Leveraging Software Innovations for Automation and Control of Mammalian Cell Culture Bioprocesses

Authors:

Saso Kveder[1], Sebastian von Rotz[1], Kay Eilers[1], Hiroaki Yamanaka[2], Seong-Yeon Song[2], Yasuhito Murato[2], Paul Cizdziel[2]

[1]*Securecell*

[2]*Yokogawa Life Science*

Abstract

A critical requirement in biopharmaceutical development is the integration and automation of process equipment and analytical instruments used in the laboratory. Bioprocess labs with multiple lab-scale bioreactors often execute cultivation experiments in parallel for research or process development purposes.

As part of a collaboration between Securecell (Zurich, SW) and Yokogawa Life Science (Tokyo, Japan), this application note demonstrates the effective use of the Lucullus® Process Information Management System (Lucullus®) to assist in the control of three Advanced Control Bioreactor Systems (BR1000) to study glucose utilization of CHO cells for optimal monoclonal antibody productivity.

Introduction

In biopharmaceutical drug production, there are multiple manufacturing control variables that are considered critical process parameters (CPP). The required control of these parameters is optimized in process development and implemented in manufacturing to keep product quality within defined, acceptable limits. Many of the critical quality attributes (CQA) of the drug being produced are dependent on complex intracellular biochemical equilibriums that are naturally modulated in response to *in vitro* environmental conditions within the bioreactor vessel. One such CPP is the availability of an energy source, such as glucose, in the nutrient-rich medium used for mammalian cell cultivation.

Figure 1. The Yokogawa BR1000 Advanced Control Bioreactor System.

The optimization of glucose concentration and feed dynamics for specific production cell lines is required for high-performance fed-batch bioreactor culture. Following are the results of an investigation of glucose feed dynamics on monoclonal antibody yields using a DG44 IgG-expression CHO cell line (K1SP).

Previous experimental data suggested the optimal glucose concentration for achieving peak cell density in early-stage culture under fed-batch conditions was 3g/L. However, since the expression of recombinant antibodies becomes maximal after the achievement of peak viable cell density (VCD), it was important to understand whether glucose requirements could be further optimized in stationary or late stage culture conditions.

To investigate, parallel bioreactor cultivations were performed using three BR1000 bioreactors controlled by Lucullus® software. Additional analytical instruments and other lab equipment were also connected via Ethernet cables to the Lucullus® server.

BR1000 Advanced Control Bioreactor System

Using in-line sensors for near-infrared (NIR) and dielectric impedance spectroscopy, the BR1000 bioreactor system (Figure 1) monitors, in real time, three critical process parameters: glucose, lactate, and viable cell density (VCD). When employing a user-derived custom calibration model, the BR1000 model predictive control algorithms are designed to automate the supplemental feeding of glucose to maintain a precise setpoint value concentration. Pertinent to this report, the glucose concentration can also be dynamically controlled at any point during the cell culture timeline.

Lucullus® Process Information Management System

Lucullus® PIMS is a software for enterprise-wide bioprocess control and data management. It allows the integration of bioprocess technology equipment, such as bioreactors, probes, or analytical devices, and further allows monitoring and control based on the exchanged data. Every step along a typical bioprocess, from design, preparation, execution, and evaluation, can be facilitated with Lucullus® PIMS, resulting in a single software solution for managing all data for integrated bioprocesses.

Materials and Methods

Lucullus® Software Information Network

On a standard English Windows-based laboratory personal computer (PC) in the Yokogawa cell culture laboratory, a licensed copy of the Lucullus® PIMS was installed. The PC was then connected via Ethernet cable to a network hub that was also connected to each of the three BR1000 bioreactors (LP6, LP7, and PP4), the Roche Cedex Bio Analyzer, the Beckman Vi-CELL XR™ analyzer, and a laboratory label printer, as shown in Figure 2. Because the connection to the bioreactor systems is bidirectional, Lucullus® performed both the necessary control actions and data collection as well.

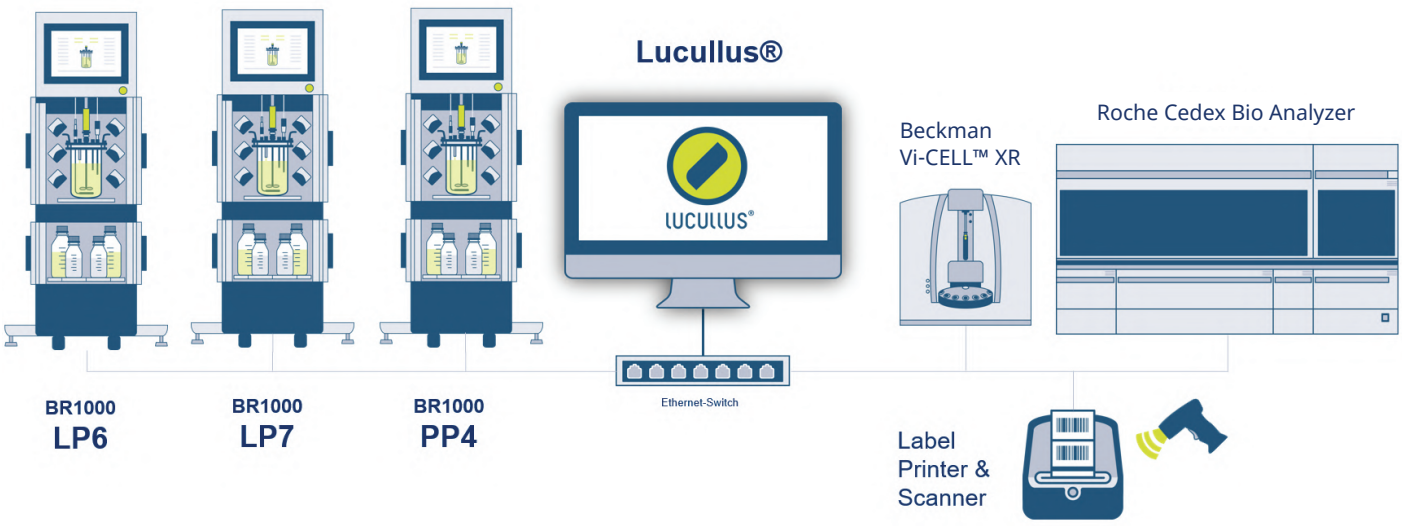

Figure 2. Integrated bioprocess technology setup consisting of three Yokogawa BR1000 bioreactors, a Beckman Vi-CELL™ XR, a Roche Cedex Bio Analyzer, and a label printer.

Prior to daily sample collection from the bioreactors, bar-coded labels were printed and attached to culture tubes that were then used for sample transferral to the analytical instruments. Based on the printed sample labels and corresponding barcodes, a reference identification was created to assign offline measurements with the corresponding process and process time. Scanner units on the instruments were employed to properly collect and track the data and transfer information from the Beckman Vi-CELL™ XR system directly to the Lucullus® database for process analytics, and made further available for comparison against the estimated value based on the model predictive control algorithms integrated on the BR1000 bioreactors.

The same automated data transfer was achieved with the Roche Cedex Bio Analyzer and corresponding lactate, glucose, and IgG concentration measurements. This highly integrated setup allowed the transfer of measurements and corresponding calculation of CPPs in a fully-automated manner, hence no additional middleware was required for transporting the analytical results.

Cell Culture

An IgG expression clonal CHO cell line (K1SP) was obtained from an external source. Cultivation was performed in FUJIFILM Irvine Scientific CHO cell medium with a starting concentration of 3g/L of supplemented glucose for all bioreactor runs. Stirred-tank glass vessel designs were employed with a starting volume of 1500mL and seeded at 150,000 cells/mL with the same seed stock. Standard pH, temperature, gases, and agitation were programmed according to previously optimized parameters and automatically sensed and controlled by the BR1000 bioreactor system. Growth was allowed to proceed in parallel for 14 days.

The three bioreactors used in the experiment were designated PP4, LP6, and LP7. Bioreactor PP4 was the experimental control with a steady-state concentration of glucose at 3g/L maintained throughout the entire cultivation timeline. The other two bioreactors were controlled by Lucullus® software to automatically shift the glucose concentration when the cultures achieved a VCD of 16 million cells/mL. Bioreactor LP6 was shifted up to 5 g/L and bioreactor LP7 was shifted down to 1 g/L glucose as illustrated in Figure 3. The feed solution employed was the Irvine Scientific CHO cell medium supplemented with 28g/L glucose.

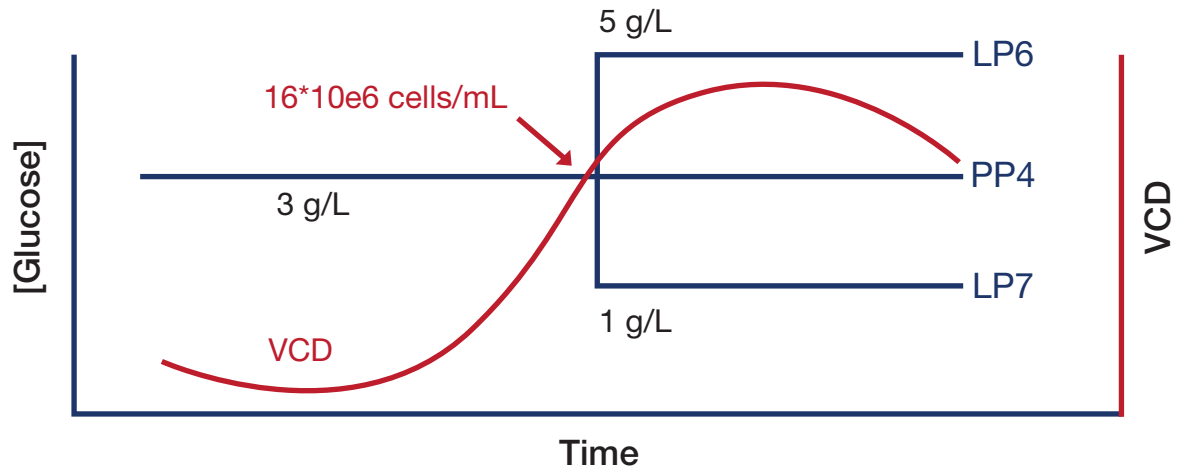

Figure 3. Glucose concentration shift dynamics executed in this study.

Sampling and Offline Analysis

Immediately after setup and daily thereafter, a 10mL sample of the bioreactor culture suspension was extracted for offline analytical testing. Of the sample, 1mL was mixed with a staining reagent according to protocol and analyzed with the Beckman Vi-CELL™ XR instrument to determine total cell count and VCD. The remaining portion was centrifuged to pellet cells and a portion of the supernatant was analyzed with the Roche Cedex Bio Analyzer for glucose, lactate, and IgG concentration. Another aliquot of the sample was placed in storage at -20°C (-4°F) for possible future characterization of the secreted antibody product.

Results

Cell Growth Dynamics

Across the three bioreactors, cell growth was consistent with all cultures achieving a peak cell density of about 20 million cell/mL on about the eighth day of the culture timeline (Figure 4). Due to the setpoint concentration and culture demands for glucose, the feed volume varied, resulting in significant differences in the resultant final volume.

The LP6 bioreactor with a 5g/L glucose setpoint concentration finished over 2.3 times higher than the original starting volume. Other bioreactors were progressively less in the final volume but in a manner consistent with the glucose setpoint (Figure 5).

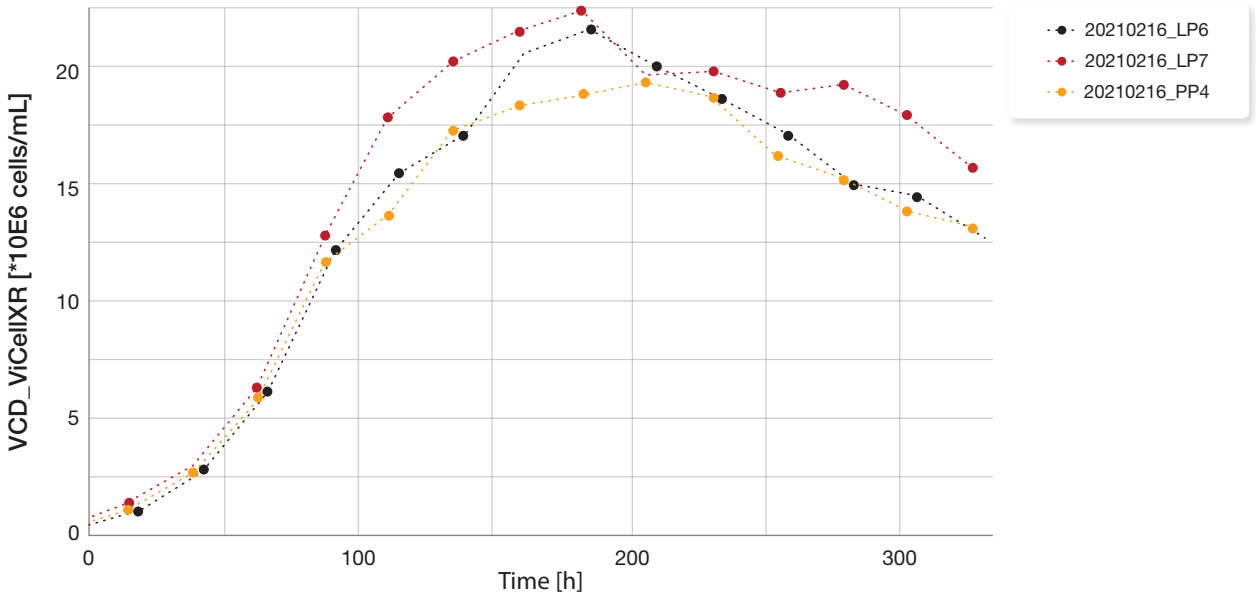

Figure 4. All bioreactor cultures demonstrated reasonably consistent cell growth dynamics.

Peak Cell Density and Peak Day (Vi-CELL™ XR)

Bioprocess	VCD (x10e6)	Day (#)
LP6 (5 g/L)	21.9	8
PP4 (3 g/L)	19.6	8
LP7 (1 g/L)	22.7	9

Total Volume (mL)

Bioprocess	Start (mL)	Finish (mL)
LP6 (5 g/L)	1400	3273
PP4 (3 g/L)	1400	2932
LP7 (1 g/L)	1400	2445

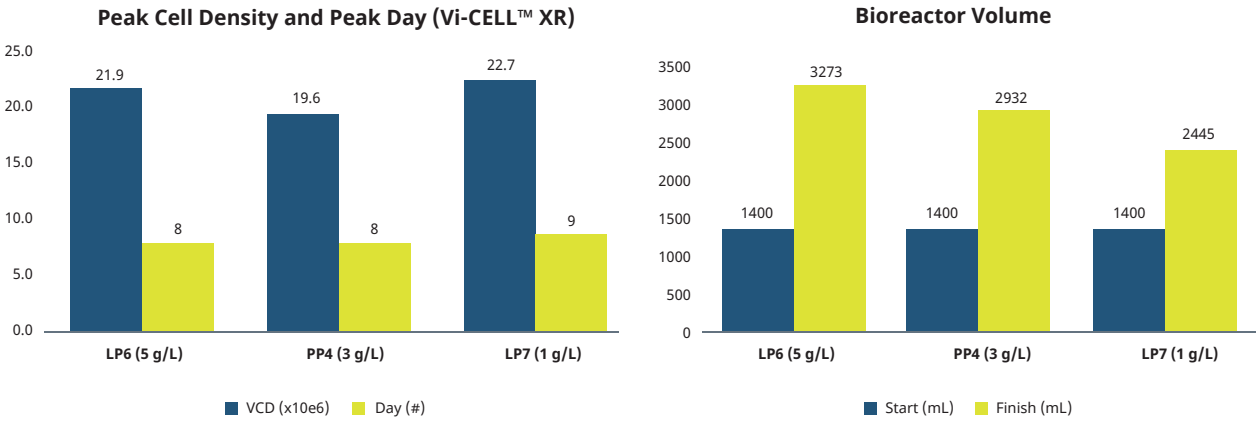

Figure 5. Peak cell densities and final culture volumes are indicated for each bioreactor.

Comparison of Estimated and Offline Parameters

Across the three bioreactors, the viable cell density, glucose, and lactate concentrations were measured with offline analysis and estimated based on the integrated MPC. The overlay graph in Figure 6 compares the corresponding signals and indicates already high agreement between estimated and offline values.

A descriptive statistical comparison was conducted based on an integrated script in Lucullus® and is discussed in the next section.

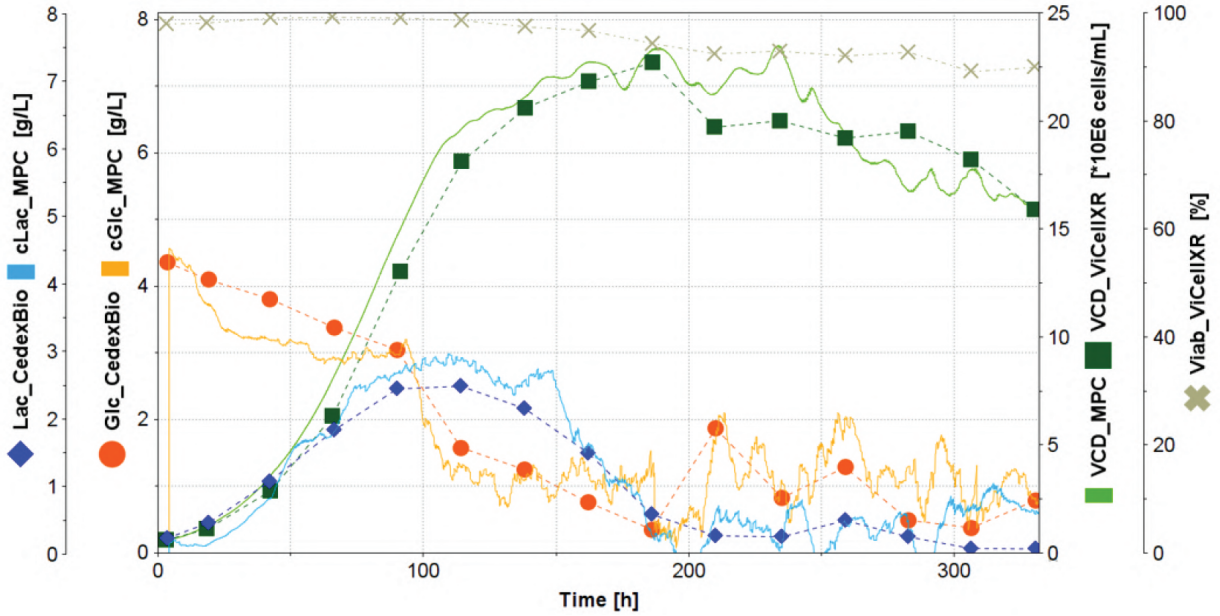

Figure 6. An overlay of the at-line reference measurements and corresponding estimations with the MPC algorithm shows agreement between the signals of LP7 data.

Data Reliability

The glucose, lactate, and VCD data were monitored and recorded using in-line sensor data predicted by the model predictive control (MPC) algorithm and measure on an offline cellular (Beckman Vi-CELL™ XR), and a biochemical analyzer (Roche Cedex Bio Analyzer). Under ideal circumstances, these two data sets are expected to be in close alignment. Generally, the offline data is considered the true "reference data," as the assays have been used extensively for years throughout the industry and display high reliability. Control of the bioreactor, however, is managed using the soft-sensor MPC data derived from the BR1000 in-line sensor systems. To determine the degree of similarity between the MPC data and the reference data, the values were compared using the root mean squared error of prediction (RMSEP) statistic according to the formula shown in Figure 7.

$$RMSEP = \sqrt{\frac{\sum_{k=1}^{n} (a_k - b_k)^2}{n}}$$

RMSEP = sqrt [{ $(a_1 - b_1)^2 + (a_2 - b_2)^{2+} (a_3 - b_3)^2$ • • • $(a_n - b_n)^2$ } / n]

Inline data (MPC data) = { a_1, a_2, a_3 • • • a_n}

Offline data (reference data) = { b_1, b_2, b_3 • • • b_n}

Figure 7. The formula used to characterize the alignment of MPC data with offline analytical assay results.

To calculate RMSEP, Lucullus® was programmed with a custom script according to the formula in Figure 7. The script provided a continuous update of the underlying statistical calculation and a final single value for each of the soft-sensor parameters on all bioreactors, as seen in Figure 8.

RMSEP value			
Bioprocess	**Glucose**	**Lactate**	**VCD**
LP6 (5 g/L)	**0.568**	**0.376**	**2.332**
PP4 (3 g/L)	**0.604**	**0.800**	**5.342**
LP7 (1 g/L)	**0.447**	**0.320**	**1.534**

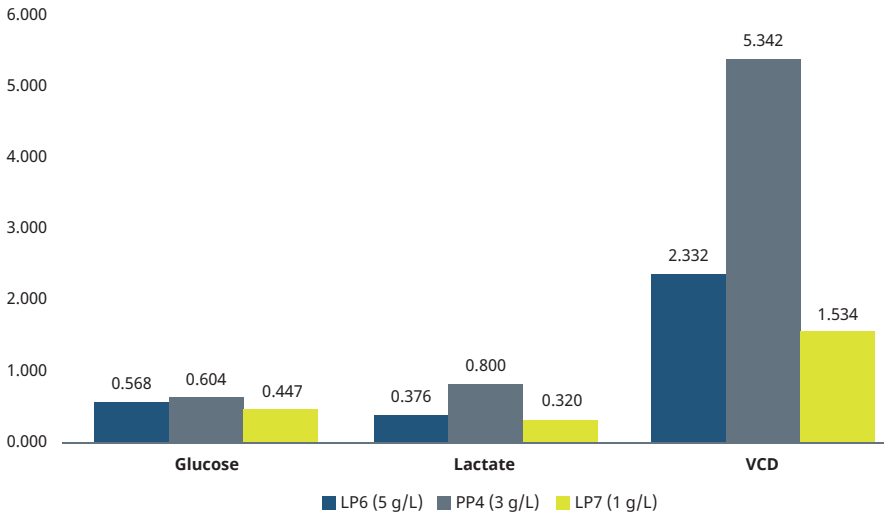

Figure 8. RMSEP values calculated by Lucullus® for glucose, lactate, and viable cell density reported and compared.

Antibody Yields

Along with the other critical culture parameters, the concentration of IgG antibody produced was assayed daily using the Roche Cedex Bio Analyzer. The IgG antibody concentrations varied only slightly in the final culture media upon a comparison of all bioreactors. However, the final volumes varied significantly, and as a consequence, the overall yields showed a range, with the least amount produced by the LP7 (1gm/L) bioreactor culture, as shown in Figure 9.

Antibody Yield		
Bioprocess	**Conc. Max (mg/L)**	**Total Yield (mg)**
LP6 (5 g/L)	**1707**	**5554.5**
PP4 (3 g/L)	**1868**	**5429.2**
LP7 (1 g/L)	**1722**	**4161.3**

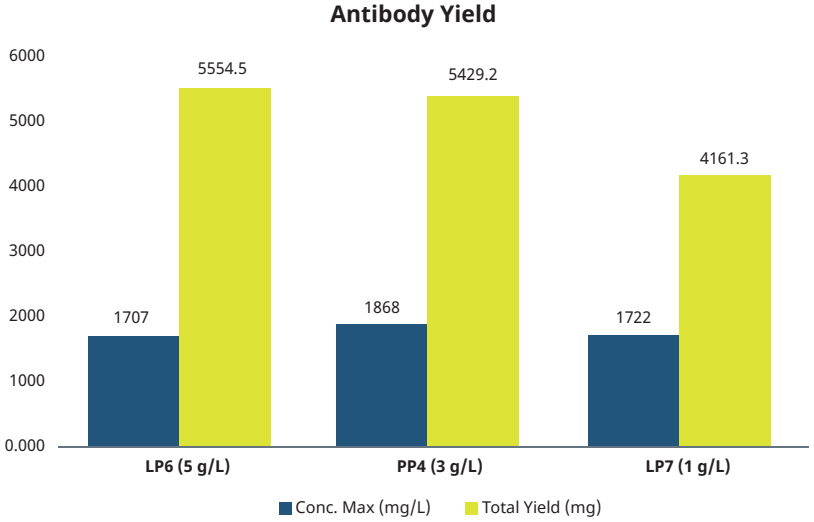

Figure 9. Antibody concentrations were determined by the Roche Cedex Bio Analyzer with an IgG-specific immunoreagent assay. Results are shown for the final day (Day 14).

Discussion

The reproducibility of cell culture performance across multiple independent bioreactors in this experiment is a critical requirement to interpret the resulting data properly. Monitoring reproducibility was addressed through the RMSEP value calculation scripts. These determinations revealed the NIR sensing was calibrated and performed well enough to provide continuous concentration estimates for glucose and lactate concentrations that appeared consistent with the actual offline determined measurements. The reported values are calculated as the cumulative alignment of all samples taken across the entire culture timeline. The RMSEP values ranged from 0.320, representing the closest observed alignment (LP7, Lactate), to 0.800, representing the poorest aligned case (PP4, Lactate). A perfectly aligned comparison would have a theoretical value of 0.000. Overall, regarding these NIR-based RSMEP values, we can conclude that the glucose feed process worked consistently across the three independent bioreactor runs.

The conducted experiment was programmed to trigger a dynamic shift upon achievement of VCD of 16 million cells/mL in two of the cultures (LP6, LP7). The monitoring and execution of the glucose shifts at the precise VCD was carried out by creating another embedded program (execution script) within Lucullus®. This was done to ensure that the concentration shifts would be synchronized properly and initiate independent of human involvement, thereby eliminating any possible user error.

Each of the cultures appeared to reach very similar peak viable cell densities, from 19.6 million cells/mL (PP4, 3g/L glucose) to 22.7 million cells/mL (LP7, 1g/L glucose). Although the RMSEP values (VCD) for the three cultures demonstrated a higher reported overall number and greater variability, a comparison to other RMSEP parameters such as measurements for glucose and lactate is inappropriate due to different measurement units and scales. Furthermore, the RMSEP values (VCD) are calculated from the cumulative alignment across the entire culture timeline, which is less significant to our considerations than the achieved peak VCD, which showed little variation. Based primarily on the RMSEP values of glucose and peak VCDs, it can be concluded that the bioreactor performance of the three BR1000 units was consistently good overall, and variation in the observed final yield of antibody is likely not an artifact of glucose feeding or bioreactor-induced cell growth discrepancies.

Interestingly, the LP7 (1g/L glucose) bioreactor displayed the highest VCD achievement but the lowest total yield of monoclonal antibodies. The other two cultures, PP4 (3g/L glucose) and LP7 (5g/L glucose) both achieved about 20% greater total yields of mAb. It is also noteworthy that the feeding regiment employed was using a 28% glucose solution delivered in a complete (sterile) CHO cell media preparation. As such, cultures maintained at a higher glucose concentration were provided greater feed volumes of glucose and all fresh nutrient components, ending with the greatest final total volume (LP6, 5g/L glucose, 3273mL).

If a greater feed volume of nutrients other than glucose was responsible for increased antibody yields, one might expect to see that reflected in the achievement of higher cell densities into later stages of the culture timeline. However, there appears to be no such differences or correlations observed between increased VCD and increased feeding. In fact, the LP7 culture (1g/L glucose) actually showed the highest VCD maintenance, but conversely had the lowest mAb yield.

Conclusion

Dual Scientific Purposes

This experiment had the dual purposes of being both scientifically objective and subjective. The objective purpose was to study and optimize glucose requirements for maximal monoclonal antibody productivity. The subjective purpose was to explore the potential benefits provided by combining the use of multiple BR1000 bioreactor units with the process information management capabilities of Lucullus®.

Regarding the glucose optimization, the highest yields of monoclonal antibody resulted at concentrations of 3 to 5 g/L glucose in stirred tank fed-batch culture with the BR1000 bioreactor system, with the overall yield difference between these two concentrations being negligible.

The possibility of any qualitative differences between the monoclonal antibody produced at these concentrations was not explored, especially with respect to any antibody critical quality attributes (CQA). Typical CQA might include glycosylation profiles, charge variant profiles, or other biochemical parameters. From the daily collected samples, aliquots were measured (i.e., mAb, VCD, glucose, lactate), and some material was frozen for possible future analysis (TBD). Until such time that the samples can be further characterized, it is reasonable to conclude that a consistent concentration of 3g/L glucose is optimal and more economical for the cultivation of the K1SP CHO cell line to maximize mAb production.

Regarding the more subjective assessment of the BR1000 bioreactor and Lucullus® combination, the following can be concluded:

- **Consolidated Operations**
  The ability to network and control multiple laboratory instruments from one human-machine interface is a key design feature of Lucullus®. For that purpose, it worked extremely well, allowing users to monitor, start, and control three bioreactors simultaneously with process parameters of each available on one screen.

- **Sample Planning and Tracking**
  Labels were planned and printed on a typical office printer and used the barcodes with the Roche Cedex Bio Analyzer, where it worked well. The Beckman Vi-CELL™ XR unit that was employed unfortunately did not have a barcode reader capability.

- **Customized Control Automation**
  Programming the glucose shift in two of the bioreactors at a designated VCD milestone executed automatically and flawlessly during off hours with no human monitoring.

- **Calculation Scripts for Process Insights**
  Programming the RMSEP calculation was simple and the data was a good control for comparing multiple bioreactor units. Only this one script was employed, although there are ideas for several useful others.

- **Real-Time Convenient Data Display Graphics**
  Lucullus® provided great flexibility to sort, cut, and display data in many different graphical formats with data from a single bioreactor or multiple bioreactors on one graph.

- **Remote Monitoring and Control**
  Internet programming and control of the bioreactor units from Securecell engineers in Switzerland during this experiment clearly demonstrated the remote access and control possibilities.

- **Data Backup and Integrity**
  The bioreactor data was routinely stored in each of the BR1000 bioreactor units, while also automatically saved by Lucullus® to yet another location designated by the user.

In conclusion, the BR1000 Advanced Control Bioreactor System from Yokogawa Life Science exhibited effective CHO cell models and performance within and across the three bioreactor cell cultures, while the Lucullus® Process Information Management System from Securecell provided laboratory instrument data management and control capabilities, including flexible programming, that created benefits for efficient execution of process development experiments plus data integrity and storage.