Application note

Enhancing Monoclonal Antibody Yield and Quality Through Automated Multi-Component Feedback Control Loops Using the MarqMetrix All-In-One Process Raman Analyzer

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Industry/Application:

Biopharma PAT / Upstream Bioreactor

Products used:

Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer, Thermo Scientific™ MarqMetrix™ Performance BallProbe™ Sampling Optic, Thermo Scientific™ TruBio™ software

Goals:

This study implemented an advanced carbon control strategy in a bioreactor by simultaneously monitoring glucose and lactate concentrations using feedback control from a process Raman analyzer. The approach boosted titer production and enhanced both product quality and cell viability. The success of this multi-analyte approach demonstrates how process Raman analysis enables many other sophisticated, multi-component feedback control loops to improve both product yield and consistency across batches.

This work also establishes that a process Raman analyzer is a exceptionally effective process analytical technology (PAT) tool that has high integrability with diverse processes, offering substantial time and cost savings as well as paving the way for modern automation and AI-driven manufacturing workflows.

Key Analytes/Features:

Glucose, lactate, viable cell density, titer, quality attributes, automation, advance carbon control logic

Key Benefits:

- The Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer delivers real-time, high-fidelity data that enables the deployment of effective control strategies to optimize product yield, quality, and batch consistency.
- Its ability to perform non-destructive, multi-analyte monitoring in a single scan offers a significant advantage over traditional technologies, supporting advanced feedback control strategies and informed decision-making.
- As a data-rich platform, the process Raman analyzer is ideally suited for integration into any process monitoring and control for automated manufacturing systems.

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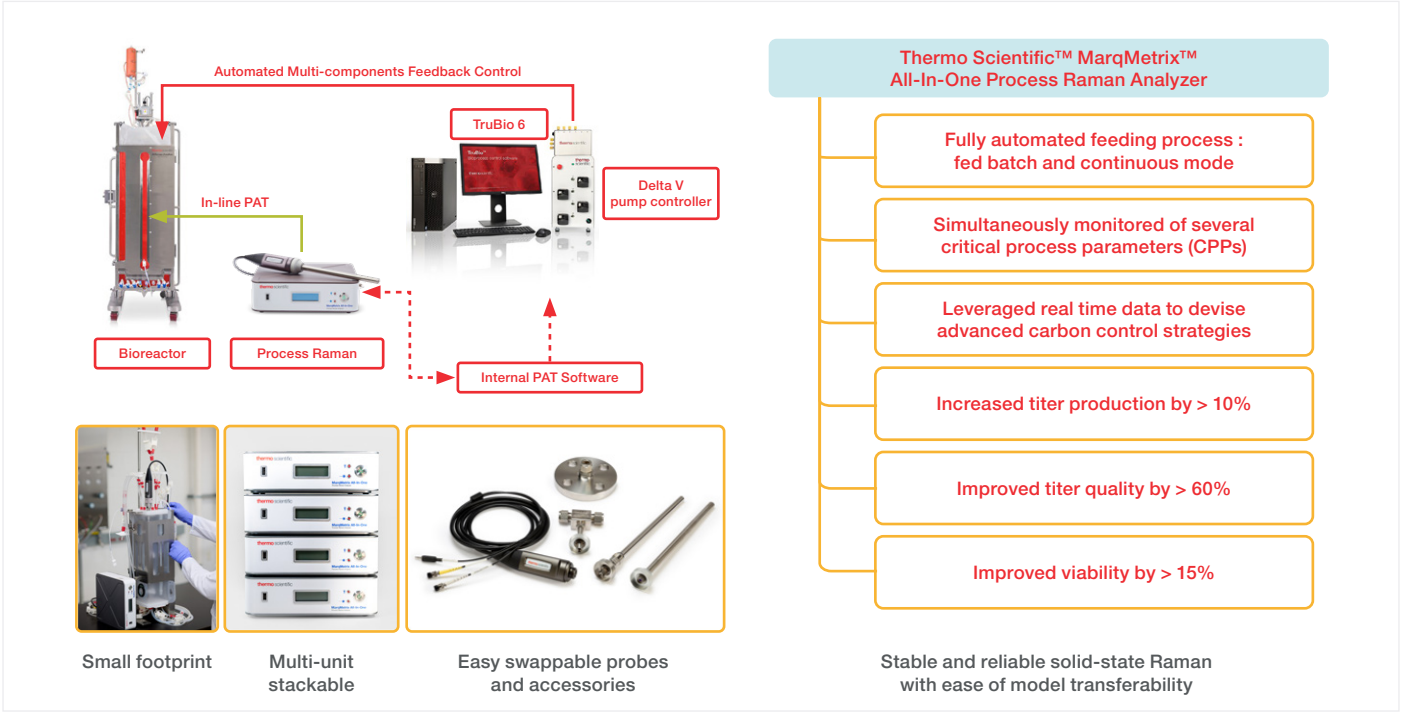

Figure 1. Benefits of the MarqMetrix All-In-One Process Raman Analyzer (shown integrated with a 5L DynaDrive Bioreactor, (bottom left). The system enables real-time, in-line monitoring of multiple analytes to support intelligent, automated bioprocess control.

Introduction

The application of Raman spectroscopy for real-time monitoring and control of bioreactors marks a significant advancement in bioprocessing technology. Over the past decade, its adoption has enabled tighter control of critical process parameters (CPPs), offering early indicators of process performance to ensure consistency and improve product quality. Raman spectroscopy leverages the unique vibrational signatures of molecules, allowing for highly specific detection even in complex biological matrices. This enables simultaneous, real-time monitoring of multiple CPPs, including nutrient concentrations, metabolic by-products, and cell density. As a result, users gain deep, real-time insights into cellular metabolism and can implement more dynamic, adaptive feeding strategies based on metabolic network understanding.

Previous studies have demonstrated the negative impact of high lactate on cell health and quantity and quality of titer produced.[2] In this study, we demonstrate the successful implementation of process Raman analyzer for simultaneous multi-component feedback control of glucose and lactate in a bioreactor. By continuously monitoring both analytes, the feedback control loop dynamically maintained a constant total carbon concentration (glucose + lactate) at a setpoint of 2 g/L.

This advanced carbon-source-based control strategy ensured that cellular metabolic demands were met while promoting lactate consumption toward the end of the run. As a result, lactate accumulation was significantly reduced, leading to substantial improvements in titer yield, product quality, and cell viability compared to standard bolus feeding strategies (Figure 2).

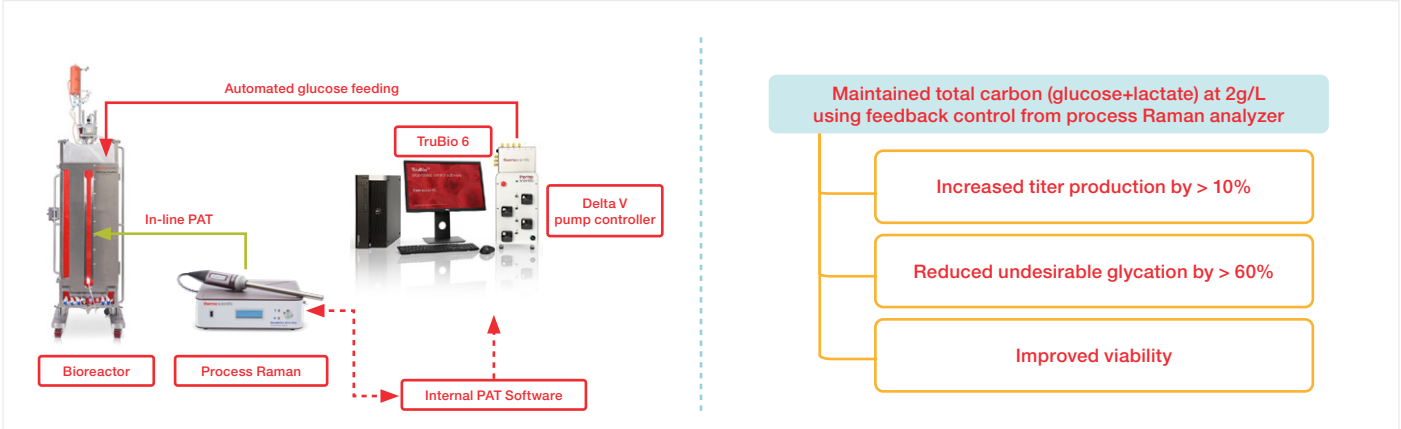

Figure 2. Demonstrating advance total carbon (glucose +lactate) control using inline process Raman leading to enhancement in titer production by more than 10%, reduction in glycation by more than 60%, and improved percent viability.

The outcomes of this work underscore the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer as a key Process Analytical Technology (PAT) tool-enabling real-time, multi-analyte control for any biomanufacturer seeking to engage in intelligent, automated biomanufacturing.

Experimental Details

1. **Chemometric model development.** The details of glucose and lactate chemometric Partial Least Square (PLS) models are previously described.[3] Briefly, three spectral regions were selected for the glucose regression model: 1065-1232 cm^{-1}, 1595-1863 cm^{-1}, and 2704-3078 cm^{-1}. The spectral region of 1065-1232 cm^{-1} includes the characteristic Raman peak of glucose at ~1125 cm^{-1}, attributed to the stretching vibrational modes of CO and CC and the in-plane bending of COH bonds (ν(CO), ν(CC), β(COH)). The spectral region of 1595-1863 cm^{-1} is associated with the symmetric bending of water molecules, while the spectral region of 2704-3078 cm^{-1} includes Raman peaks assigned to the symmetric and antisymmetric stretching vibration modes of CH_2 and CH bonds of glucose. Other biomolecules in the bioreactors also contribute to the Raman signature in the CH stretching region. Extracting glucose-specific information from the CH stretching region in the PLS latent variables improved the model's specificity for glucose and its predictive performance across different cell lines, media, cell density, and bioreactor scales.[4]

The selected spectral regions for glucose were preprocessed in the following order:

- Savitzky-Golay (Sav-Gol) filter (1st derivative, order = 2, window width = 13)
- Standard Normal Variate (SNV)
- Mean centering

The Savitzky-Golay filter removed unwanted baseline information, SNV normalized all spectra to have a mean of zero and a standard deviation of one and mean-centering removed the mean feature from all spectra.

To minimize overfitting, a leave-one-out cross-validation (LOOCV) strategy was used such that each dataset for a given bioreactor run was left out once during the cross validation. The root means squared error of cross-validation (RMSECV) was calculated and used to determine the appropriate number of latent variables (LVs). The optimal number of LVs was selected by minimizing the root mean square error of calibration and cross validation while maintaining their ratio close to 1.

The lactate model was developed using a similar strategy, except a spectral region of 800 to 1750 cm^{-1} was used and SNV was substituted with L1 norm as shown in Table 1.

All data management, cosmic ray removal, averaging, and timestamp alignment were performed in an internally developed Python platform. The data were then processed in Python as well as a commercially available software package SOLO 9.3.1 (2024, Eigenvector Research. Inc. Manson, WA USA 98831)

Analyte	Model Type	Region Selection cm^{-1}	Preprocessing
Glucose	PLS	1065-1232; 1595-1863; 2704-3078	Sav-Gol filter (1st Derivative; order = 2; Window width = 13) + SNV + Mean Center
Lactate	PLS	800 - 1750	Sav-Gol filter (1st Derivative; order = 2; Window width = 11) + L1 Norm (Area = 1 for 1540-1750 cm^{-1}) + Mean Center

Table 1. Showing spectral region and preprocessing used for model development.

2. **Cell culture and bioreactor methods.** CHO-K1 GS cells were inoculated in Efficient-Pro™ medium (Gibco) supplemented with 1.5 mg/L insulin and 1% anticlumping agent at a density of 0.75 million cells/mL in a 5 L glass bioreactor. The bioreactor was operated at a temperature of 37°C, pH 7 ± 0.2, and dissolved oxygen (DO) maintained at 40%. The pH was controlled by the addition of CO_2 for high pH and sodium carbonate for low pH adjustments. The cells were grown in standard fed-batch bioreactors, in duplicate, using a Thermo Fisher Scientific platform process with daily feeding starting day 3. A volume specific feed rate of Efficient-Pro feed 3 and Enhancer (Gibco) was used, fed either by bolus or continuous addition for a total run duration of 14 days. Glucose additions were controlled using the conditions outlined in Table 2. The MarqMetrix All-In-One Process Raman Analyzer with a Thermo Scientific™ MarqMetrix™ Performance BallProbe™ Sampling Optic was used for the continuous control conditions (Figure 3). The Raman data were acquired using 785nm laser with the acquisition setting of power = 450 mW, integration time = 3000ms, and averages = 20.

Real-time predictions of glucose and lactate were sent to the reactor control system (TruBio; Figure 4) where automated glucose supplementation was enabled using the logic outlined in Table 2. Bioreactor runs with automated continuous glucose control and automated fed batch standard glucose control were also performed using the feedback from process Raman analyzer.

Figure 3. MarqMetrix All-In-One Process Raman Analyzer and MarqMetrix Performance BallProbe Sampling Optic. Also shown is Thermo Scientific™ MarqMetrix™ FlowCell™ Sampling Optic.

Condition	Glucose control strategy
Automated continuous glucose control	Continuous control glucose target 8 g/L
Automated fed-batch standard glucose control	Daily bolus when daily glucose reading < 3 g/L feed to 6 g/L
Automated total carbon source (glucose + lactate) control	Total carbon source (glucose + lactate) control at 2 g/L

Table 2. The three different modes of bioreactor and control logics used in this study.

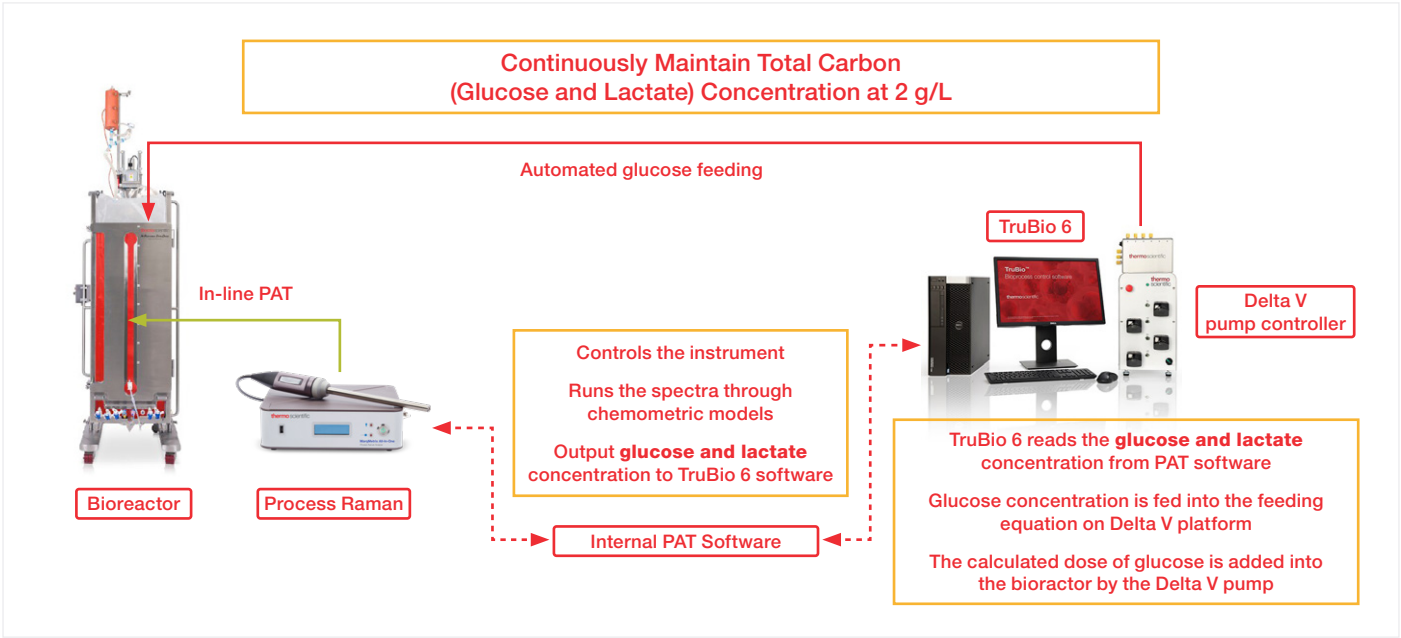

Figure 4. Illustration of the workflow to maintain the total carbon (glucose + lactate) at 2 g/L using feedback control logic. Similar workflow was implemented for other two control logics listed in Table 2.

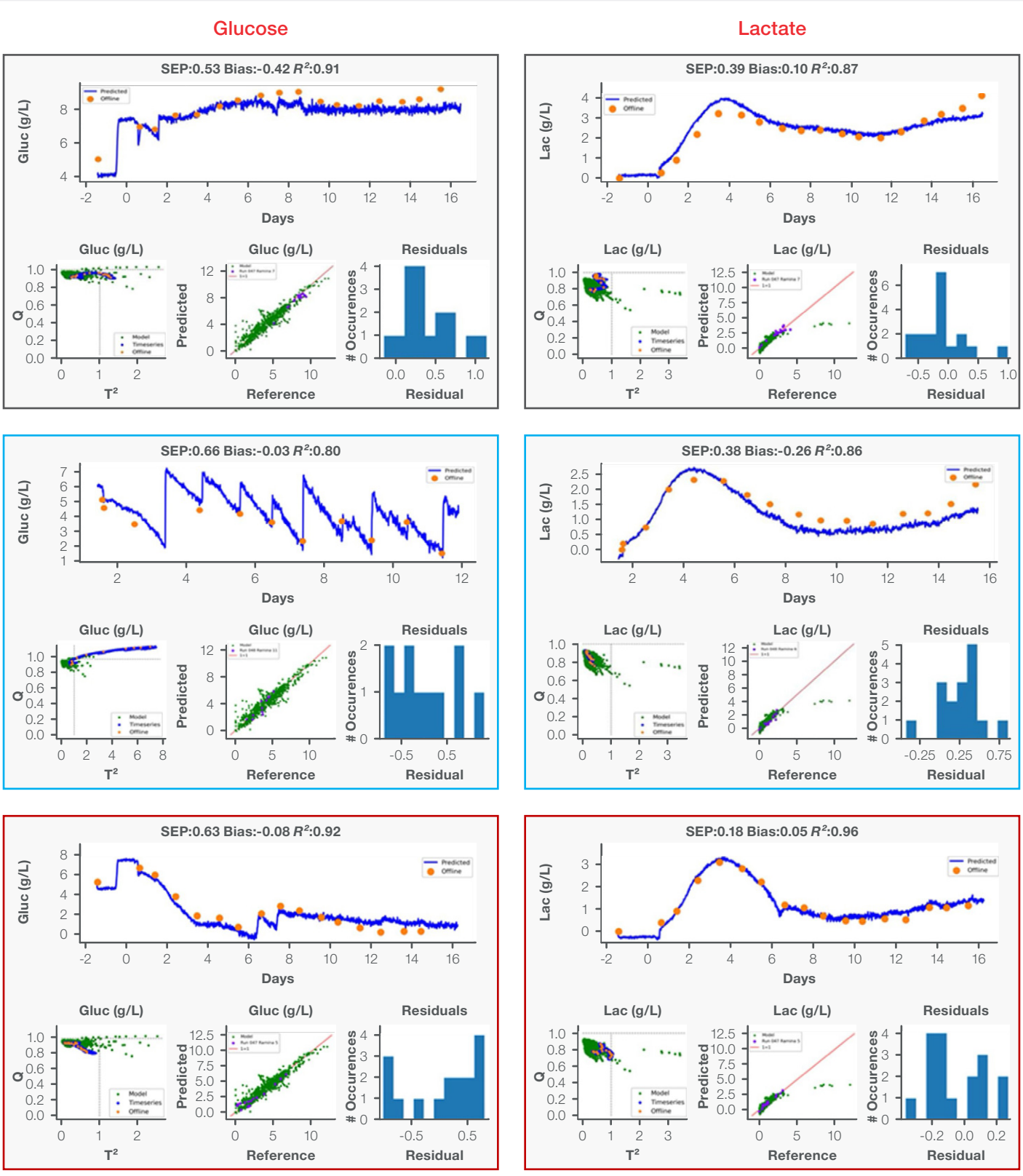

Figure 5. Results for full automated bioreactor runs under the feedback control of process Raman analyzer carried out using three different control logics highlighted in Table 2. Three different glucose controls were compared: Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate) control maintaining at 2 g/L.

Results and discussions:

The predicted glucose and lactate concentrations for the three different bioreactor runs with distinct control logics (listed in Table 2) are shown in Figure 5. The left panel depicts the glucose predictions, while the right panel shows the lactate predictions. The results are summarized as follows:

i. **Automated continuous glucose control (top; black outlined):** As previously demonstrated and reiterated in this study, automated feedback control using process Raman analyzer was employed to maintain a constant glucose concentration of 8 g/L.[5] The lactate production peaked at a concentration of 2.5 g/L on day 4 and remained approximately at the same level for the entire run. This experiment design served as a control to evaluate the impact of lactate accumulation on cell health and titer quality and quantity.

ii. **Automated fed-batch standard glucose control (middle; blue outlined):** In this conventional fed-batch setup, glucose was fed in boluses to maintain concentrations between 3 g/L and 6 g/L. The methodology for automating bolus glucose feeding in the fed-batch run was described in our previous work.[3] Under this control strategy, lactate peaked at 2.5 g/L on day 4, then decreased and stabilized at approximately 1 g/L.

iii. **Automated total carbon (glucose + lactate) control (bottom; red outlined):** In this run, predicted glucose and lactate concentrations from process Raman analyzer were used as feedback to maintain a total carbon concentration of 2 g/L. When the predicted lactate concentration was high, less glucose was added to the reactor to maintain the total carbon concentration. This strategy enhanced lactate consumption, thereby reducing overall lactate accumulation in the bioreactor.

We evaluated cell growth and viability for the three control strategies mentioned above. As shown in Figure 6, the cell growth curve, expressed as viable cell density and viability percentage, was similar for both automated continuous glucose control and automated total carbon control. For automated fed-batch standard glucose control, the viable cell density was slightly lower, while the viability percentage was comparable to the other two runs. The percent viability was higher throughout the bioreactor runs when the total carbon (glucose + lactate) was maintained at 2 g/L.

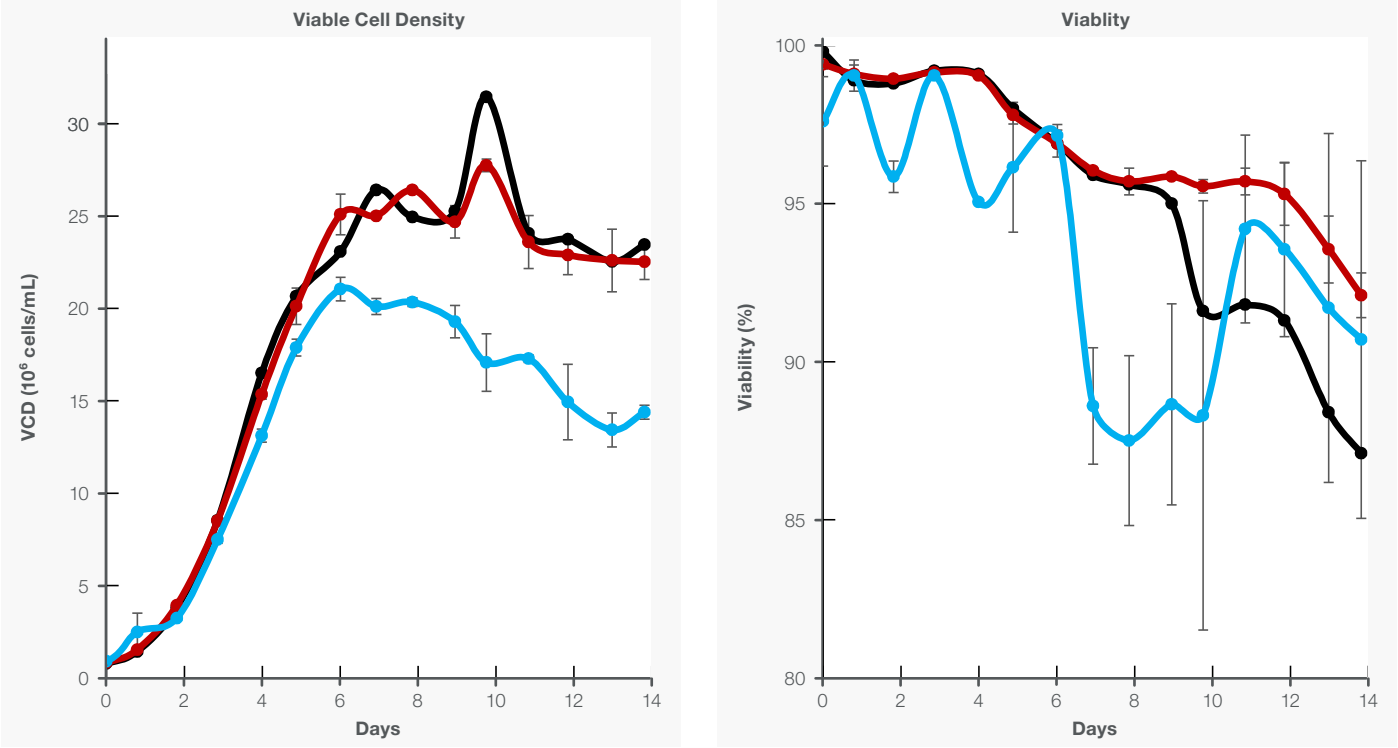

Figure 6. Showing viable cell density (VCD) and viability from reference measurement. Three different glucose controls were compared: Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate) control maintaining at 2 g/L.

Next, we compared the effect of these three control strategies on total titer production and titer quality. As shown in Figure 7, the automated total carbon control strategy increased titer production by 10%. Additionally, the extent of glycosylation, an attribute of protein quality, is shown in Figure 8. Glycation, an undesirable product resulting from the condensation of the aldehydic functional group of glucose with the free amine of monoclonal antibodies, was 2% in the automated total carbon control bioreactor. In contrast, glycation was 12% and 6% in the automated continuous glucose control and fed-batch glucose control bioreactors, respectively. Thus, automated total carbon control strategy improved the titer quality by 83% and 66% when compared with automated continuous glucose control and fed-batch glucose control bioreactors, respectively.

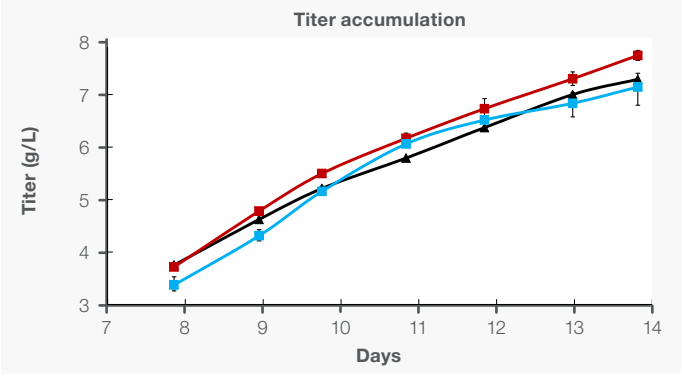

Figure 7. Showing enhancement in titer production by implementing advanced total carbon control through feedback control form process Raman. Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate) control maintaining at 2 g/L.

Conclusion

1. **Unmatched Real-Time Control Capability:**
   Maintaining a constant total carbon concentration (glucose + lactate) of 2 g/L in dynamic bioreactor environments demands precise, simultaneous analyte monitoring. The MarqMetrix All-In-One Process Raman Analyzer delivers near real-time, high-fidelity data every two minutes, empowering advanced control strategies that are simply not feasible with conventional technologies. This makes it an indispensable PAT tool for modern bioprocessing.

2. **Proven Impact on Product Yield and Quality:**
   The Raman-enabled carbon control strategy resulted in a >10% increase in titer and a reduction in glycation by 83% and 66% compared to continuous and fed-batch glucose control, respectively. These outcomes underscore the analyzer's transformative impact on both product quantity and quality.

3. **Versatile Automation Across Bioprocesses:**
   All three bioreactor runs—each employing distinct control logics (Table 2)—were successfully managed through automated feedback using the process Raman analyzer. Its flexible design, including easily swappable probes, supports seamless adaptation to other critical metabolites such as amino acids, making it a universal solution for bioprocess control.

4. **Foundation for Intelligent Manufacturing:**
   With its real-time, data-rich insights and seamless integration into automated systems, the MarqMetrix All-In-One analyzer is not just a monitoring tool; it is a cornerstone technology for intelligent, AI-driven biomanufacturing. It enables consistent, high-quality production while reducing time, cost, and variability.

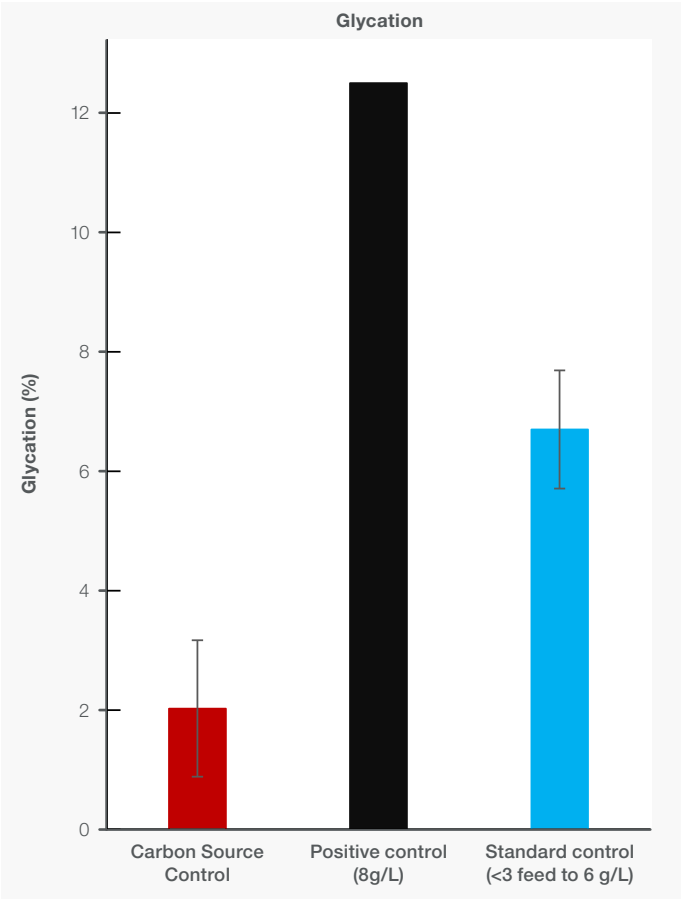

Figure 8. Significant reduction in glycation by maintaining the total carbon (glucose+ lactate) at 2 g/L. Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate) control maintaining at 2 g/L.

References:

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