	0.06	1.00	1.01	0.01	13.00	13.24	0.24
1.00	1.11	0.11	1.00	1.07	0.07	5.00	4.98	0.02	1.00	1.00	0.00
1.00	0.97	0.03	5.00	5.09	0.09	1.00	1.01	0.01	1.00	1.02	0.02
5.00	5.02	0.02	1.00	1.03	0.03	1.00	1.05	0.05	1.00	1.00	0.00
1.00	1.03	0.03	1.00	0.98	0.02	1.00	0.90	0.10	7.50	7.48	0.02
1.00	0.91	0.09	1.00	1.09	0.09	2.50	2.63	0.13	1.00	1.02	0.02
1.00	1.06	0.06	2.50	2.50	0.00	1.00	1.02	0.02	1.00	1.02	0.02
2.50	2.43	0.07	1.00	0.95	0.05	1.00	0.91	0.09	1.00	1.00	0.00
1.00	1.09	0.09	1.00	0.94	0.06	1.00	0.97	0.03	4.00	4.14	0.14
1.00	0.97	0.03	1.00	1.02	0.02	2.00	2.18	0.18	1.00	1.15	0.15
1.00	1.02	0.02	2.00	2.03	0.03	1.00	1.07	0.07	1.00	1.06	0.06
2.00	2.06	0.06	1.00	0.96	0.04	1.00	1.01	0.01	1.00	1.06	0.06
5.00	4.63	0.37	1.00	0.95	0.05	1.00	0.52	0.48	20.00	20.68	0.68
7.50	7.11	0.39	2.00	1.75	0.25	10.00	10.17	0.17	1.00	1.48	0.48
10.00	9.77	0.23	2.50	1.95	0.55	7.50	7.39	0.11	4.00	4.51	0.51
1.00	0.68	0.32	5.00	4.66	0.34	5.00	4.90	0.10	7.50	7.00	0.50
2.00	2.14	0.14	7.50	7.45	0.05	2.50	2.48	0.02	13.00	13.25	0.25
2.50	2.21	0.29	10.00	10.20	0.20	2.00	2.01	0.01	16.50	17.01	0.51
1.00	0.88	0.12	5.00	5.14	0.14	1.00	0.84	0.16	20.00	20.05	0.05
2.00	2.07	0.07	7.50	7.45	0.05	10.00	9.88	0.12	1.00	0.93	0.07
2.50	2.46	0.04	10.00	10.06	0.06	7.50	7.57	0.07	4.00	3.98	0.02
5.00	4.97	0.03	1.00	0.95	0.05	5.00	5.01	0.01	7.50	7.50	0.00
7.50	7.54	0.04	2.00	1.98	0.02	2.50	2.69	0.19	13.00	12.98	0.02
10.00	10.05	0.05	2.50	2.49	0.01	2.00	1.88	0.12	16.50	16.58	0.08
1.00	0.99	0.01	1.00	1.13	0.13	5.00	4.14	0.86	20.00	20.03	0.03
2.00	2.02	0.02	10.00	10.05	0.05	7.50	7.44	0.06	1.00	1.27	0.27
2.50	2.47	0.03	7.50	7.45	0.05	10.00	9.93	0.07	4.00	3.93	0.07
5.00	4.90	0.10	5.00	5.10	0.10	1.00	0.89	0.11	7.50	7.56	0.06
7.50	7.55	0.05	2.50	2.46	0.04	2.00	1.99	0.01	13.00	12.86	0.14
10.00	9.93	0.07	2.00	1.95	0.05	2.50	2.52	0.02	16.50	16.85	0.35
1.00	0.96	0.04	1.00	1.03	0.03	10.00	9.97	0.03	13.00	13.03	0.03
2.00	1.95	0.05	10.00	10.16	0.16	1.00	1.12	0.12	16.50	16.43	0.07
2.50	2.59	0.09	7.50	7.53	0.03	2.00	2.03	0.03	20.00	20.46	0.46
5.00	4.96	0.04	5.00	5.11	0.11	2.50	2.49	0.01	1.00	0.75	0.25
7.50	7.44	0.06	2.50	2.49	0.01	5.00	4.87	0.13	4.00	3.98	0.02
10.00	9.96	0.04	2.00	1.98	0.02	7.50	7.53	0.03	7.50	7.50	0.00

Fructose			Glucose			Ethanol			Sucrose		
Actual	Predicted	Error	Actual	Predicted	Error	Actual	Predicted	Error	Actual	Predicted	Error
2.00	2.04	0.04	10.00	9.98	0.02	2.50	2.49	0.01	7.50	7.47	0.03
1.00	1.00	0.00	7.50	7.46	0.04	2.00	2.09	0.09	13.00	12.90	0.10
10.00	10.04	0.04	5.00	4.98	0.02	1.00	1.03	0.03	16.50	16.50	0.00
7.50	7.49	0.01	2.50	2.62	0.12	10.00	8.36	1.64	20.00	20.36	0.36
5.00	4.84	0.16	2.00	1.82	0.18	7.50	7.27	0.23	1.00	0.83	0.17
2.50	2.44	0.06	1.00	1.09	0.09	5.00	4.96	0.04	4.00	4.05	0.05
10.00	10.03	0.03	2.00	1.92	0.08	2.50	2.53	0.03	7.50	7.45	0.05
7.50	7.49	0.01	1.00	0.96	0.04	2.00	2.06	0.06	13.00	12.73	0.27
5.00	5.02	0.02	10.00	9.93	0.07	1.00	1.00	0.00	16.50	16.31	0.19
2.50	2.47	0.03	7.50	7.61	0.11	10.00	9.85	0.15	20.00	20.09	0.09
2.00	2.04	0.04	5.00	5.09	0.09	7.50	7.52	0.02	1.00	1.19	0.19
1.00	0.98	0.02	2.50	2.45	0.05	5.00	4.99	0.01	4.00	3.95	0.05
10.00	9.96	0.04	2.50	2.50	0.00	2.00	1.88	0.12	7.50	7.47	0.03
7.50	7.53	0.03	2.00	1.99	0.01	1.00	1.01	0.01	13.00	12.99	0.01
5.00	5.02	0.02	1.00	1.13	0.13	10.00	9.97	0.03	16.50	16.57	0.07
2.50	2.69	0.19	10.00	9.77	0.23	7.50	5.85	1.65	20.00	20.15	0.15
2.00	2.03	0.03	7.50	7.44	0.06	5.00	5.05	0.05	1.00	0.78	0.22
1.00	1.04	0.04	5.00	5.04	0.04	2.50	2.52	0.02	4.00	3.97	0.03
10.00	9.99	0.01	2.50	2.62	0.12	2.00	1.99	0.01	7.50	7.59	0.09
7.50	7.54	0.04	2.00	2.01	0.01	1.00	0.84	0.16	13.00	13.05	0.05
5.00	4.95	0.05	1.00	0.90	0.10	10.00	10.13	0.13	16.50	16.39	0.11
2.50	2.43	0.07	10.00	10.10	0.10	7.50	5.74	1.76	20.00	20.39	0.39
2.00	1.99	0.01	7.50	7.48	0.02	5.00	4.99	0.01	1.00	0.92	0.08
1.00	0.99	0.01	5.00	5.03	0.03	2.50	2.58	0.08	4.00	4.07	0.07
2.00	2.01	0.01	7.50	7.44	0.06	5.00	4.86	0.14	16.50	16.38	0.12
2.50	2.48	0.02	5.00	5.01	0.01	2.50	2.47	0.03	20.00	19.88	0.12
5.00	4.96	0.04	2.50	2.44	0.06	2.00	2.02	0.02	1.00	0.75	0.25
7.50	7.46	0.04	2.00	2.00	0.00	1.00	0.91	0.09	4.00	3.89	0.11
10.00	9.96	0.04	1.00	0.82	0.18	7.50	7.69	0.19	7.50	7.47	0.03
1.00	0.92	0.08	10.00	10.32	0.32	10.00	10.12	0.12	13.00	12.94	0.06
7.50	7.56	0.06	2.00	1.98	0.02	5.00	4.90	0.10	16.50	16.01	0.49
5.00	4.91	0.09	2.50	2.56	0.06	2.50	2.34	0.16	20.00	20.21	0.21
2.50	2.46	0.04	5.00	4.94	0.06	2.00	2.10	0.10	1.00	1.13	0.13
2.00	2.03	0.03	7.50	7.34	0.16	1.00	1.13	0.13	4.00	4.07	0.07
1.00	1.04	0.04	10.00	9.77	0.23	7.50	7.43	0.07	7.50	7.27	0.23
10.00	9.98	0.02	1.00	1.00	0.00	10.00	10.05	0.05	13.00	13.10	0.10
7.50	7.47	0.03	5.00	4.97	0.03	2.00	2.22	0.22	16.50	16.43	0.07
5.00	5.06	0.06	2.50	2.57	0.07	2.50	2.42	0.08	20.00	19.99	0.01
2.50	2.57	0.07	2.00	1.98	0.02	5.00	4.85	0.15	1.00	1.07	0.07
2.00	1.95	0.05	1.00	0.83	0.17	7.50	7.48	0.02	4.00	4.03	0.03
1.00	1.05	0.05	7.50	7.51	0.01	10.00	10.06	0.06	7.50	7.45	0.05
10.00	10.02	0.02	10.00	10.04	0.04	1.00	1.05	0.05	13.00	12.70	0.30
7.50	7.44	0.06	5.00	4.94	0.06	7.50	7.45	0.05	4.00	4.04	0.04
5.00	5.01	0.01	2.50	2.50	0.00	10.00	9.98	0.02	7.50	7.56	0.06
2.50	2.57	0.07	2.00	2.20	0.20	1.00	0.94	0.06	13.00	12.69	0.31
2.00	1.95	0.05	1.00	1.06	0.06	2.00	1.98	0.02	16.50	16.44	0.06
1.00	0.98	0.02	7.50	7.48	0.02	2.50	2.30	0.20	20.00	20.04	0.04
10.00	10.00	0.00	10.00	9.93	0.07	5.00	5.09	0.09	1.00	0.93	0.07

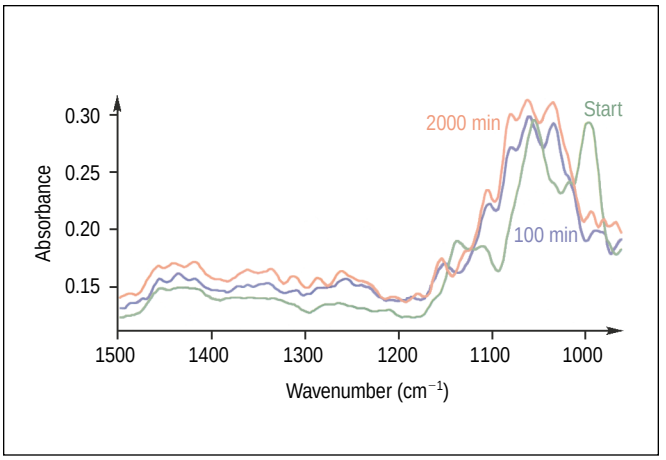

Figure 1. Comparison of spectra at the start of the experiment at 100 min and 2000 min.

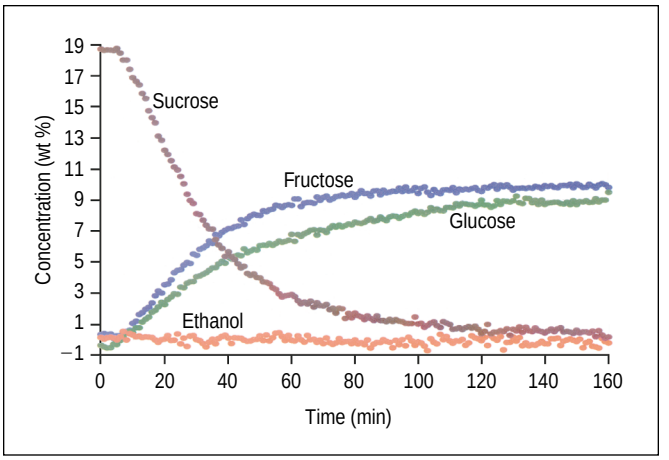

Figure 2. Time concentration profile (160 min).

trum (that is, collecting each spectrum for approximately 30 s).

Analysis. Figure 1 shows spectra in the region of 960–1500 cm^{-1} at 0, 100, and 2000 min from the addition of the yeast to the reactor. Changes in the spectrum are obvious, particularly as the sucrose peak centered at approximately 1000 cm^{-1} disappears. However, because of the strongly overlapping nature of the spectra, a simple peak area analysis is not possible.

Fairbrother and co-workers (3) gave a good, simple description of the considerations that need to be included in developing an FT-IR quantitative analysis using PLS methods. They worked with a whey fermentation in which complex mixtures of two components — lactose and lactic acid — were analyzed. In our study, three possible fermentation intermediates and products were included in the standard set of samples used for calibration: glucose, fructose, and ethanol. This gave a calibration set based on four components when the sucrose itself was included.

We determined from a factorial design that a matrix of 98 standards would generate a robust model. Sets of standard aqueous starting solutions of each component were prepared, and these were used to prepare a total of 98 standard solutions as shown in Table I. We prepared samples by mixing 1-mL aliquots of the appropriate starting solution for each component. (Note that the concentrations used for the sucrose standards were different from those used for the other three components because of differences in solubility. The integrity of the calibration was not affected.)

Calibration method. The 2000 spectra that we acquired from the fermentation experiment were transferred to Grams 32 (Thermo Galactic, Salem, NH) to build a model using PlusPlus/IQ. Two models were used: one for sucrose, fructose, and glucose, and one for ethanol. In each case, we removed undesirable variations using the mean center and autobaseline methods. The training sets were built with PLS1 calibration and cross-validation diagnostics, using three files out of rotation. Factors for the modelswereobtained by the leave-one-cross-validation technique (Table II).

Once the model was built using PLS/IQ (Thermo Galactic), the program REMPLS was used to predict the experimental data. REMPLS is particularly convenient for predicting very large data sets because it provides a simple table of concentration vs. time that can be conveniently analyzed using conventional spreadsheet techniques.

RESULTS AND DISCUSSION

Figure 2 shows the changes in concentration for four components of the fermentation mixture as it evolved during the first 160 min of the experiment. After an initial

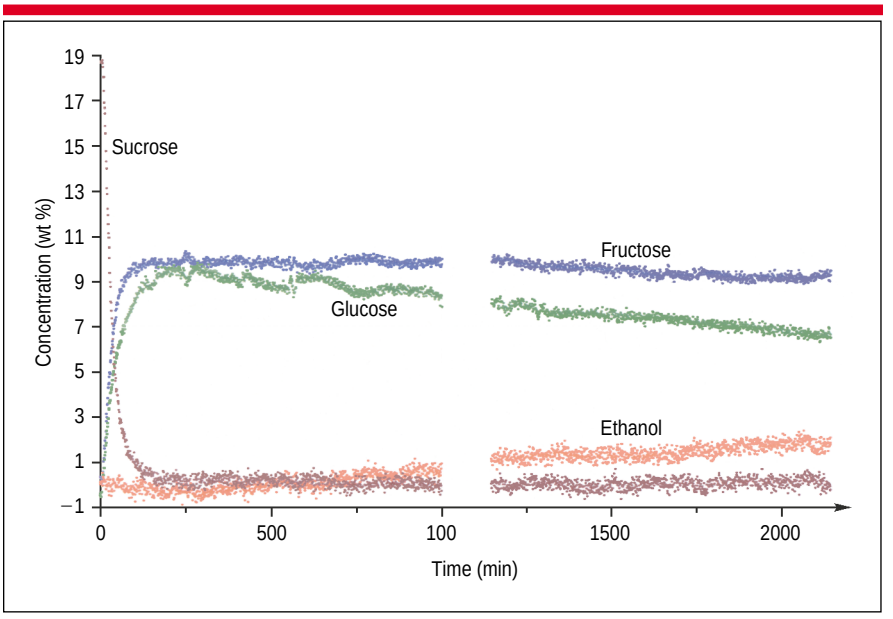

Figure 3. Time concentration profile (36 h).

Table II. Factors, correlation coefficients, and spectral regions for solution components.

Component	Factor	Correlation Coefficient	Spectral Regions
Sucrose	10	0.9994	3020–2900 cm^{-1}, 1500–930 cm^{-1}
Fructose	13	0.9985	3020–2900 cm^{-1}, 1500–930 cm^{-1}
Glucose	11	0.9983	3020–2900 cm^{-1}, 1500–930 cm^{-1}
Ethanol	14	0.998	2999–2875 cm^{-1}, 1120–999 cm^{-1}

induction period of approximately 7 min the sucrose concentration dropped rapidly. The concentrations of fructose and glucose rose correspondingly from zero until, after approximately 120 min, the sucrose concentration had dropped almost to zero, while the fructose and glucose concentrations leveled off at approximately 10 wt% and 7 wt%, respectively.

Figure 3 shows the changes in concentration that were observed when the fermentation was run for 36 h (2160 min). After about 250 min the glucose concentration began to drop slowly, while traces of ethanol began to be observed.

After 1500 min the glucose concentration dropped by about 2 wt%, and the ethanol concentration rose by a corresponding amount. Fairbrother and coworkers (3) compiled a list of the advantages of FT-IR spectroscopy for monitoring the progress of fermentation reactions that can be summed up as speed, simplicity, and versatility. The use of the fiber-optic ATR probe adds to these advantages by eliminating the need to use a sampling system such as the Circle cell, so that the method can be adapted for use in virtually any reactor without special adaptations and without perturbing the reaction conditions. The result is a method that enables true in situ monitoring of the fermentation in real time, using techniques and software that can be adapted easily to automatic or semiautomatic use and to process control applications.

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