Manual and automated sampling methodologies for monitoring bioreactor cell culture

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Abstract

The main aim of this study is to compare the analyses of bioreactor cell culture parameters in samples withdrawn manually, *in-situ,* and automatically, with an at-line instrument. The same parameters were analyzed from the samples acquired, using the same multi-analyte instrument. Strong correlations were obtained for glucose and lactate, and viable cell concentration.

Objectives

- Monitoring fed-batch cell culture in a bioreactor using *in-situ* and at-line tools
- Sampling the cell culture aseptically, using manual and automated procedures
- Measuring and comparing key parameters of the cell culture with a multi-analyte analyzer

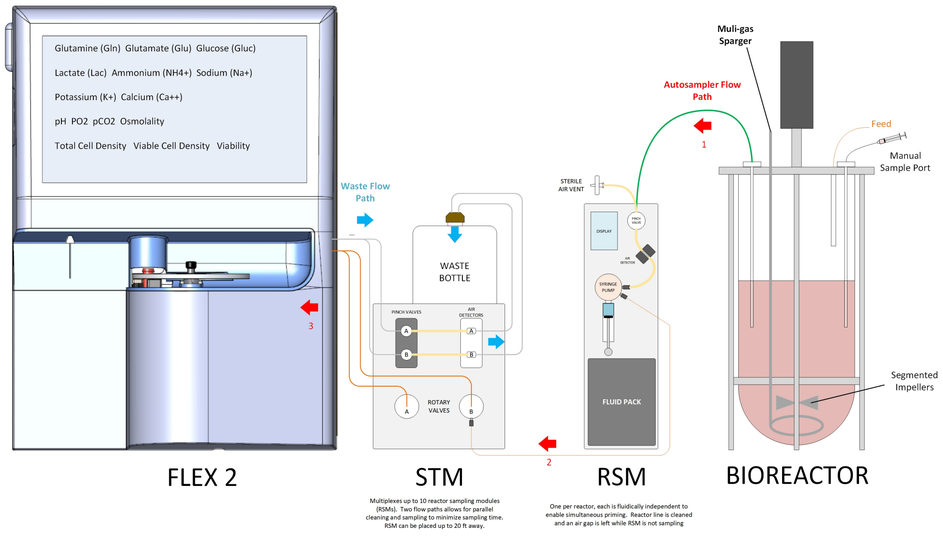

Figure 1. Automated Bioreactor Platform.

Results

For the purpose of this study, glucose and lactate concentrations, total (TCD) and viable cell densities (VCD) were monitored during the cell culture, from samples withdrawn via the automated system and manually, with a syringe. Data were plotted between that obtained with the autosampler (Y axis) versus that with the manual methodology (X axis). A linear regression of the data was generated for each of the 4 parameters and produced a coefficient of determination no less than 0.98.

Conclusion and future work

The results suggest that 1) there is a strong correlation between the data obtained via the automated and manual sampling methodologies, 2) the automated sampling device can be used instead of manual sampling for the analysis of important cell culture parameters which cannot normally be measured *in-situ*, i.e. glucose, lactate, total and viable cell densities, 3) automation of sampling can facilitate acquisition of process data around the clock, while keeping sampling time similar to the manual methodology. Future work will include the design of a methodology for process control, such as maintaining constant glucose concentration, from measurements obtained with the automated sampling platform.

Introduction

In order to make a quality product by cell culture efficiently, it is important to monitor and control the cell culture environment and the cells as closely as possible. When *in-situ* sensors are available, their direct use within the cell culture vessel (i.e. the bioreactor) is favored. This is the case of temperature, dissolved oxygen and pH sensors commonly included in the bioreactor, at all scales. However, when analytes and cell attributes cannot be measured directly *in-situ*, sampling of the cell culture is necessary and external analysis is carried out. For example, cell concentration and cell viability using Trypan Blue, glucose and lactate are typically measured in samples withdrawn manually or automatically from the bioreactor, for analysis off line or at line. There are benefits with both sampling methodologies, the latter facilitating the build-up of a richer data pool and the implementation of tighter control of the bioprocess. For both methodologies, it is essential that asepsis of the cell culture environment be preserved, and thus robust sampling procedures are needed.

Methodology

The CHO-DG44 cells were grown over a period of 9 days in a 2-L bioreactor, in complex medium (Dynamis, Thermofisher Scientific, Waltham MA), containing ~6 g/L glucose initially. Glucose decreased during the course of the experiment and was targeted to be maintained above 2 g/L, with feed addition. Multiple manual automated samples were taken daily. The bioreactor is connected to a data historian, this enabling the visualization of data in real or near-real time. For a given time point, automated sampling was initiated first and followed by manual sampling immediately after. The same multi-analyte analyzer (FLEX 2, Nova Biomedical, Waltham MA) was used for analyses of all samples. For the automated samples, the reactor sampling module (RSM) draws out 5.6 mL directly from the culture in the bioreactor and transfers it to the sample transfer module (STM). In this work, the STM multiplexes two RSMs: one is cleaned while the other one is used for sampling, allowing back-to-back analysis without waiting for the completion of the cleaning after the previous sample. From aspiration to delivery, it takes about 2-3 minutes (steps 1-3, Figure 1). Step 1 involves purging, where 3.8 mL of the 5.6 mL culture sample is taken, and the sample is moved from the bioreactor to the RSM. Step 2 involves the movement of the sample from the RSM to the STM. Step 3 is the movement of the sample from the STM to the FLEX 2. The analysis of the sample culture takes approximately 4-5 min, this happens after step 3, within the FLEX 2. After the sample is analyzed, the lines are flushed with deprotinizing solution, and purged with air to prepare for the next sample. For manual sampling, using a 3 mL syringe a void volume is first extracted, then using a 5 mL syringe 4 mL is extracted. 1.5 mL of the sample is analyzed. The manual sampling port is shown on figure 1 and denoted by a syringe. The probe of the analyzer (labeled on figure 1 as “in-situ Manual Sampling Port”) takes up 350 µL of the sample, and this is done in three replicates for each sample. The probe gets withdrawn into the machine after the sample is taken, into a wash sleeve that surrounds the probe. The deprotinizing solution is used to sanitize the segment of line between the syringe pump and pinch valve on the RSM, the line is then left dry to isolate the reactor from the syringe pump.

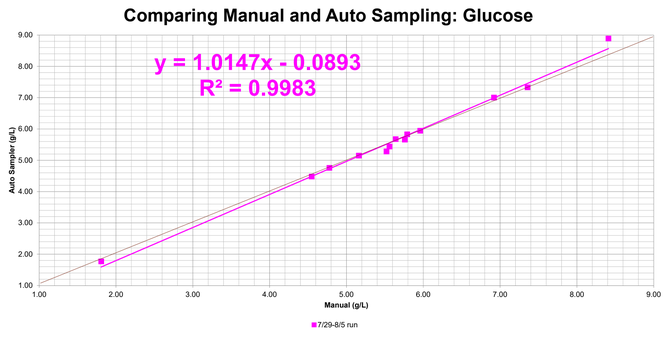

Figure 2. Comparing Manual and Auto Sampling When Glucose levels are monitored. The coefficient of determination is over 0.99, suggesting a strong correlation.

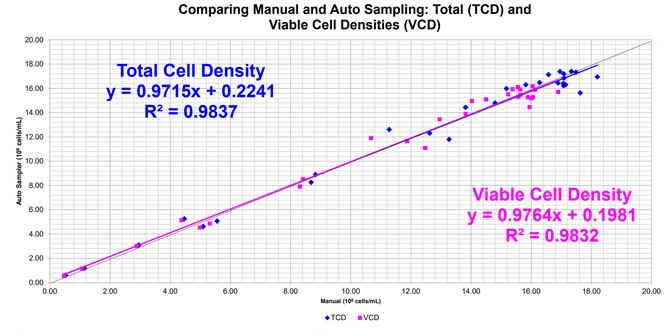

Figure 3. Comparing Manual and Auto Sampling focusing on Total Cell Density (TCD) and Viable Cell Density (VCD). The coefficient of determination is over 0.98, suggesting a strong correlation.

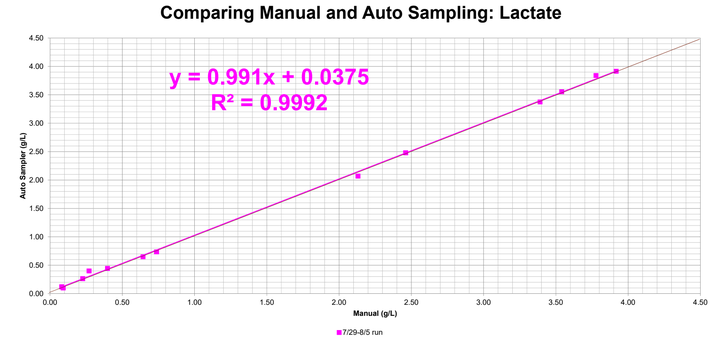

Figure 4. Comparing Manual and Auto Sampling When Lactate levels are monitored. The coefficient of determination is over 0.99, suggesting a strong correlation.

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