Multivariate Approach for the Simultaneous Determination of Total Biomass and Glucose from a Yeast Fermentation by Sequential Injection Analysis

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Key words: biomass, glucose, yeast fermentation, sequential injection analysis, bioprocess monitoring

A simultaneous determination of total biomass and glucose was performed using a simple sequential injection system with the full range of visible absorbance spectrum as the analytical signal. The assays were performed on-line with no pre-treatment of the sample. In the past, measurements on unfiltered bioprocess samples have been avoided due to anticipated problems such as cell aggregation, clogging of the flow system, cell adhesion to tubing walls and cross contamination. The sample was diluted, mixed with the glucose reagent in a stirred cell and the resulting signal was collected as a function of time. Calibration models were determined using linear partial least squares regression and tested by a cross-validation. The average difference between the model's prediction of total biomass and the dry weight determination was 5 %. The glucose model lacked quantitative predictive ability but could identify pulse spikes in the fermentation broth. The sampling frequency was 7 samples/h.

Jednoczesne oznaczanie całkowitej biomasy i glukozy przeprowadzono w prostym sekwencyjnym układzie wstrzykowym, z pełnym zakresem widma absorpcyjnego jako sygnałem analitycznym. Oznaczenia wykonywano on-line bez wstępnego przygotowania próbek. Poprzednio unikano wykonywania pomiarów w niefiltrowanych próbkach z bioprocessów, ze względu na możliwość agregacji komórek, zatkanie się układu, adhezie komórek do ścianek przewodów i w konsekwencji przenoszenie błędu kontaminacji. Próbkę rozcieńczano, mieszało z odczynnikiem do oznaczania glukozy i rejestrowano sygnał analityczny w funkcji czasu. Modele kalibracyjne wyznaczono stosując liniową cząstkową regresję metody najmniejszych kwadratów i sprawdzono je w próbie krzyżowej. Średnia różnica między zawartością całkowitej biomasy przewidywaną przez model a oznaczeniem suchej masy wynosiła 5 %. Model glukozy nie pozwalał na przewidywanie ilościowe, lecz umożliwiał identyfikację sygnału w brzeczce fermentacyjnej. Częstotliwość próbkowania wynosiła 7 próbek na godzinę.

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Two common themes in bioprocess literature are the measurement of biomass, [1–3] and glucose [4, 5]. Total biomass is an important parameter that is often used as a measure of product formation, substrate consumption and process disturbances [6]. More precise control of the bioprocess can be obtained by rapid and frequent measurement of significant parameters such as total biomass and glucose. Most total biomass determinations are indirect, based on in situ measurements of dissolved oxygen uptake or carbon dioxide evolution [6]. The traditional direct determination of biomass by measurement of dry weight is both time and labor intensive and is not suitable for on-line bioprocess control. Other methods such as total wet biomass and turbidimetry are much faster but less accurate [3, 7]. Recently, we reported a simple univariate assay for total biomass in a yeast fermentation using both 90° and 180° scattered light at 670 nm as the analytical signal [8]. Not only does this assay provide information on the total biomass in the fermentation on-line, a linear model built using one fermentation can be used to predict the total biomass in a subsequent run.

A second parameter is the concentration of the nutrient carbon source in the fermentation broth. Glucose is one of the most common nutrient carbon sources used. Glucose is traditionally monitored in filtered fermentation samples using an enzymatic/colorimetric reagent such as Trinder [9]. This assay requires 20 minutes for sample preparation and color development and uses single wavelength detection. An alternate method uses a biosensor with glucose oxidase embedded in a membrane. Both manual methods used involve at least one enzymatic reaction and rely upon formation of hydrogen peroxide which is then directly related to the concentration of glucose in the filtered broth. This method also uses single wavelength detection. The trade-off is evidently speed vs. accuracy.

Amperometry is probably the most often used method of detection for glucose biosensors. Nearly all methods are based on electron exchange from the anodic oxidation of hydrogen peroxide produced by the enzymatic reaction of GOx [10]. The YSI glucose analyzer is a very popular manual system for glucose measurement. It is, in fact, the only successful commercially available biosensor. Once again, the method relies on the generation of hydrogen peroxide via the GOx reaction:

\[
\text{glucose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + \text{H}_2\text{O}_2
\]
\[
\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-
\]

The electrons generated are then measured by the sensing electrode. This is known as a Clark electrode [11] and it is this principle that is used in the YSI glucose analyzer.

An in situ electrode can be constructed by immobilizing the GOx onto the surface of an electrode through a Schiff base [12]. An autoclavable in situ glucose electrode was developed by Brooks et al. This allowed for continuous monitoring of a fermentation without the possibility of contamination by introducing the electrode to the fermentation broth after sterilization [13]. They improved the enzyme immobilization by using a periodate-oxidized GOx and used alkylamine coating on the electrode.

For a FIA system, the GOx is either immobilized onto controlled pore glass by glutaraldehyde or sandwiched between two dialysis membranes. The GOx sensor is then attached to the sensing electrode [12] with an o-ring. Several other groups have used this method, improving on it by producing a microthin sensing layer [14],
automating the measurement to control glucose levels in a fermentation [4, 15] or by using a wall-jet-electrode, which has a fast response time, good sensitivity and a low dead volume [16]. In another method, the GOx was immobilized into graphite paste modified with the electroactive material tetracyanoquinodimethane (TCNQ). The TCNQ acts as an efficient mediator for oxidation of the reduced GOx [10].

Regardless of the advances made in the area of biotechnology, there still remains a need for an analytical method that can quickly measure or estimate the total biomass and carbon source of a system [2, 3]. It should have a wide dynamic range and a high linearity of response. Ideally, the method should sample the bioprocess directly and eliminate or minimize preparation for analysis allowing for continuous unattended real-time monitoring. Recently, a method for the determination of total biomass using sequential injection was developed [8] and this method was extended by full spectrum multivariate analysis to determine glucose simultaneously.

The total biomass/glucose assay developed in our laboratory used as a platform an aerobic fermentation of *Saccharomyces cerevisiae*. The assay accurately dilutes an unfiltered fermentation sample and collects the visible spectrum after incubation with the Trinder reagent. Although single wavelength detection has proven to be adequate for biomass monitoring [8], the glucose cannot be adequately measured in the presence of the biomass (unpublished results).

**EXPERIMENTAL**

Sequential injection analyzer

A schematic diagram of the sequential injection (SI) system is shown in Fig. 1. Figure 2 shows how the SI unit fits into the fermentation unit. The SI system consisted of a peristaltic pump (Model C4V, Alitea USA, Medina, WA) coupled to an electrically actuated 10-port selection valve (Model SD10P, Valco Instruments, Houston, TX).

![Schematic diagram of the sequential injection manifold](image)

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**Figure 1.** Schematic diagram of the sequential injection manifold that was used for this work. The peristaltic pump (Q), 10 position multi-port valve (MV) and data acquisition system are all computer controlled via QuickBASIC. HC = holding coil (75 cm), LS = light source, D = detector, S = sample, C = carrier (Trinder reagent), FO: fiber optic bundles. See text for full description of the components and Table 2 for other symbol definitions. Tubing diameter: 0.8 mm, carrier flow rate: 2.0 ml min⁻¹, $V_m$: 1.7 ml
Fermentor

Silicone tubing (3 m)

Circulating line

T-piece

Teflon tubing (11 cm)

Nonporous tubing

High-flow pump 320 ml/minute

To SI analyzer

Figure 2. Schematic diagram of the fermentation unit and the circulation line. A high flow peristaltic pump circulated fermentation broth at 320 ml min⁻¹ and the small peristaltic pump in the sequential injection analyzer would sample from this constantly flowing stream through the tee piece.

The valve was equipped with 1/16" fittings. All connections and plumbing were made with 0.8 mm i.d. Teflon tubing and fittings (Upchurch Scientific, Oak Harbor, WA). The pump tubing was Viton No. 13 (Cole Parmer, Chicago, IL) and was connected to the Teflon tubing with flow injection analysis (FIA) tubing adapters (Upchurch Scientific, Oak Harbor, WA). A flow cell served as both the reactor and detector vessel and has been described elsewhere [8]. The detector was a modified Hewlett-Packard Model HP8452A Diode Array Spectrophotometer (DAS) (Hewlett-Packard, Los Angeles, CA). Modification involved placing an optical unit in the sample chamber to direct the signal from the flow cell to the diode array through a fiber optic bundle. An external light source was necessary due to the large light loss realized through the fiber optic bundles. The light source contained a halogen projector lamp and was built in-house. The HP8452 QuickBASIC programming library was included in the control software. It was modified to remove the header information so that the data was directly compatible with both MatLab (version 4.0, The MathWorks Inc., Natick, MA) and Pirouette (version 1.2, Infometrix, Seattle, WA) in ASCII format before saving to disk. The HP library was also used for selecting other parameters, including integration time (1 s), spectra collection time (4 s), spectral resolution (2 nm) and range (402–800 nm) and shutter operations (open).

The pump and valve were controlled by a data acquisition board (Model ADA-1100, Real Time Devices, State College, PA) from an IBM compatible computer (Model 486DX, Comtrade, City of Industry, CA). The control software was written in-house by the authors in QuickBASIC (version 4.5, Microsoft, Redmond WA) and controlled the movements of the pump and valve as well as call the spectrometer to collect and save data. All data analysis was done in MatLab and Pirouette.

Glucose-biomass assay

The assay is initiated by rinsing the flow cell at a flow rate of 2.0 ml min⁻¹ for 25 s with the Trinder reagent that served as the carrier solution.

Next, the valve rotates to the sample position and pulls enough sample to fill the sample line (200 µl). At this point, a blank signal is collected from the spectrophotometer. The valve then rotates to the auxiliary waste port to rinse any sample that may be in the holding coil (500 µl). The system is now ready to accept the sample from the fermentation unit and the valve rotates to the sample port again and draws 50 µl of a fermentation sample into the holding coil. Next, the sample is propelled to the dilution flow cell (235 µl) and the flow and stirring is stopped for 3.5 minutes (the incubation period for the Trinder reaction). At the end of the incubation period, the flow and stirring resume for 20 s (650 µl) in order to dilute the sample sufficiently such that the signal will not be too high for the detector. The flow and stirring are stopped again and four spectra are collected over the range of 402–800 nm (with 2 nm resolution). Finally, the flow and stirring are restarted and the flow cell is rinsed with 6 ml (3.5x the
Sequential injection analysis in total biomass and glucose monitoring

volume of the flow cell) of carrier to prepare it for the next sample. The entire assay for both total biomass and glucose is complete in less than 8 min. The assay was performed every 30 min for the first 32 h of the fermentation and every hour thereafter on-line for the duration of the fermentation run. Three sequential fermentations (numbered I, II and III) were used in the presentation of the assay for this paper.

Fermentation

Three liters of fermentation broth were prepared using the recipe in Table 1. The glucose feed, water for the acid and base feeds, the fermentor vessel containing the broth and all tubing lines were autoclaved for 45 min at 121°C. Upon cooling, the appropriate amount of acid and base were added to their respective flasks. The fermentation vessel was cooled to 30°C before inoculation. The pH was maintained at 4.5 by the automated acid and base feeds. The glucose feed was automated through a program written in-house for the Bioflo III 5 L fermentation unit (New Brunswick Scientific, Edison, NJ). Acid, base and glucose feed rates as well as agitation, temperature and %O₂ were recorded and saved to disk.

Table 1. Parameters of fermentation process

<table>
<thead>
<tr>
<th>Fermentation broth</th>
<th>Feeds</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>glucose</td>
<td>50%(m/m)</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>NH₃ sq</td>
<td>20%(V/V)</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>H₂SO₄</td>
<td>10%(V/V)</td>
</tr>
<tr>
<td>MgSO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antifoam</td>
<td>glucose feed</td>
<td>0–30 g l⁻¹</td>
</tr>
</tbody>
</table>

Samples for the manual determination of total biomass and glucose concentrations were collected automatically into 60 ml poly(tetrafluoroethylene) (PTFE) bottles at the time of inoculation (time zero) and every two hours thereafter by a refrigerated autosampler designed in our laboratory. Typically, fermentations would run from 56 to 72 h before one of the feeds was exhausted. Samples were obtained directly from a fed-batch aerobic fermentation of *Saccharomyces cerevisiae* and were not washed, filtered or otherwise pre-treated prior to the assay.

Chemicals

Yeast extract, agar and peptone were obtained from Difco (Detroit, MI). All other chemicals were reagent grade or higher and used without further purification. All solutions were prepared in 17.6 MΩ cm⁻¹ deionized water (Barnstead NANOpure II, Dubuque, IA). The carrier solution was Trinder reagent which was obtained from Sigma (cat. no. 315-500, St. Louis, MO) and prepared per manufacturer’s instructions.

Dry weight measurements

The total biomass was determined by the dry weight method [17, 18]. Five or ten ml of fermentation sample was accurately delivered to a tared centrifuge tube and the weight recorded. The sample was centrifuged for 3 min and the broth saved in a separate vial for manual glucose measurement. The biomass pellet was spun again to insure all of the broth was removed. The pellet was resuspended in distilled water and centrifuged 2 or 3 times. The final washed pellet was resuspended in water and poured into a tared aluminum weighing pan, dried at 85°C for 20–24 h, cooled to ambient temperature and weighed to give the dry weight. All measurements were performed in duplicate and the average reported.

Glucose assay

Glucose concentration was determined independently using the Trinder reagent and standard addition. The standard addition was necessary due to the strong matrix effect of the fermentation broth. The standards were prepared by diluting a stock solution of 100 g l⁻¹ glucose and their concentrations confirmed on a Glucose Analyzer (YSI Model 27, Yellow Springs Instrument Co., Yellow Springs, OH).
The analyzer was calibrated with a blank and a 200 mg dl\(^{-1}\) standard purchased from YSI (part no. 2355). Linearity was confirmed with a 500 mg dl\(^{-1}\) standard (part no. 2356). Standard (100 \(\mu\)l) and fermentation sample with biomass removed by centrifugation (200 \(\mu\)l) were combined with 2.0 ml Trinder reagent and the absorbance at 504 nm was recorded after a seven minute incubation at room temperature (23\(^{\circ}\)C).

**Multivariate analysis**

A set of fermentation samples were taken for manual determination of total biomass (dry weight method) and glucose (Trinder with standard addition). This calibration set was used to develop partial least squares (PLS) models for calculating the total biomass and glucose concentrations in the unknown set. Before the spectra were analyzed, they were mean-centered and smoothed with a 25 point smoothing function available within the Pirouette software package.

**RESULTS AND DISCUSSION**

**Determination of flow cell volume**

The theory governing the relationship between peak height and width in a flow injection system using a gradient flow chamber has already been established [19], and in detail by Tyson [20–23] and Pardue and Fields [24, 25]. Garn et al. examined dispersion when extensive sample dilution was required [26]. There is good agreement that the most important parameter in such a system is the dispersion coefficient since all the mathematical relationships ultimately depend upon the magnitude of dispersion achieved in the gradient chamber. All symbols used in the discussion are defined in Table 2 and illustrated in Fig. 1.

The relationship that describes the concentration of the sample in the stirred flow cell after a given time \(t\) is [19]:

\[
C_A = C_{A0} e^{\frac{-tQ}{V_m}}
\]

where

\[
C_{A0} = \frac{S_v C_A^0}{V_m}
\]

Therefore, when \(t = 0\) (the sample has just completely entered the gradient chamber/flow cell), \(C_A = C_{A0}\).

**Table 2. List of symbols**

<table>
<thead>
<tr>
<th>Parameter or variable</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample concentration, g kg(^{-1})</td>
<td>(C_A^0)</td>
</tr>
<tr>
<td>Sample concentration in the cell after all sample has entered</td>
<td>(C_{A0})</td>
</tr>
<tr>
<td>Elapsed time after all sample has entered the flow cell, min</td>
<td>(t)</td>
</tr>
<tr>
<td>Sample aspiration time</td>
<td>(t_s)</td>
</tr>
<tr>
<td>Injected sample volume, ml</td>
<td>(S_v)</td>
</tr>
<tr>
<td>Flow cell volume, ml</td>
<td>(V_m)</td>
</tr>
<tr>
<td>Flow rate, ml min(^{-1})</td>
<td>(Q)</td>
</tr>
<tr>
<td>Sample concentration in the cell at time (t)</td>
<td>(C_A)</td>
</tr>
<tr>
<td>Total system dispersion = (C_A/C_{A0})</td>
<td>(D)</td>
</tr>
</tbody>
</table>
The parameter determining whether the signal peak is contained within the linear range of the assay is $C_{A0}$. This represents the maximum concentration that will be in the flow cell during the measurement and its actual value will be determined by the dispersion coefficient, $D$:

$$D = \frac{1}{1 - \exp(-S_v/V_m)}$$

(5a)

or since the approximation $S_v \ll V_m$ is valid ($S_v$ is at least 25x$V_m$)

$$D = \frac{V_m}{S_v}$$

(5b)

Thus, the dispersion can be easily manipulated by varying either $S_v$ or $V_m$.

There will be a biomass concentration over which the peak maximum signal will be outside the linear range of the detector. Using the flow injection system described previously [8], it was established that the value of $C_{A0}$ must be less than two for the peak maximum signal to be within the linear range of the assay. Variations in pathlength of the flow cell may raise or lower this value.

From (4), it can be seen that the value of $C_{A0}$ can be adjusted to the proper value by varying $D$ ($S_v$ and $V_m$). Due to the relationship between the dispersion coefficient and $C_{A0}$, a simple formula can be derived to determine the proper volume of the gradient dilution chamber for any range of sample concentrations and finely tuned by selecting sample volume for the biomass assay under consideration from

$$C_{A0} = \frac{C^0}{D}$$

(6)

If $C_{A0}$ is assumed to be 2.0, then the value of the dispersion coefficient at the peak maximum must be half of the maximum expected concentration of biomass. For example, if a fed-batch fermentation typically reaches a maximum biomass of 100 g l$^{-1}$, the dispersion coefficient at the peak maximum should be no less than 50. Figures 3a and 3b show how the dispersion and maximum sample concentration vary with flow cell and sample volume. The practical aspects and characterization of the stirred flow cell have been discussed previously [8].

Raw data

A typical set of spectra for a set of calibration standards is shown in Fig. 4. Data collection for these measurements are atypical for a flow injection type of measurement. Rather than collecting a single wavelength flow injection signal representing a continuous range of dilutions of the sample in the flow cell [8], a single dilution of the sample was selected by stopping the flow and the spectra were collected over a short period of time and averaged.
For each fermentation there was a calibration set and an unknown set of samples. Those in the calibration set had the total biomass determined independently by the dry weight method and the glucose concentration by a manual Trinder assay. This calibration set used to calculate the principle component regression (PCR) and partial least squares calibration models for a comparison of the methods. Principle component analysis (PCA) was used to identify outliers. For each fermentation, the PLS model was superior and required one or more fewer principle components for the best fit model over the PCR model. Therefore, only the PLS models will be presented and discussed. This was done for all three fermentations discussed in this paper.

The spike which is consistent from sample to sample and between fermentation runs is due to the spectrophotometer adjustment. The 666 nm diode is masked so that the intense spike coming from the halogen lamp does not cover the absorbance spectrum of the sample. The internal lamp in the DAS did not have the intensity necessary for the fiber optic configuration so an external source was used and some of the halogen spike is detected by the spectrophotometer.
Sequential injection analysis in total biomass and glucose monitoring

Figure 4. Raw data after the 25 point smoothing was applied from fermentation II. Most of the samples shown here only exhibit the characteristic spectrum of biomass. However, three of the samples also show a characteristic visible spectrum of the Trinder reagent after reaction with glucose and are indicated (8, 12 and 48 h samples). Each spectrum represents one sample used to calculate the models used to determine total biomass and glucose in unknown samples.

Biomass

The fermentations that were run during the development and refinement of the glucose/biomass assay were designed to have high biomass yields and nominal glucose concentrations. The maximum biomass concentration for any given fermentation ranged from 50 to 75 g kg⁻¹. High biomass concentrations provided a greater challenge for the glucose monitoring and tested the robustness of the sampling system. In a previous paper [8], a univariate method for total biomass was introduced and had proven to be the least complicated method for total biomass determination and prediction. For total biomass prediction, the multivariate method discussed in this paper does not provide superior results. The benefit of this multivariate approach is in the simpler data collection, evaluation and determination of the regression model for subsequent predictions.

The final model that was used to predict total biomass in unknown fermentation samples as well as future fermentation runs came from fermentation II. The optimal number of factors (PC's) was determined using a leave-one-out cross validation, thus each sample in the calibration set had also been treated as an unknown. The standard error of variance (SEV) was computed for accumulating principle components (PC) and used to determine the number of PC's to use in the model. The minimum SEV typically indicated the best answer for the number of PC's to use in the model without overfitting. For the total biomass model, the first 4 PC's were used. Examination of
the loadings vectors gave a good indication of what the model was representing. The first PC was flat, indicating that all the variables (wavelengths) are equal contributors to the model. Since the signal for total biomass is light scattering, this is expected. The second PC looks like the visible spectrum for the reagent used for the glucose assay. Therefore, even though the spectrum is not always readily apparent in the raw data, the model is able to separate the light scattering signal (PC1) from the glucose signal (PC2). The last two PC's are similar in appearance to the regression vector of the biomass model. These PC's are probably modeling differences in particle size and size/cell density differences. In the final model, there were no samples that had high leverage or studentized residual values. The regression model is shown in Fig. 5a and contained 10 samples in the calibration set The prediction line which plots the predicted biomass (using the model in Fig. 5a) against the measured biomass is shown in Fig. 5b.

The model described above was used on the fermentations I and III to test it's ability to predict total biomass in other fermentation runs in which the total biomass values had previously been determined by the dry weight method. The comparison of the dry weight measurement for total biomass with the PLS prediction using the model from fermentation II is shown in Fig. 6. The PLS prediction of the calibration
Figure 6. Comparisons of the total biomass model and the dry weight biomass calibration set for the three fermentations used in this work: a) fermentation I, b) fermentation II, c) fermentation III; (—) dry weight method, (●) PLS model
set of fermentation II is included as a comparison. Figure 7 shows the prediction of the unknown samples in fermentation II along with the calibration set and the dry weight measurement. Overall, the model determined using the calibration set had excellent predictive ability when the total biomass concentration was greater than 1.0 g kg\(^{-1}\) biomass. Averaging the error of the samples with a total biomass greater than 1 g kg\(^{-1}\) biomass results in an average error of 6.15 % for fermentation I, 3.68 % for fermentation II and 3.97 % for fermentation III. Even when the most precise dry weight method is used for total biomass determination, the error associated with the results when the fermentation sample contains less than 1 g kg\(^{-1}\) total biomass is on the order of 10 % relative standard deviation.

![Figure 7. The total biomass model used to predict unknown samples in fermentation II; (—) dry weight method, (+) training set for the PLS model, (○) unknown set predicted by model](image)

**Glucose**

The glucose determination for both the automated sequential injection method and the manual method was based on the glucose oxidase (GOx)/peroxidase (POx) reaction. The assay is based on the following reactions:

\[
glucose + H_2O + O_2 \xrightarrow{glucose oxidase} \text{gluconic acid} + H_2O_2
\]  

(7)
Sequential injection analysis in total biomass and glucose monitoring

H₂O₂ + 4-aminoantipyrine + p-hydroxybenzene sulfonate

peroxidase → quinoneimine dye + H₂O

Therefore, the concentration of glucose is directly proportional to the absorbance maximum of the quinoneimine dye. This method is generally used as a univariate assay for the determination of glucose off-line. In this study, it has been implemented as an on-line assay without prior filtration of the sample.

The raw data were handled in the same manner as it was for the total biomass assay. By inspecting the raw data (Fig. 4) it is apparent that only a few of the samples resemble the spectrum of a colored, particulate free solution. These are the 8, 12 and 48 hour samples. The final model that was used to predict unknown samples for glucose concentration was determined from fermentation III. As before, outliers were eliminated using PCA and a leave-one-out cross validation was used to build the model. A minimum value of the SEV was obtained using the first three PC's and it is this model that was used for prediction. The first two PC's represent the biomass and the glucose, while the third PC probably represents the turbidity effect on the glucose assay. The final model used 20 samples and had only one sample with both the leverage and studentized residuals being large. The regression model is shown in Fig. 8a. This is probably the most convincing evidence that the model is actually

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Figure 8. a) A plot of the glucose model used in this work. The contribution (y axis) is the value each variable (wavelength) contributes to the model. b) Comparison plot of the predicted glucose concentration and the measured glucose by the manual univariate Trinder assay.
Figure 9. Comparisons of the glucose model and the manual Trinder assay calibration set for the three fermentations used in this work. a) fermentation I, b) fermentation II, c) fermentation III, (---) dry weight method, (●) PLS model.
measuring glucose within the high background signal of total biomass. It has a shape which is very similar to the visible spectrum of the Trinder reagent after reaction with glucose, with a maximum contribution at 504 nm, the wavelength maximum for the assay. The prediction line which plots the predicted glucose concentration (using the model in Fig. 8a) against the measured glucose concentration is shown in Fig. 8b. The prediction plot indicated that this model (Fig. 8a) will probably not be as good a predictor as the total biomass model. However, the model allowed predictions when the glucose concentrations were below 10 mg dl⁻¹ and it is such a lower concentration range that is of interest to routine industrial fermentations (Zymogenetics, Seattle, WA, personal communication). With the aid of this model it was also possible to detect the presence of concentration spikes of glucose in the broth during fermentation, although it was unable to accurately predict the magnitude of the spike. Since the model only included samples in the range of 1–10 mg dl⁻¹, it should not be expected to accurately predict glucose concentrations outside its range of calibration.

The model described above was then applied to the unknown sample set from the same fermentation (III) as well as the calibration sets from the previous two fermentations (I and II). A comparison of the glucose concentration made with the univariate manual Trinder assay method and the predicted values using the model developed using fermentation III for all three fermentations is shown in Fig. 9. As was noted before, the model does not give a precise representation of the glucose concentration, although it does distinguish between high and low glucose levels. Figure 10 shows
the prediction of the unknown samples from fermentation III along with the calibration set and the manual glucose measurement. When the glucose concentration is within the calibration range of the model, the predictive ability of the model is good. It confirms that the fermentation broth has experienced a spike in the glucose concentration but does not yield a precise value of the spike maximum.

Conclusion

Principle component analysis allowed this multivariate assay to distinguish between the response of biomass and glucose in unfiltered fermentation samples. PC 1 primarily described the biomass component, while PC 2 described the glucose component. PLS was used to build models to predict total biomass and glucose in raw fermentation samples. The model for total biomass had good predictability, while the glucose model is best used to distinguish between high baseline levels of glucose and spikes in the glucose concentration. Even though the glucose model has weaknesses, it provides a reasonably quick estimate of the total glucose availability to the yeast, a feature that is currently not available by any other technique. The measurement is available within 6 minutes after sampling. In contrast, off-line measurements are always biased low since the yeast continue to consume glucose even when stored at low temperatures. Versatile, highly reproducible dilution provided by the sequential injection technique allows optimal reaction and measurement conditions as well as precise time control which allowed fully automated biomass and glucose monitoring to be carried simultaneously on untreated samples of fermentation broth. The results of this work confirm that combination of flow injection with spectroscopy and chemometrics is a powerful tool for monitoring of biotechnological processes.

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Sequential injection analysis in total biomass and glucose monitoring


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