Compendium

PAT-enabled scaling and optimization of upstream bioprocesses

Process Raman spectroscopy and bioreactor application note compendium

thermo scientific

Introduction

Scaling up a bioprocess is a complex, multi-variable challenge. Designing processes with scalable parameters is essential for ensuring confidence when transitioning from the research laboratory to clinical and production operations.

The application notes in this compendium show how modern bioreactors and process Raman technology can enhance bioprocess development and enable advanced process control strategies from pilot to production scale.

Table of contents

PAT-enabled advanced process control for cell culture

Raman spectroscopy as a PAT tool in a continuous perfusion run 5

Real-time metabolite monitoring with process Raman 14

Monitor and maintain glucose concentrations in real-time 17

Enhancing monoclonal antibody yield and quality 22

Fully automated perfusion bioreactor under multi-modal feedback control 30

Process Raman as a platform solution 38

Additional upstream processing resources

Streamlining seed train scale-up through utilization of the turndown ratio of a single use bioreactor 49

Part 1: Intuitive bioprocess scale-up from bench scale to pilot scale 55

Demonstration of scalability for multiple cell lines 62

Chemometric model transferability across process Raman instruments 69

Measure key variables with process Raman for enhanced control 73

Product solutions 77

PAT-enabled advanced process control for cell culture

Using Raman spectroscopy as a process analytical technology tool in a 50-day continuous perfusion run

Authors

Mike Bates[1], Kevin Broadbelt[1], Jan Ott[2], Vivian Ott[2], Regine Eibl[2], Lin Chen[1], David Kuntz[1], Lin Zhang[1], Mathew Zustiak[1], and Sue Woods[1] (Thermo Fisher Scientific)[1]

School of Life Sciences and Facility Management, Institute of Chemistry and Biotechnology, ZHAW Zurich University of Applied Sciences[2], 8820 Wädenswil, Switzerland

Highlights

- Implementation of Process Raman as a PAT tool for in-line and real-time monitoring of critical process parameters, including titer, cell densities, and nutrient concentrations in mAb-producing, high-cell-density perfusion CHO cell culture, is discussed.
- The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer with Thermo Scientific™ Lykos™ PAT Software, with 21 CFR Part 11 compliance, is used for in-line, real-time, *calibration-free* monitoring of a bioreactor during a 50-day continuous perfusion run.

Summary

The biopharmaceutical industry is driven by the need to increase production and reduce costs while maintaining product quality. One effective way to achieve this goal is to streamline the monitoring process of biologics production, thus allowing more effective control of production parameters. In this application note, we introduce a case study using Process Raman for in-line, real-time monitoring of specific critical process parameters (CPPs) in high-density mammalian perfusion cell cultures reaching 100–130 million cells mL^{-1}. The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer System is shown to enable in-line measurements of CPPs, including glucose, lactate, ammonium, product titer, and cell viability, over the course of a 50-day continuous perfusion bioreactor run.

Background

In recent years, Raman spectroscopy has gained popularity as a process analytical technology (PAT) tool that enables real-time monitoring and control of critical bioprocessing parameters that are key to the successful production of therapeutic drugs. The product portfolio of biologics is broadening, and implementation of different spectroscopic PAT tools can address the limitations of traditional off-line analytical methods for such products. The production of biotherapeutic products requires high process efficiency while ensuring product quality and minimized manufacturing costs[1]. Implementing PAT tools in biopharmaceutical manufacturing is a critical priority identified by the FDA, with the goal of allowing rapid development and access to novel therapeutics and existing medications without compromising product quality[2,3].

Cell culture processes are labor intensive due to the frequent sample analyses required. When operating bioreactors in fed-batch mode, nutrients are periodically supplemented via a bolus feeding strategy using predetermined volumes of concentrated nutrients. While batch production is a well-vetted manufacturing method, this production strategy can be slow and inefficient. Recent advances in biomanufacturing processes involve continuous processing. Continuous bioprocessing technologies, whether upstream or downstream, can increase speed while decreasing the costs of producing these essential biologics[5]. Perfusion cell culture is a process that uses filters to keep cells in a bioreactor while continuously exchanging culture medium. Fresh medium replenishes nutrients and carbon sources, while cellular waste and medium depleted nutrients are removed. Key advantages of bioreactors operated in perfusion mode include flexibility, lower cost, improved quality, and greater speed.

In this study, we focused on the continuous perfusion operation mode for suspension cell culture. The goal of continuous perfusion is to develop a process that maintains a steady state in which productivity and product quality can be sustained long-term with minimal variability, with bioreactors running for 30 – 90 days.

Raman spectroscopy is a laser-based method for generating a chemical fingerprint of a sample[2,3]. A key advantage of Raman spectroscopy is its ability to measure numerous analytes in a non-destructive manner, *in situ*, and with low interference from water. Fortuitously, numerous analytes with distinct Raman fingerprints enable monitoring of CPPs such as nutrient feed levels, cell metabolites, cell growth profiles, product levels, and product quality attributes[1,2]. The MarqMetrix All-In-One Process Raman Analyzer with Thermo Scientific Lykos PAT Software is designed to offer accurate, reliable, real-time identification and quantification of numerous CPP analytes. Process Raman is extremely advantageous when adopted into a continuous perfusion culture system. Utilizing an immersed Raman optical sensor provides real-time process information about the CPPs, unlike traditional monitoring systems, which require manual sampling and off-line analysis[1,2]. Noted advantages include an increase in data acquisition frequency, the opportunity for rapid correction of any detected process parameter deviations, and reduction of contamination risk due to a reduced offline sampling.

This application note describes the integration of the MarqMetrix All-In-One Process Raman Analyzer with the 50L Thermo Scientific™ DynaDrive™ bioreactor to perform in-line measurements of CPPs in a continuous perfusion run (See Figures 1-3). Here, we highlight the integration of the MarqMetrix All-In-One Process Raman Analyzer System to perform in-line measurements of glucose, lactate, ammonium, viable cell density (VCD), total cell density (TCD), and titer. This PAT tool provides accurate prediction models for several parameters and metabolites and shows high correlations with offline measurements. The simultaneous measurements of metabolites, product titer, and protein concentration allow for real-time process control, demonstrating the effectiveness of process Raman spectroscopy as a PAT tool in biopharmaceutical industries.

Materials and methods

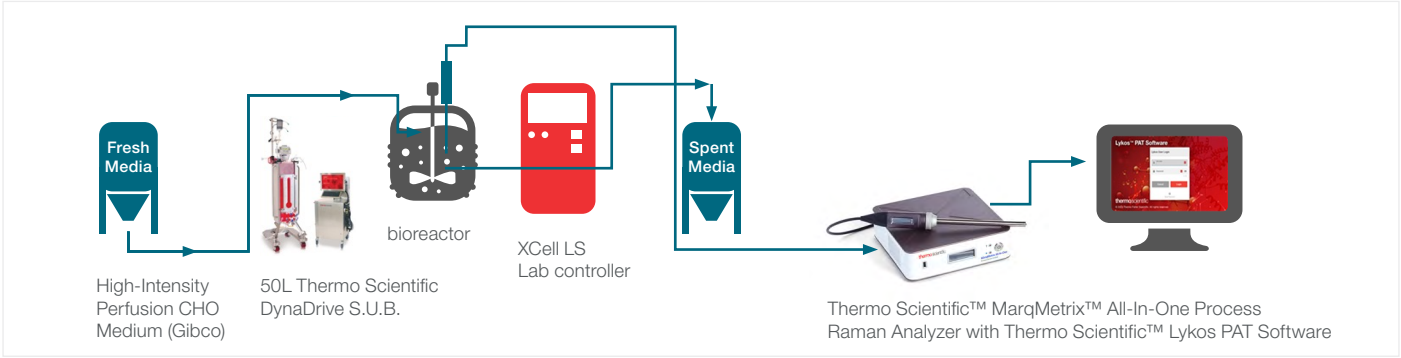


Figure 1. Scheme of perfusion process configuration and integration of an in-line Raman sensor (MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Software).

Cell line and medium

For the two 50 L perfusion cultivations, a trastuzumab-producing CHO K1 cell line was used. The basal medium was 0.66x concentrated High-Intensity Perfusion CHO medium (Gibco™), whereas 1x concentrated medium was used as the feed medium. Both media were supplemented with 4 mmol L^{-1} l-glutamine (Gibco™) and 1% Anti-Clumping Agent (Gibco™).

Inoculum production

Inoculum production was carried out over a period of 10 days. The first three passages were carried out at a shake flask scale. A 10 L wave-mixed bioreactor (Cellbag 10 L, Cytiva) with a working volume of 5 L was used for the last passage.

Perfusion cultivations at 50 L scale

For the 50 L perfusion cultivations, the ATF version of the DynaDrive S.U.B. (Thermo Scientific) with the corresponding G3Pro Bioprocess Controller was used. Cell retention was realized with Repligen's XCell ATF6 single-use version and the corresponding XCell LS controller at an average ATF flow rate of 17 L min^{-1}. The cultivation was started with a VCD of $3x10^5$ cells mL^{-1}. Perfusion was started after an initial 3-day batch phase. The perfusion rate was limited to 1 d^{-1}. The bleed was controlled with the permittivity probe IncyteArc (Hamilton Bonaduz) to keep a viable cell volume of 100–130 mm^3 mL^{-1} constant. To keep the bioreactor volumes constant at 50 L, the harvest pump was controlled by the bioreactor weight. Glucose concentration was either kept constant at 2 g L^{-1} with a CIT Sens Bio glucose sensor (C-CIT) for the first run or was manually controlled for the second run. To prevent foaming, a 1:10 diluted antifoam C emulsion (Sigma Aldrich) was added quasi-continuously. In all cultivations, the pH was controlled to a value of ≤7.15 by adding CO_2 via the drilled hole sparger. DO was controlled at 40% by adding N_2, air and O_2 using the corresponding sparger. An overlay gassing rate of 0.05 vessel volumes min^{-1} with air was chosen. The stirrer speed was set to 136.5 rpm (corresponding to 40 W m^{-3}) in the DynaDrive.

Analytics

Once a day, cell-specific parameters, such as the VCD, viability, and cell diameter, were determined with a Cedex HiRes analyzer (Roche Diagnostics, Basel, Switzerland). The Cedex Bio analyzer (Roche Diagnostics) was used to check the substrate and metabolite concentrations of glucose, lactate, glutamine, and ammonium as well as the IgG titer.

Figure 2. 50L Thermo Scientific DynaDrive S.U.B. for cell culture applications.

Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer measurements

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer System, with the optical Bioreactor ball probe directly immersed in the bioreactor (50L). Each Raman spectra resulted from an average of 60 measurements, with an integration/exposure time of 1 sec and a laser power setting of 450 mW. The total acquisition time per data spectra was 2 minutes, with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and offline instrument analysis to build the model.

Figure 3. MarqMetrix All-In-One Process Raman Analyzer with optical MarqMetrix Bioreactor BallProbe™ immersed in a 5L glass bioreactor.

Chemometric model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzers and numerous bioreactors were used to create models for each analyte. The training datasets were collected from 12–24 samples per bioreactor to create each chemometric model. In-line and at-line measurements were aligned using timestamps between the MarqMetrix All-In-One Process Raman Analyzer and the at-line instrument, the Nova Flex II. All data was reviewed before building the models. In addition, an algorithm was implemented to remove data spikes in the spectra caused by cosmic rays. The spectral region of interest was selected, and multiple spectra were averaged to increase signal-to-noise ratios such that each measurement corresponded to a ten-minute read-time. The spectra were pre-processed to remove differences in the baseline due to fluorescence and other effects. Spectra were also normalized to remove differences in absolute intensity between various bioreactor types. Partial Least Squares (PLS) models were created for each analyte of interest, and leave-out-one-run cross-validation was performed to test the optimization of each model. Analytes of interest include glucose, lactate, ammonium, VCD, TCD, and titer.

Additionally, an augmentation approach was used to improve the models. Data from two ZHAW perfusion runs was collected. One run was used to augment training data to improve the prediction accuracy for the other. The run used to augment the training data was weighted equally to the rest of the training data despite having far fewer samples because this data was assumed to have greater similarities to the prediction run. In this manner, the model was able to learn from the bulk data in the regular training set but was fine-tuned specifically to give the best predictions on ZHAW data. This approach optimizes the benefits of using both a broad general dataset and a specifically targeted but much smaller dataset.

Results

To analyze the chemometric modeling results, let us first define the key figures of merit: bias and RMSEP. Bias is the average difference between predicted values and reference values. The Root Mean Squared Error of Prediction (RMSEP) is the combined error of bias and precision, where precision is the randomness (noise) around the mean of the predicted values, assuming there is no bias. Furthermore, the quality of a chemometric model is typically evaluated using the Q-residuals and Hotelling's T-squared values. The Q-residuals are used to quantify how well the model fits the raw data. Q-residuals should typically be less than 1, which indicates that the model accounts for all the variance in the spectra. A high Q-residual value indicates an observation not well explained by the model, suggesting it may be an outlier. Hotelling's T-squared values measure the distance of each observation from the model's center in the space of the retained components. A high T-squared value expresses an observation far from the model's center, which could also suggest it as a potential outlier. Both Q-residuals and Hotelling's T-squared values are important tools for model diagnostics in chemometrics.

While the results of applying generalized chemometric models, based on bolus-fed CHO cell lines, produced prediction errors of approximately 1 g L^{-1} for glucose and lactate, these generalized models produced poor results when predicting ammonium, titer, VCD and TCD. In contrast, the augmentation of the generalized models using one ZHAW run to predict the other ZHAW run resulted in prediction errors of 0.36 g L^{-1} for glucose and 0.37 g L^{-1} for lactate. Furthermore, the augmentation of the generalized model enabled accurate predictions for ammonium (RMSEP = 0.95 mmol L^{-1}) and titer (RMSEP = 0.36 g L^{-1}) while also providing prediction accuracy for VCD & TCD of ≈10 million cells mL^{-1}, which translates to +/- 10% of the stationary phase concentrations of ≈100 million cells mL^{-1}.

Figures 4-9 show the correlation between offline data and predictions made by applying the chemometric models to the spectra collected using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer. In each Figure, the predicted vs. offline data is plotted in panel A. These figures demonstrate the ability to monitor changes in various CPPs in real-time with a high degree of accuracy. Panel B in each figure speaks to the quality of the chemometric model, as the Q-residuals and Hotelling's T-squared values for each analyte model exhibit very low values; this indicates a good fit of the model with negligible unaccounted variance or outliers.

Panel C in each figure demonstrates the linearity of the CHO-cell training data, augmented with a weighted ZHAW run (green) and a ZHAW run used as a prediction data set (purple). One reason for applying the weighted-ZHAW augmentation to the independent training data set was the very small linear range for many analytes in these perfusion runs. In contrast to the small linear range of CPPs such as glucose, lactate, ammonium, and titer, the cell density values, VCD and TCD, exhibited a wide range from 20–130 million cells mL^{-1}. The results demonstrate excellent correlations between the model prediction data obtained from the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer and the offline data for numerous CPPs (Table 1).

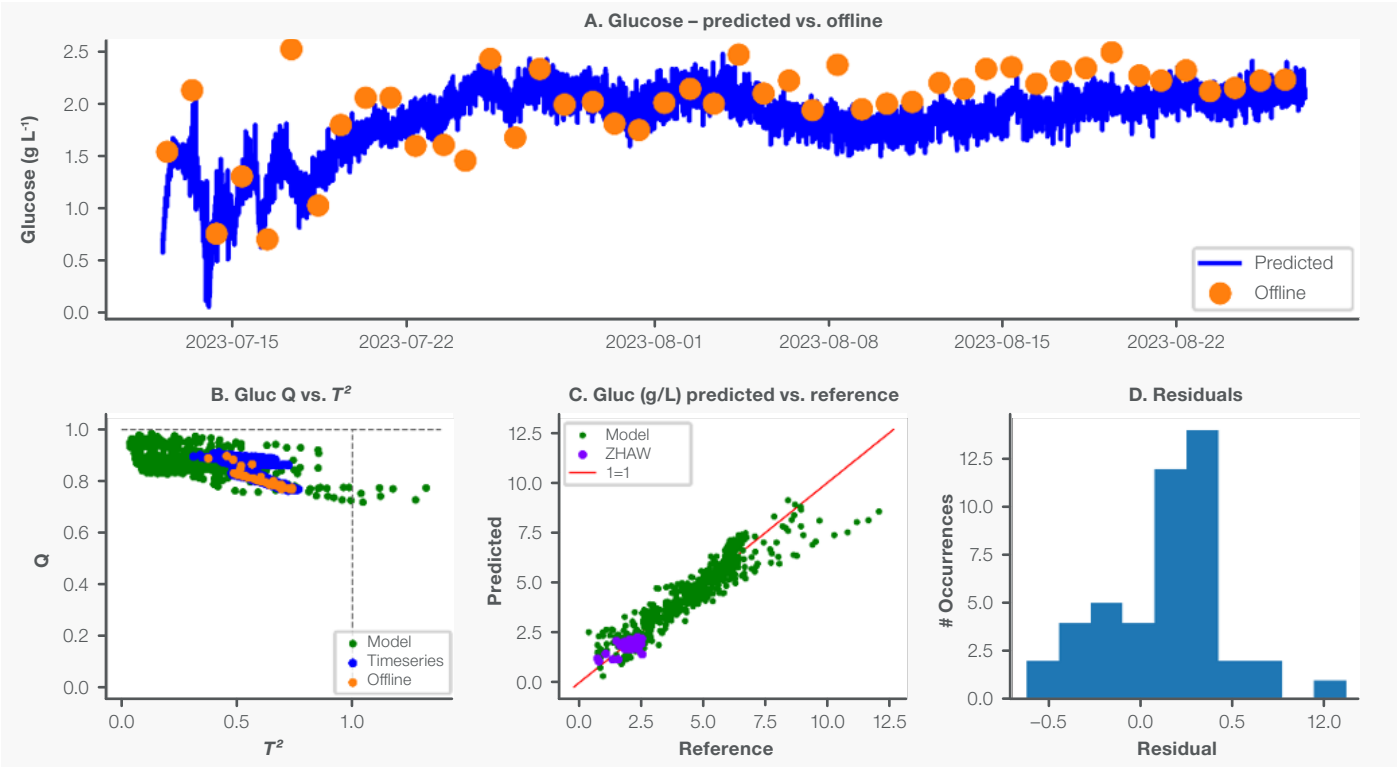


Figure 4. Modeling results for glucose monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

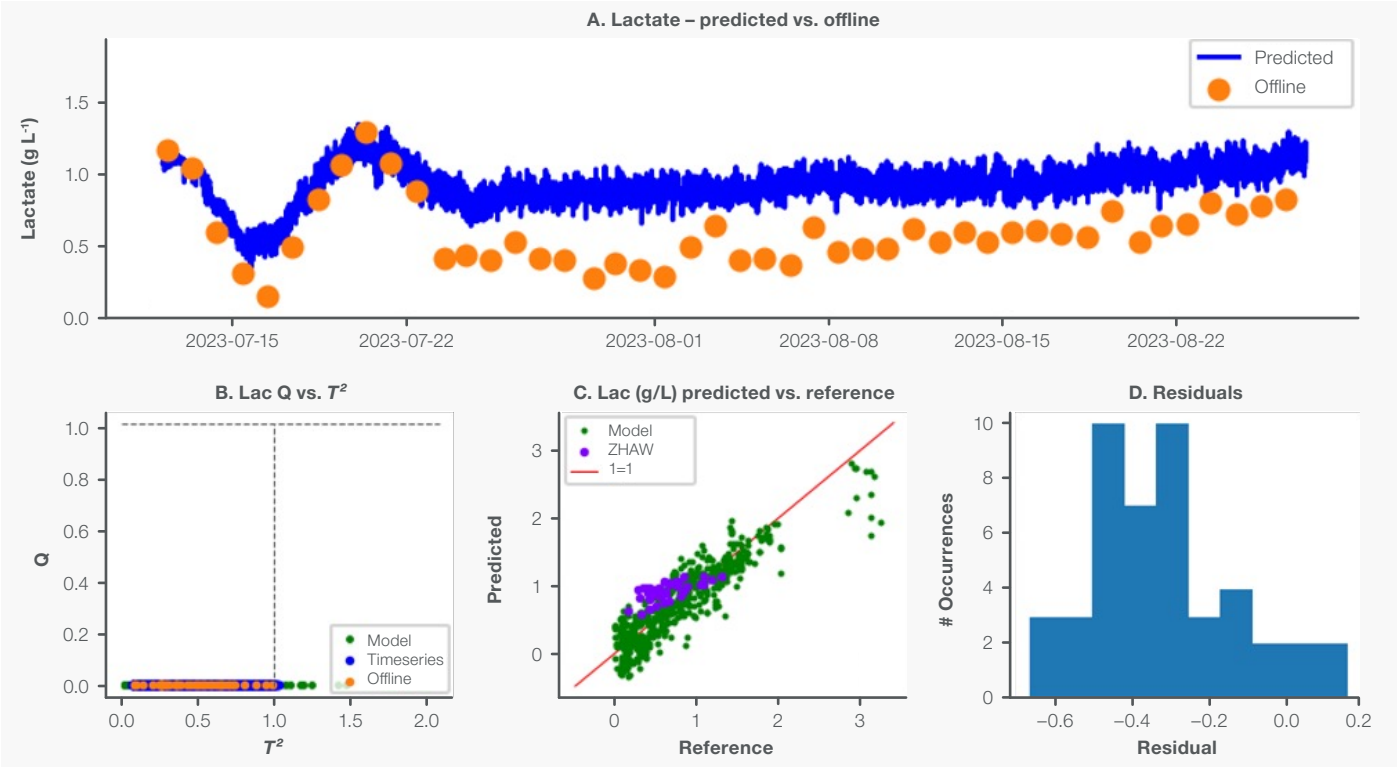


Figure 5. Modeling results for lactate monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

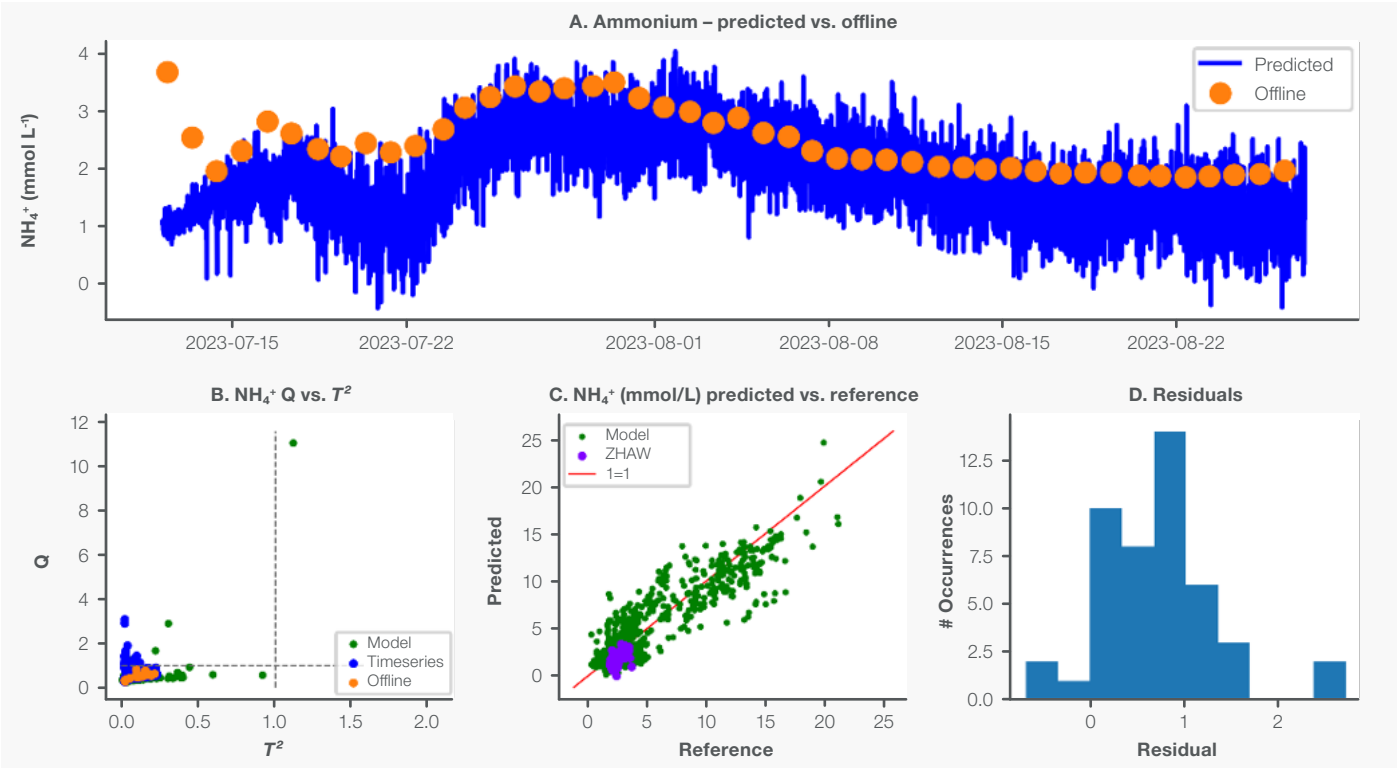


Figure 6. Modeling results for ammonium monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

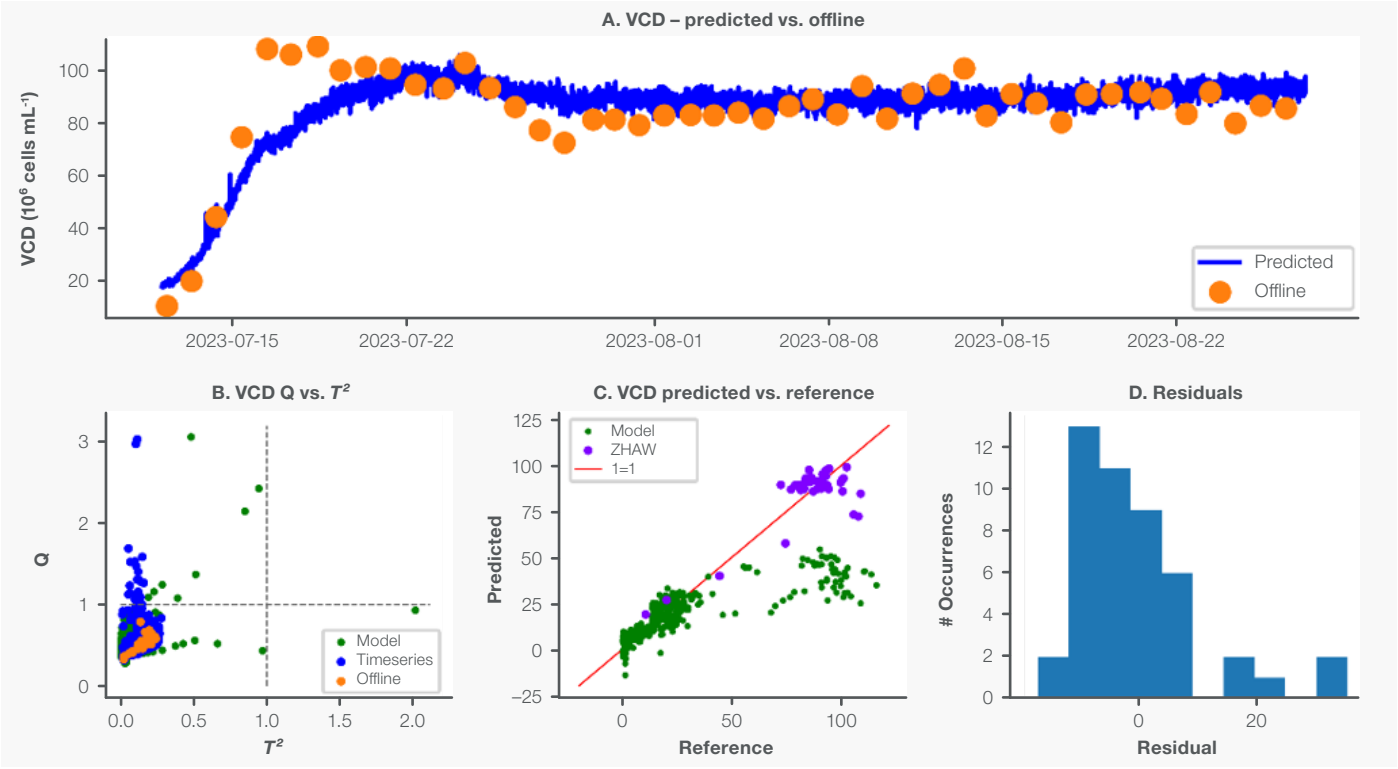


Figure 7. Modeling results for Viable Cell Density (VCD) monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

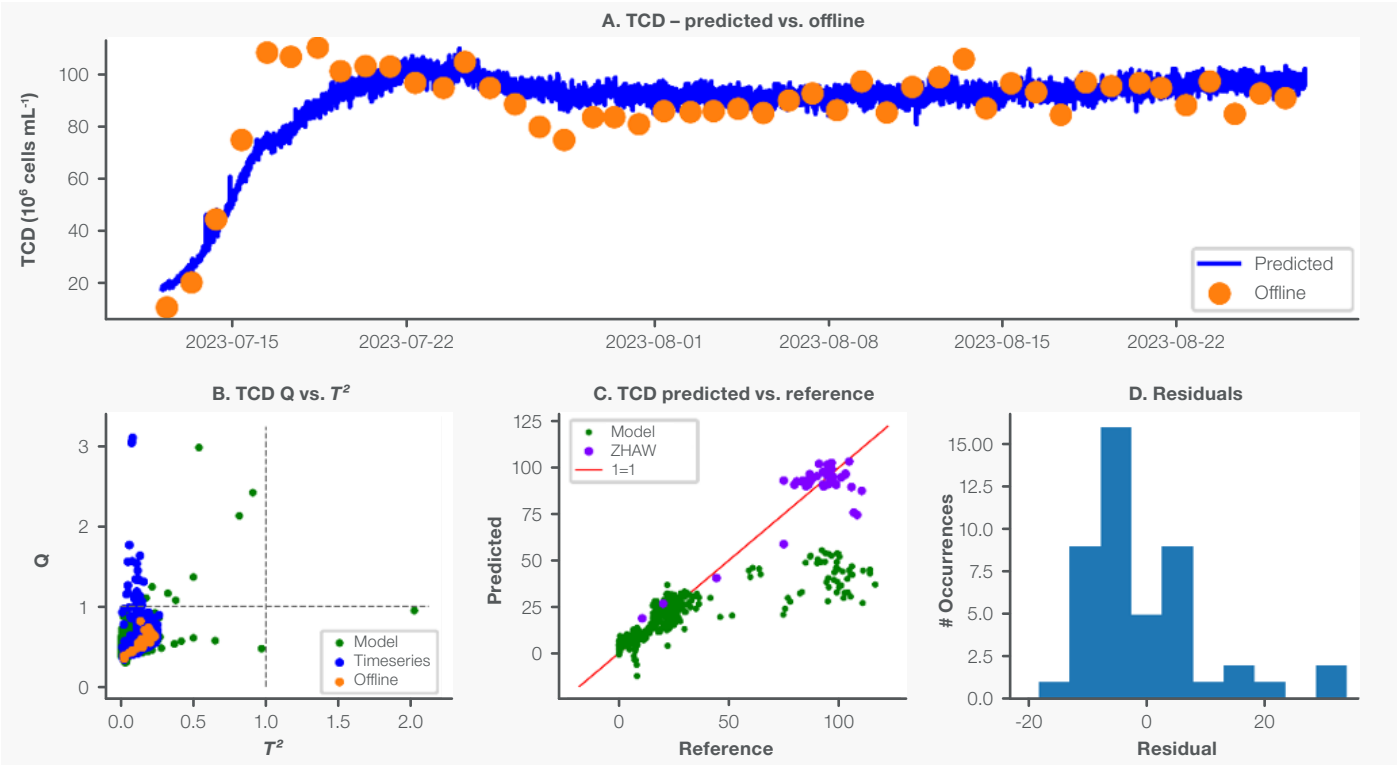


Figure 8. Modeling results for Total Cell Density (TCD) monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

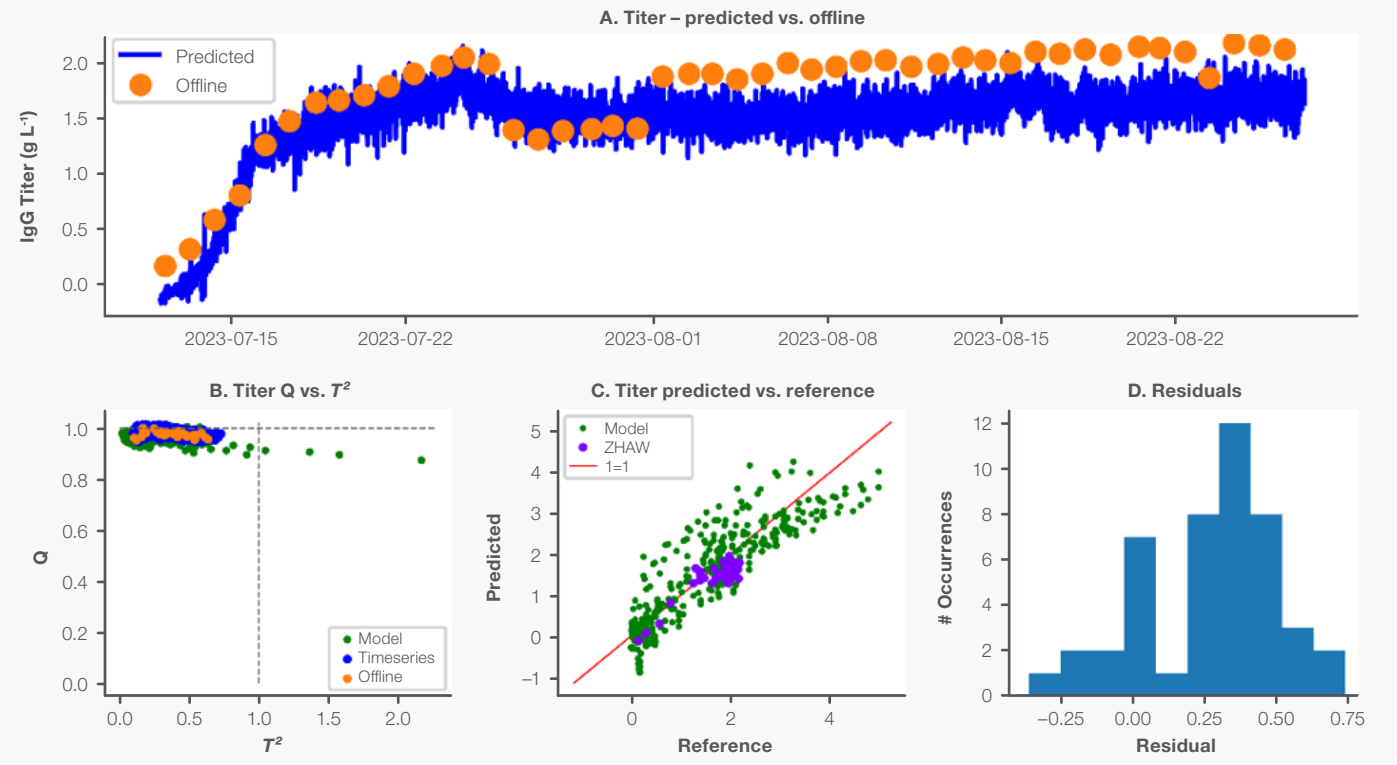


Figure 9. Modeling results for titer monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the Process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

Analyte	Units	RMSEP	Bias
Glucose	g L^{-1}	0.36	-0.15
Lactate	g L^{-1}	0.37	0.32
Ammonium	mmol L^{-1}	0.95	-0.71
VCD	million cells mL^{-1}	10.78	0.47
TCD	million cells mL^{-1}	10.59	0.62
Titer	g L^{-1}	0.36	-0.27

Table 1. Summary of Chemometric Modeling Results for this 50-day Perfusion Run.

Conclusion

The study in this application note highlights an approach to using Raman spectroscopy, specifically Process Raman, for in-line, real-time monitoring of a high cell density mammalian cell culture run in a bioreactor operated in perfusion mode The MarqMetrix All-In-One Process Raman Analyzer provides exceptional data quality, which, when combined with multivariate PLS modeling, enables in-line, real-time monitoring of CPPs glucose, lactate, ammonium, titer, and cell density measurements. The ability to obtain accurate cell density measurements in a wide range from 20-130 million cells mL^{-1} showcases the robustness and utility of these chemometric models and the efficacy of utilizing the MarqMetrix All-In-One Process Raman Analyzer to collect high-quality spectral data. Great data lead to robust and accurate chemometric models, thereby unlocking the potential of Process Raman as a critical PAT tool in biopharmaceutical processes.

The simultaneous measurements of metabolites and product titer are of special interest as they will allow for greater process control of cultivation and purification parameters within continuous biomanufacturing processes. Additionally, Process Raman greatly enhances the operator's understanding of the CHO cell perfusion process as these CPPs are measured repeatedly in short intervals in a non-destructive manner. This study demonstrates the capability of Process Raman spectroscopy as a PAT tool that can pair seamlessly with automation systems to improve yields and enhance product quality.

The results of the perfusion cultivations discussed in this application note have been published in detail, https://www.mdpi.com/2227-9717/12/4/806.[6]

References

1. Research, C. for D. E. and. *PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*. U.S. Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance (accessed 2023-03-21).
2. Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. Nicholas R Abu-Absi [1], Brian M Kenty, Maryann Ehly Cuellar, Michael C Borys, Sivakesava Sakhamuri, David J Strachan, Michael C Hausladen, Zheng Jian Li. Biotechnol Bioeng. 2011 May;108(5):1215-21. doi: 10.1002/bit.23023. Epub 2010 Dec 22
3. Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Brandon N Berry [1], Terrence M Dobrowsky [1], Rebecca C Timson [1], Rashmi Kshirsagar [1], Thomas Ryll [1], Kelly Wiltberger [2] Biotechnol Prog 2016 Jan-Feb;32(1):224-34. doi: 10.1002/btpr.2205. Epub 2015 Dec 21.
4. Modernizing the Way Drugs Are Made: A Transition to Continuous Manufacturing | FDA, Sau (Larry) Lee, PhD, Deputy Director of the Office of Testing and Research, and Chair of the Emerging Technology Team, Office of Pharmaceutical Quality, CDER
5. Integrated Continuous Pharmaceutical Technologies – A Review. András Domokos, Brigitta Nagy, Botond Szilágyi, György Marosi, and Zsombor Kristóf Nagy. *Organic Process Research & Development* **2021** *25* (4), 721-739 DOI: 10.1021/acs.oprd.0c00504
6. Scaling Fed-Batch and Perfusion Antibody Production Processes in Geometrically Dissimilar Stirred Bioreactors. Vivian Ott, Jan Ott, Dieter Eibl, Regine Eibl. Processes. 2024, 12(4), 806.

Application note

Process Raman analysis

Real time metabolite monitoring using the MarqMetrix All-In-One Process Raman Analyzer and the 500L Dynadrive Single-Use Bioreactor (S.U.B.)

Authors

Juan Villa, Matthew Zustiak, Elizabeth Amoako, David Kuntz, Lin Zhang, Kevin Broadbelt and Sue Woods (Thermo Fisher Scientific)

Background

The capability of Raman spectroscopy to reflect small changes in complex aqueous systems has expanded the application of this technique to analyze biopharmaceutical processes such as cell growth in bioreactors. When the Raman spectrometer is utilized as a continuous process analyzer of these complicated chemical systems, this technology can monitor biopharmaceutical production processes real-time, in-situ and non-destructively. The ability of Raman Spectroscopy to detect changes of numerous metabolites during bioreactor processes has elevated this technology to a robust Process Analytical tool.

Thermo Scientific™ MarqMetrix™ Single-Use Bioreactor BallProbe™ Sampling Optic with TouchRaman™ immersion technology

Reusable Raman Spectroscopy analysis optical probes offer benefits such as improved process repeatability and reliability by reducing run-to-run variability. With the MarqMetrix All-In-One Process Raman Analyzer, there are a wide range of probes available. The MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic is designed to meet the requirements of the bioprocess industry and can be used with the MarqMetrix All-In-One Process Raman Analyzer. These probes are quick and easy to swap and connect, are durable and can handle sterility practices including offline autoclaving.

Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), for perfusion cell culture applications

The DynaDrive Single-Use Bioreactor (S.U.B.), the latest advancement in S.U.B. technology, offers improved performance and scalability for large volume bioproduction. The cuboid-shaped tank offers several key advantages over legacy S.U.B. designs including superior mixing and mass transfer capabilities as well as improved scalability.

This application note describes the integration of the MarqMetrix All-In-One Process Raman Analyzer with the 500L DynaDrive Single-Use bioreactor to perform in-line measurements of critical process parameters (CPPs). Utilizing continuously generated spectral data throughout a cell growth culture run, accurate prediction models for several parameters and metabolites were developed using this integrated system.

Materials and methods

Cell culture and feeding strategy

Cell culture was performed in a 500L DynaDrive S.U.B, containing a working volume of approximately 320L of cell culture medium, and inoculated with 0.5*106 cells/mL at a temperature of 36.5 °C, pH= 6.9+/- 0.3, DO = 50%). The pH level was controlled by CO_2 gassing and sodium carbonate additions, as needed. The cells were grown in a chemically defined medium and fed daily with a two-step feeding process, starting at day 3. The first feed media was added at 4% by weight based on the starting volume and the second feed media was added at 0.4%. The temperature was shifted to 33 °C on day 6. The run terminated after 14 days. The bioreactor was covered to protect from stray light. After autoclaving, the MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic was inserted into the DynaDrive S.U.B. during the run for in-line, real-time spectral Raman data generation.

Figure 1. 500L Thermo Scientific DynaDrive S.U.B. for cell culture applications.

MarqMetrix All-In-One Process Raman Analyzer measurements

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer, with the MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic of the MarqMetrix All-In-One Process Raman Analyzer directly immersed in the bioreactors (500L) D. Each Raman spectra was the result of an average of 20 measurements with an integration/exposure time of 3 sec, and laser power setting at 450 mW. The total acquisition time per data spectra was 2 minutes, with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and off-line instrument analysis to build the model.

Chemometrics, model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzer instruments, probes, and bioreactor types were used to create models. The training datasets were collected from 45 samples per bioreactor to create each chemometric model. The spectral data was reviewed, and outlier spectral spikes caused by cosmic rays were removed. The spectral region of interest was selected, and the spectra were pre-processed to remove the baseline and maximize signal to noise.

Figure 2. Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer.

Many pre-processing techniques were tested, including the Savitzky Golay filter with derivatives, Automatic Whitaker Smoothing, Extended Multiplicative Scatter Correction, SNV, and mean centering. The best pre-processing techniques used varied, based on which specific parameter of interest was modelled. Partial Least Squares (PLS) models were created for each property of interest and cross-validation was performed to test the optimization of each model. Properties of interest included glucose, lactate, glutamine, glutamate, TCD, VCD, and other common metabolites generated during the bioreactor culture run.

Results

In this work, continuous in-line Raman spectroscopy was applied to a fed-batch CHO cell culture process. The in-line spectral data was correlated to the offline analytical data acquired for parameters of interest. The use of Raman spectroscopy to monitor process parameters first requires chemometric model building with an externally calibrated data set (independent offline data). To assess the accuracy of the MarqMetrix All-In-One Process Raman Analyzer predicted values, bioreactor samples were collected daily and analyzed for comparison. The root mean square error of calibration (RMSEC), root mean square error of cross validation (RMSECV) and root mean square error of prediction were calculated for each parameter (RMSEP). The error was averaged based upon the prediction of the model to identify the RMSECV which is used to construct the model. The RMSEP is used to test the model against “new” data that the model has not seen. The coefficient of variation, R^2, was recorded for each PLS model. The value is used to determine the amount of variation of the Y variable which the model predictors (X variables) can explain.

It is important to note that the combined use of several large, independent data sets from bioreactor runs of the same CHO culture process produced predictive chemometric models that are more accurate and robust. For this study, five independent datasets from previous bioreactor runs were combined to train a large chemometric model. The calibration model was then applied to the spectral data obtained during this DynaDrive S.U.B run. The data indicates that the model was able to accurately predict this new dataset, and that model predictions were highly correlated with data measurements collected offline for numerous metabolites as shown in Table 1.

Metabolite Predicted	R^2 Predicted	RMSEC	RMSECV	RMSEP
Glucose (g/L)	0.98	0.43	0.49	0.40
Lactate (g/L)	0.92	0.15	0.18	0.25
Glutamine (mmol/L)	0.92	0.42	0.48	0.58
Titer	0.92	0.21	0.25	0.37
Cell Viability (%)	0.94	1.72	2.29	1.83

Table 1. Correlation of model prediction with offline data analysis.

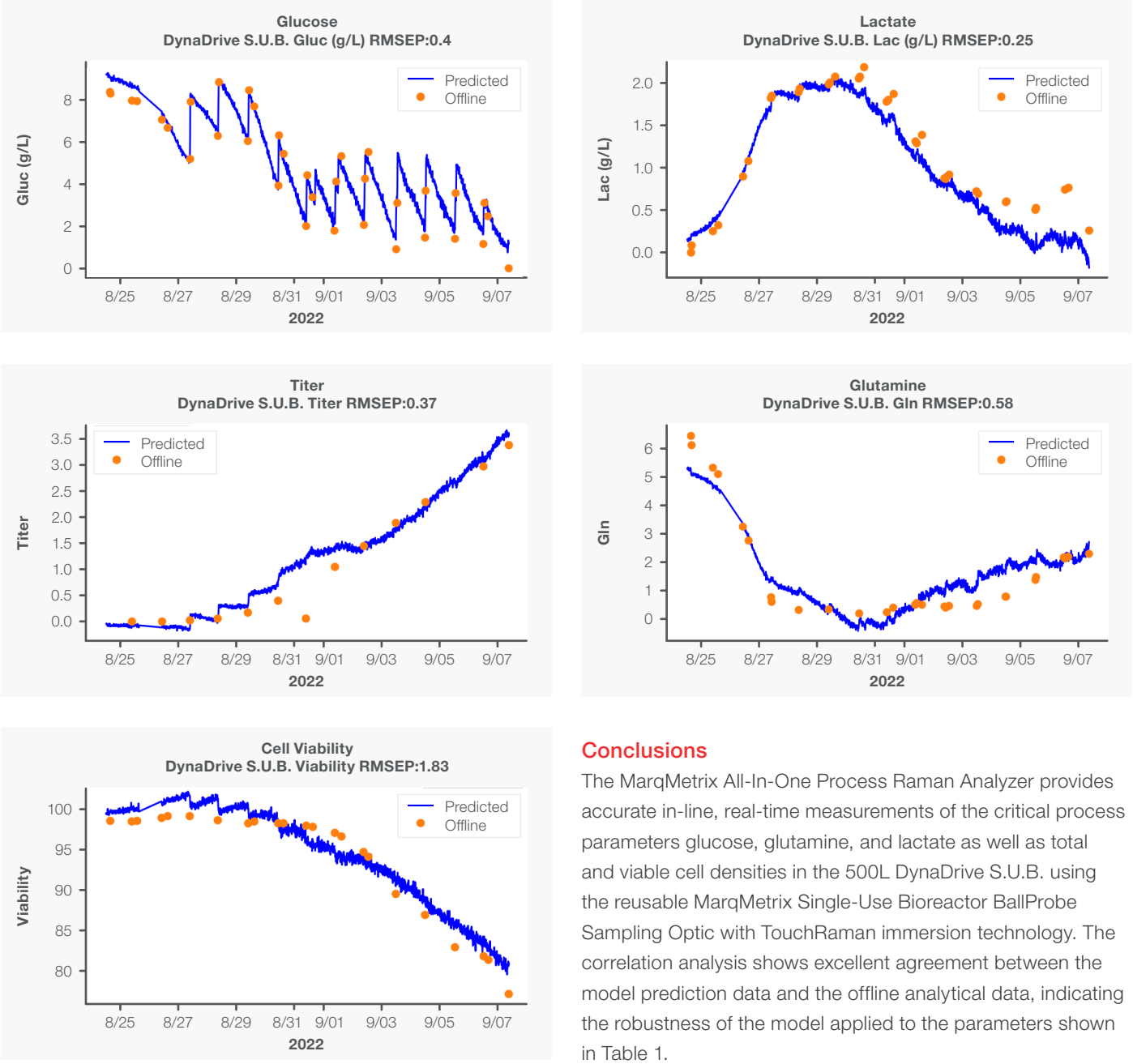


Figure 3. Thermo Scientific DynaDrive S.U.B. Chemometric Model Plots- Comparison of Raman Model vs Offline Analytical Data for Important Bioreactor Parameters.

Conclusions

The MarqMetrix All-In-One Process Raman Analyzer provides accurate in-line, real-time measurements of the critical process parameters glucose, glutamine, and lactate as well as total and viable cell densities in the 500L DynaDrive S.U.B. using the reusable MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic with TouchRaman immersion technology. The correlation analysis shows excellent agreement between the model prediction data and the offline analytical data, indicating the robustness of the model applied to the parameters shown in Table 1.

Use of Lykos and TruBio software programs for automated feedback control to monitor and maintain glucose concentrations in real time

Authors

Juan Villa, Matthew Zustiak, David Kuntz, Lin Zhang, Nimesh Khadka, Kevin Broadbelt and Sue Woods
(Thermo Fisher Scientific)

Summary

In this study, the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer with Thermo Scientific™ Lykos™ PAT Software and Thermo Scientific™ TruBio™ 6.0 Bioprocess Control Software was used to provide in-line feedback control of glucose in both Fed-Batch and Perfusion CHO cell culture processes without the need for operator intervention. This ability to monitor and maintain the desired glucose concentration in real time leads to improved process consistency and product quality, supporting crucial mAb post-translational modification of the product (e.g., glycosylation). An OPC-UA (Open Platform Communications United Architecture) connection between the Lykos PAT software and the TruBio 6.0 bioprocess control software was used for feedback control of one of the integrated pumps on the G3Lab controller supplying a concentrated glucose solution. Both Fed-Batch and Perfusion cultures were maintained automatically without operator process intervention.

Introduction

Process analytical technology (PAT) enables manufacturers to measure and control a process based on the product's critical quality attributes (CQAs) in real time. Enhanced control of critical process parameters (CPPs) optimizes quality while reducing the cost and time of product development and manufacturing. With PAT, these CPPs and, in some instances, CQAs can be measured in real time, therefore leading to gains in essential real-time process information and the ability to create a quality-by-design workstream. In recent years, process Raman spectroscopy has gained popularity as a PAT tool that enables real-time monitoring and control of critical bioprocessing parameters which are key to the successful production of therapeutic drugs. Successful production implies high process efficiency, high and consistent product quality, and minimized manufacturing costs[1]. Implementing PAT tools in biopharmaceutical manufacturing continues to receive much interest, allowing rapid development and access to therapeutics and existing medications without compromising high quality[5,6].

Complex, multivariate, and univariate instrument data are interpreted, and based on this information, critical process parameters are predicted and, where necessary, adjusted to optimize the outcome of the process. Analytical results make it possible to predict the quality of the end material and understand how altering CPPs will affect the process and product. In turn, by executing experiments with real-time quality predictions, the relationships between the CPPs and CQAs can be established to develop true process understanding. Armed with this knowledge, it is possible to 'close the loop' and control the process using those quality predictions. Integration of continuous monitoring into a bioprocess and the application of analytical data is crucial for understanding the process and proactively addressing challenges. A key challenge is in-line monitoring of critical quality attributes such as glycosylation that affect the bioproduct's stability, immunogenicity, safety, and potency. Maintaining the glucose concentration at a steady level in the bioreactor is extremely important to ensure consistent glycosylation of the product while optimizing product yields[1,2]. This online continuous monitoring and control also allow for the reduction of manual sampling and feeding of the bioreactor, which are costly and inefficient and increase the risk of contamination each time the sterile system is accessed.

This application note describes the use of the MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Raman Software to monitor glucose levels in a bioreactor and to trigger automated addition of feed so as to maintain the desired concentration. Communication between Lykos and the TruBio 6.0 control software was executed using OPC-UA[9]. A system that incorporates a sensitive Raman Process Analyzer and an established complex feedback loop allows for real-time monitoring of a critical parameter while maintaining stable concentrations. This system frees the operator from manual sampling and feeding, thereby eliminating the risks of contamination to the health of the cells.

Materials & methods

Bioreactor run summaries	
Run mode: fed-batch	**Run mode: perfusion**
• 1 Bioreactors (5L glass vessel)	• 1 Bioreactor (3L glass vessel)
• Inoculation: $0.6*10^6$ cells ExpiCHOs	• Inoculation: $0.6*10^6$ cells ExpiCHOs
• Initial media: ExpiCHO SPM	• Initial media: HipCHO
• Feed media, EFC+	• Feed media: HipCHO (perfusion)
• Run time: 14 days	• Run time: 10 days

Table 1. Media and Cell Line Summaries.

Bioreactor control system and process parameters

Process monitoring and control were performed using the MarqMetrix All-In-One Process Raman Analyzer and TruBio 6.0 Bioprocess Control Software powered by the Emerson DeltaV Distributed Control System. Cell culture was performed in a HyPerforma Glass reactor and a HyPerforma G3Lab Controller.

Fed-batch strategy

A 3L reactor was prepared with an initial working volume of 1.5L of SPM (ExpiCho stable production media + 6mM glutamine + 1 g/L pluronic) culture medium and inoculated with ExpiCho cells at $0.6*10^6$ cells/mL. Bioreactor environmental control parameters were set at a temperature of 36.5°C, a pH of 7.0, and a dissolved oxygen content of 40% air saturation. The pH level was controlled as needed using CO_2 gassing and 1M sodium carbonate additions.

Feeding strategy

The cells were grown in a chemically defined medium and were fed using a continuous feeding process starting on day 3. The feed media (2X EFFICIENT FEED C+) was added at 3% reactor weight daily. The run was terminated after 14 days. The bioreactor was covered to protect it from stray light.

Perfusion strategy

Fresh basal medium addition was initiated on day 3 at 0.5 reactor volumes/day while spent media was removed through a proprietary perfusion device at the same rate, thereby maintaining the reactor volume at a constant level. The perfusion rate was increased stepwise up to 2 volumes per day by day 5 and held at that rate for the duration of the culture.

Glucose control strategy

Glucose feedback control was initiated on day 3 for the fed-batch culture and day 0 for the perfusion culture, using the glucose value provided by Lykos to the TruBio 6.0 Bioreactor Control Software, to control the glucose concentration in the bioreactors at 3.0 g/L for the fed-batch and 4.0 g/L for the perfusion cultures.

MarqMetrix All-In-One Process Raman Analyzer monitoring

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer, with the optical BioReactor Ball Probe of the MarqMetrix All-In-One Process Raman Analyzer directly immersed in the bioreactors[7,8]. Each Raman spectrum was the result of an average of 20 measurements with an integration/exposure time of 3000 milliseconds and laser power set at 450 mW. The total acquisition time per data spectra was 2 minutes (1 min dark spectrum correction and 1 min sample spectra) with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and offline instrument analysis to align the online and offline results. Measurements were taken every 2 minutes, and based on these measurements, the pump rate was adjusted automatically.

LyKos PAT software

The Lykos provided user access to the MarqMetrix All-In-One Process Raman Solution and data access for the stored Raman spectra. As part of this application note, two new capabilities were developed: A process analysis engine that applies a chemometric model to generate a process value, and an OPC-UA server which allows automated data access.

Process control integration

Glucose addition rate control by the TruBio 6.0 Process Control System was based on process measurements from the Lycos PAT Software via OPC-UA.

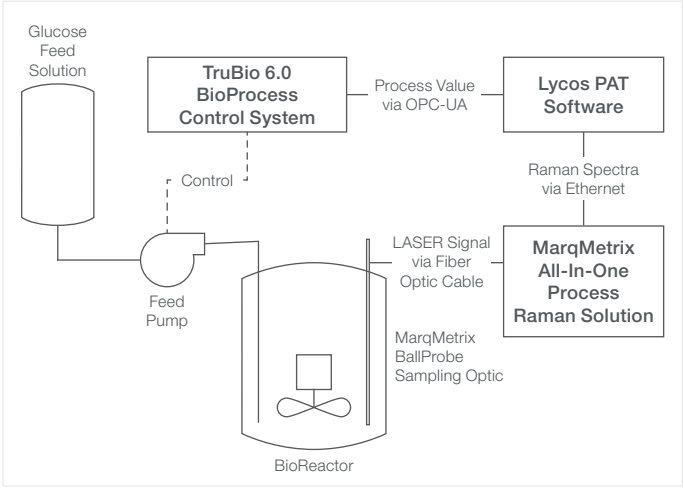


Figure 1A. Process control diagram.

Chemometric model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzers and 3 bioreactors were used to create models. The training datasets were collected from 12–24 samples per bioreactor to create each chemometric model. In-line and at-line measurements were aligned using timestamps between the MarqMetrixAll-In-One Process Raman Analyzer and the at-line instrument, a Flex2 cell culture analyzer from Nova Biomedical. All data was reviewed before building the models. In addition, an algorithm was implemented to remove data spikes in the spectra caused by cosmic rays. The spectral region of interest was selected, and each measurement corresponded to ten minutes. The spectra were pre-processed to remove the baseline and, maximize the signal-to-noise ratio, and correct for path length differences. Partial Least Squares (PLS) models were created for each property of interest, and leave-out-one-run cross-validation was performed to test the optimization of each model. Properties of interest included glucose, lactate, and titer generated during the bioreactor culture run.

Lykos PAT Software communicated directly with the bioreactor-controlling software to send the glucose concentration measured in the bioreactor. A MarqMetrix All-In-One Process Raman Analyzer and Lykos PAT Software were integrated with TruBio 6.0 Biorprocess Control Software (powered via the DeltaV Distributed Control Platform from Emerson). Raman spectroscopy was used to determine the glucose concentration in the cell culture.

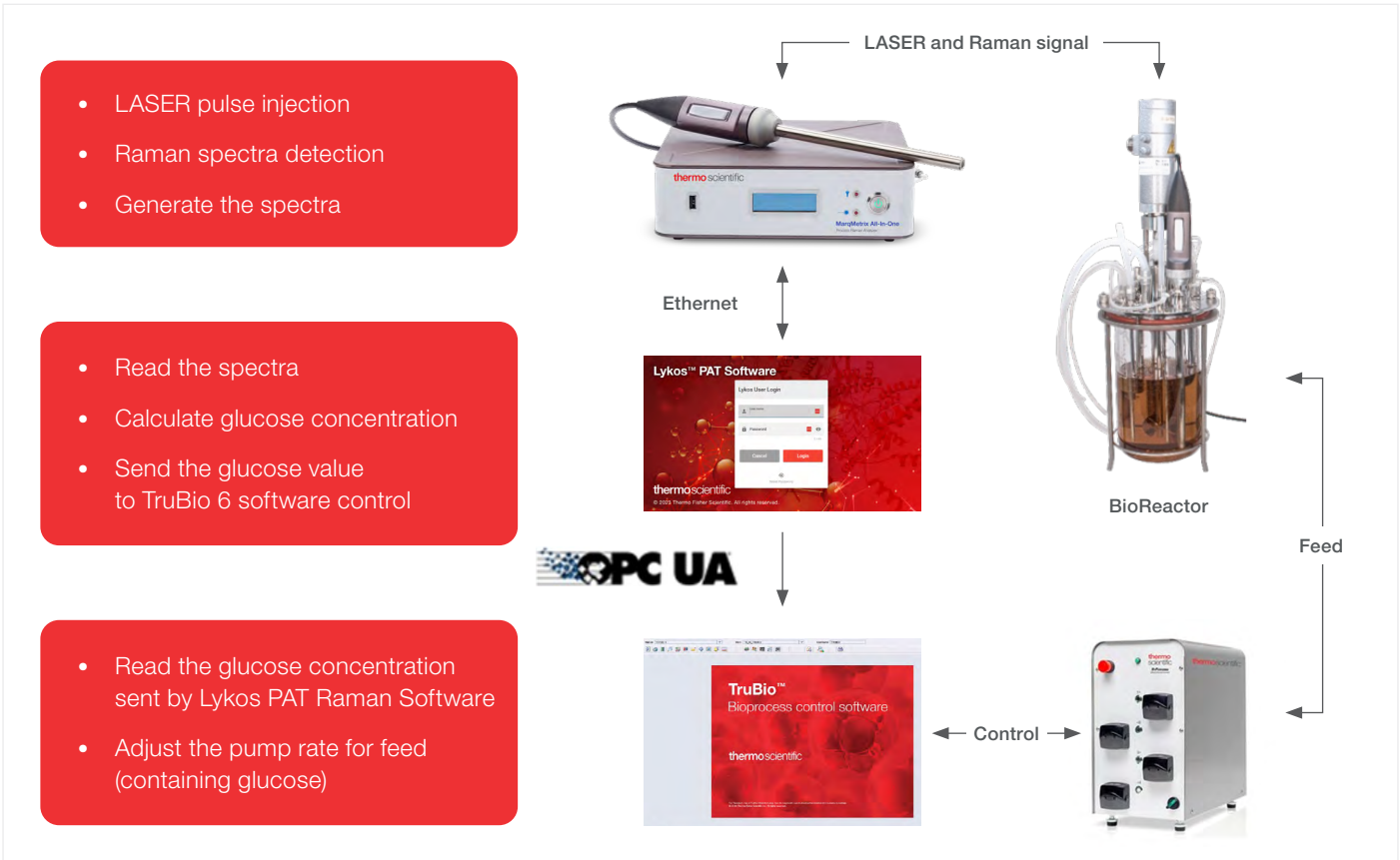
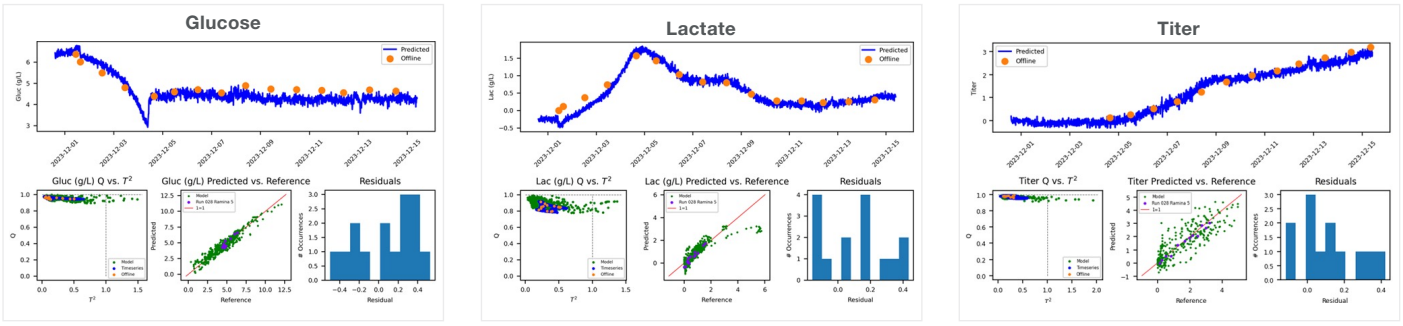


Figure 1B. Process monitoring & control.

Results

During the cell culture process, glucose was consumed by the cells, and concentration levels stabilized at around 3 g/L for the fed-batch culture and around 4 g/L for the perfusion reactor. Figures 2A & B show that once the target glucose concentration was reached, the glucose concentrations were precisely maintained at 3 g/L and 4 g/L for the fed-batch and perfusion cultures, respectively. Traditional offline samples were collected to measure the glucose and lactate concentrations as controls to compare the accuracy of the Raman analyzer measurements.

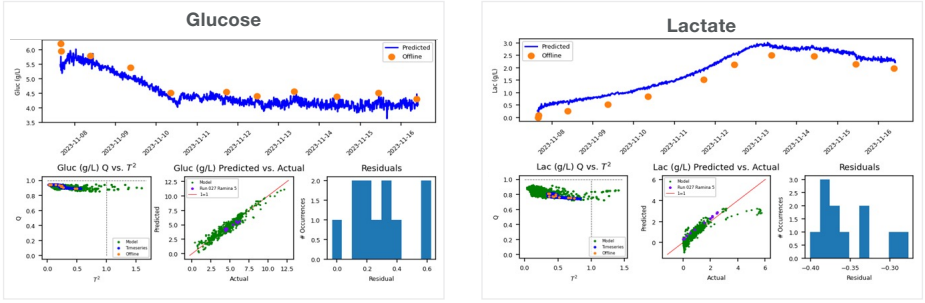


Correlation of model prediction with offline data

Metabolite/variable predicted	R^2 predicted	RMSEP
Glucose (g/L)	0.890	0.315
Lactate (g/L)	0.929	0.223
Titer (g/L)	0.980	0.200

Figure 2A. Fed-batch

Data from real-time control of glucose and monitoring of lactate and titer for a fed-batch culture are presented here.



Correlation of model prediction with offline data

Metabolite/variable predicted	R^2 predicted	RMSEP
Glucose (g/L)	0.95	0.34
Lactate (g/L)	1.00	0.36

Figure 2B. Perfusion

Data from real-time control of glucose and monitoring of lactate for a perfusion culture are presented here.

Discussion

In the above-described experiments, process data was monitored by Lykos PAT software and easily integrated with TruBio bioprocess control software via an OPC-UA protocol. The control loop demonstrated a very stable glucose concentration, which was achieved along with accurate measurements by the MarqMetrix All-In-One Process Raman Analyzer. Automating the process, as demonstrated, significantly reduced the intervention of the operator, who typically must sample the bioreactor for offline or at-line analyses. Because of this automation, the risk of contamination and batch rejection relating to operator error and other deviations is significantly reduced. In this application note we demonstrated a culture environment that can ensure maximum productivity in a reproducible manner using an automated feedback control loop. With real-time process monitoring by Thermo Fisher Scientific's MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Raman Software and feedback control enabled by TruBio 6.0 Bioprocess Control Software, we have demonstrated a real-world application of PAT and the ability to implement a process developed with a quality-by-design approach.

References

1. Ghaderi D, Zhang M, Hurtado-Ziola N, et al. Production platforms for biotherapeutic glycoproteins. Occurrence, impact, and challenges of non-human sialylation. Biotechnol Genet Eng Rev. 2012; 28:147–75.
2. Durocher Y, Butler M. Expression systems for therapeutic glycoprotein production. Curr Opin Biotechnol. 2009; 20:700–7.
3. Swiech K, Picanco-Castro V, Covas DT. Human cells: new platform for recombinant therapeutic protein production. Protein Expr Purif. 2012; 84:147–53.
4. Research, C. for D. E. and. PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. U.S. Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance (accessed 2023-03-21).
5. Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. Nicholas R Abu-Absi, Brian M Kenty, Maryann Ehly Cuellar, Michael C Borys, Sivakesava Sakhamuri, David J Strachan, Michael C Hausladen, Zheng Jian Li. Biotechnol Bioeng. 2011 May;108(5):1215-21. doi: 10.1002/bit.23023. Epub 2010 Dec 22.
6. Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Brandon N Berry , Terrence M Dobrowsky , Rebecca C Timson , Rashmi Kshirsagar, Thomas Ryll, Kelly Wiltberger Biotechnol Prog 2016 Jan-Feb;32(1):224-34. doi: 10.1002/btpr.2205. Epub 2015 Dec 21.
7. Real-time metabolite monitoring using the Thermo Scientific™ Ramina™ Process Analyzer System. Juan Villa, Mathew Zustiak, Elizabeth Amoako, David Kuntz, Lin Zhang and Kevin Broadbelt (Thermo Fisher Scientific).
8. Real-time metabolite monitoring using the Thermo Scientific™ Ramina™ Process Analyzer System and the Thermo Scientific™ 500L HyPerforma™ Dynadrive™ Single-Use Bioreactor (S.U.B.). Juan Villa, Mathew Zustiak, Elizabeth Amoako, David Kuntz, Lin Zhang and Kevin Broadbelt (Thermo Fisher Scientific).
9. Lange, J et al., Frank Iwanitz, and Thomas Burke, OPC From Data Access to Unified Architecture, 4th rev. Ed., OPC Foundation – Softing (VDE Verlag GMBH, 2010.

Application note | AN1506

Enhancing monoclonal antibody yield and quality through automated multi-component feedback control loops using the MarqMetrix All-In-One Process Raman Analyzer

Authors

Juan Villa,[1]
PhD, Bioprocess Scientist

Nimesh Khadka,[2]
PhD, Senior Application Specialist

Lin Zhang,[2]
PhD, Manager of Algorithm and Chemometric Development

David Ramirez,[1]
PhD, Staff Engineer

Matthew Zustiak,[1]
PhD, Director of Bioproduction Collaboration Center

[1] Thermo Fisher Scientific, St. Louis, Missouri USA
[2] Thermo Fisher Scientific, Tewksbury, Massachusetts USA

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.

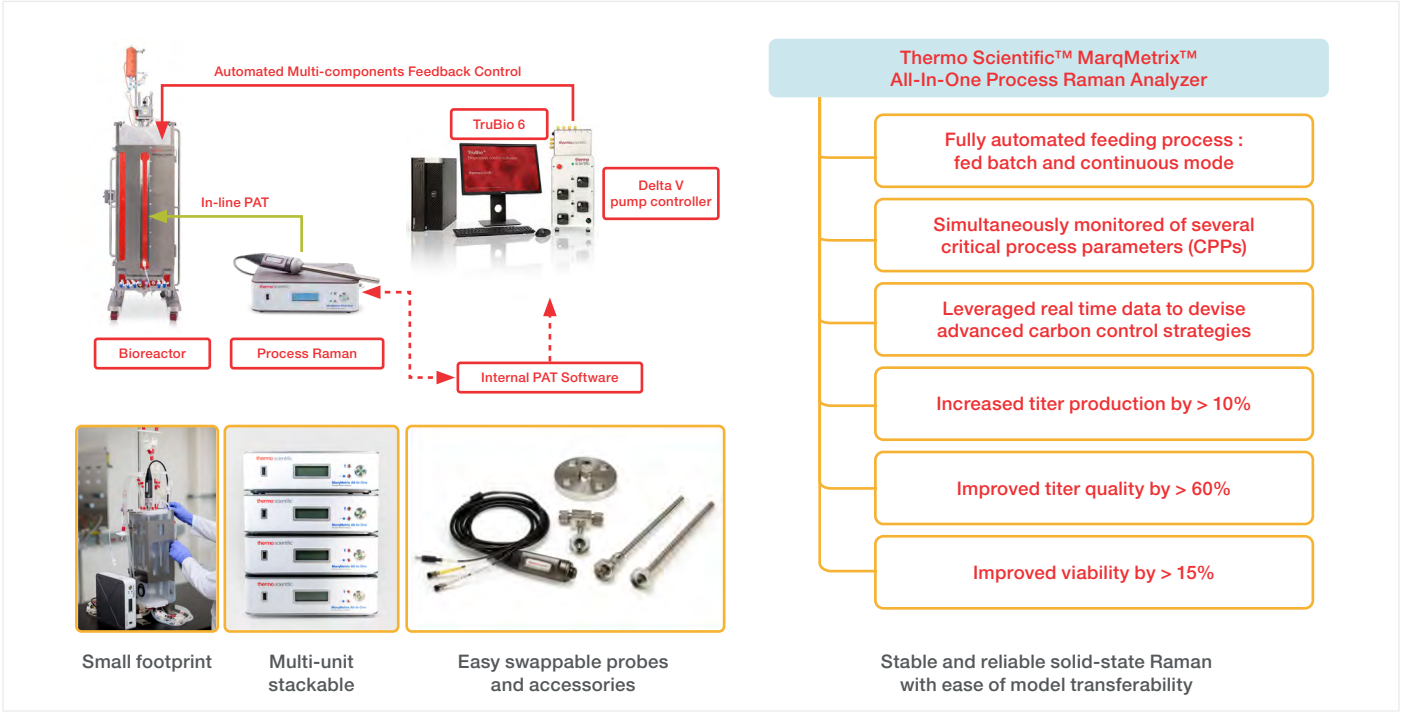


Figure 1. Benefits of the MarqMetrix All-In-One Process Raman Analyzer (shown integrated with a 5L DynaDrive Bioreactor, (bottom left). The system enables real-time, in-line monitoring of multiple analytes to support intelligent, automated bioprocess control.

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).

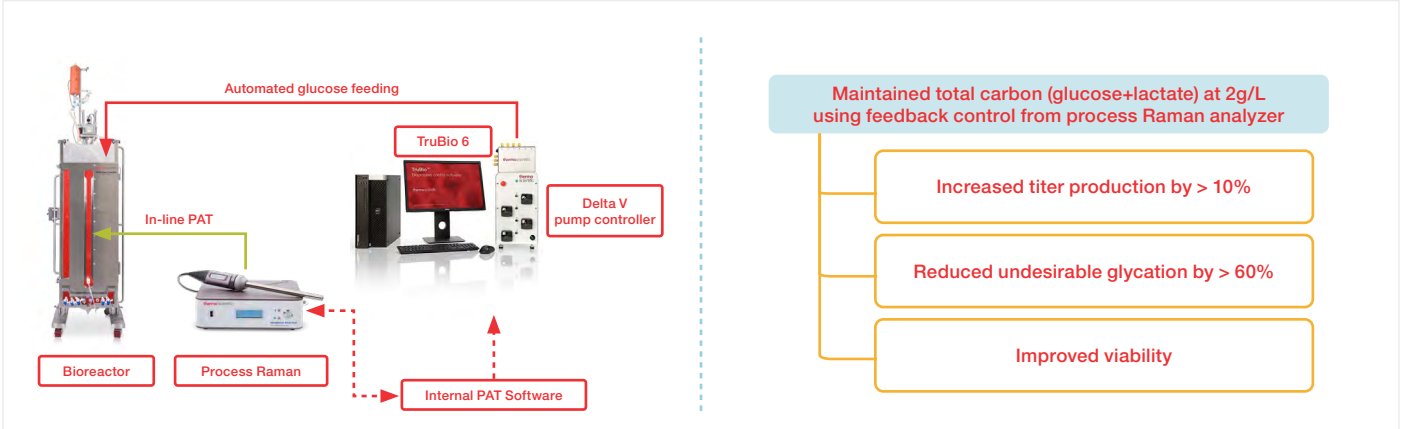


Figure 2. Demonstrating advance total carbon (glucose +lactate) control using inline process Raman leading to enhancement in titer production by more than 10%, reduction in glycation by more than 60%, and improved percent viability.

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.

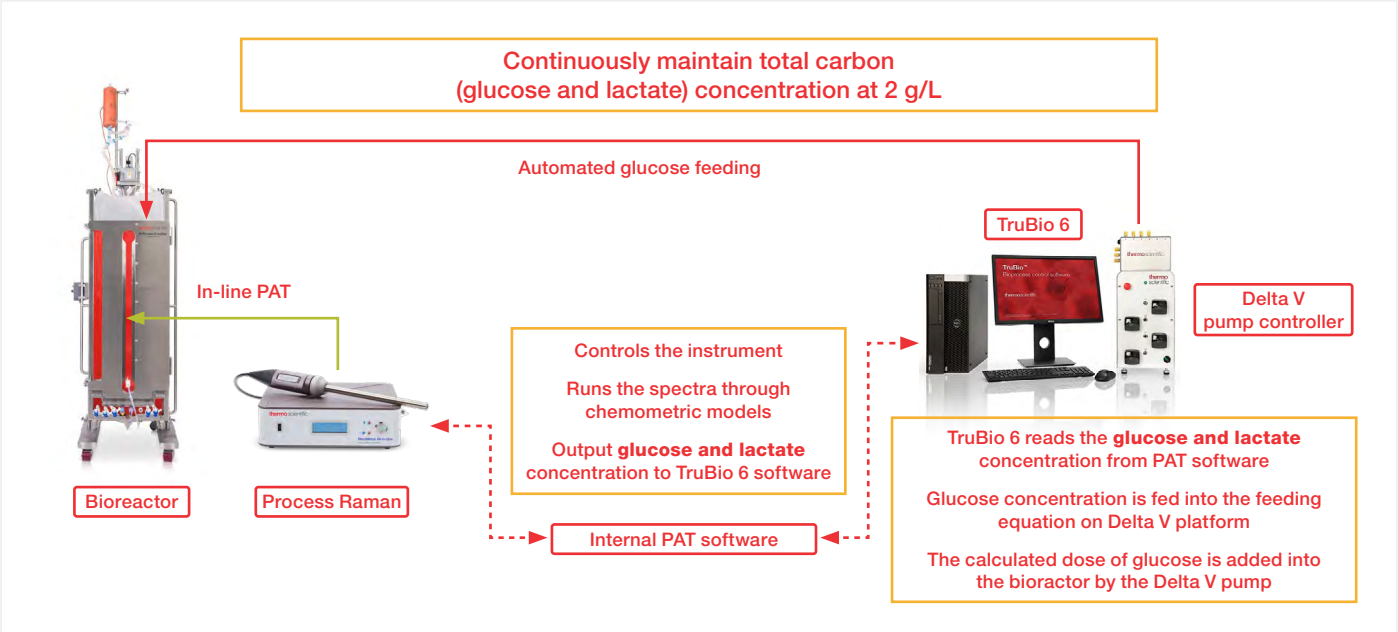


Figure 4. Illustration of the workflow to maintain the total carbon (glucose + lactate) at 2 g/L using feedback control logic. Similar workflow was implemented for other two control logics listed in Table 2.

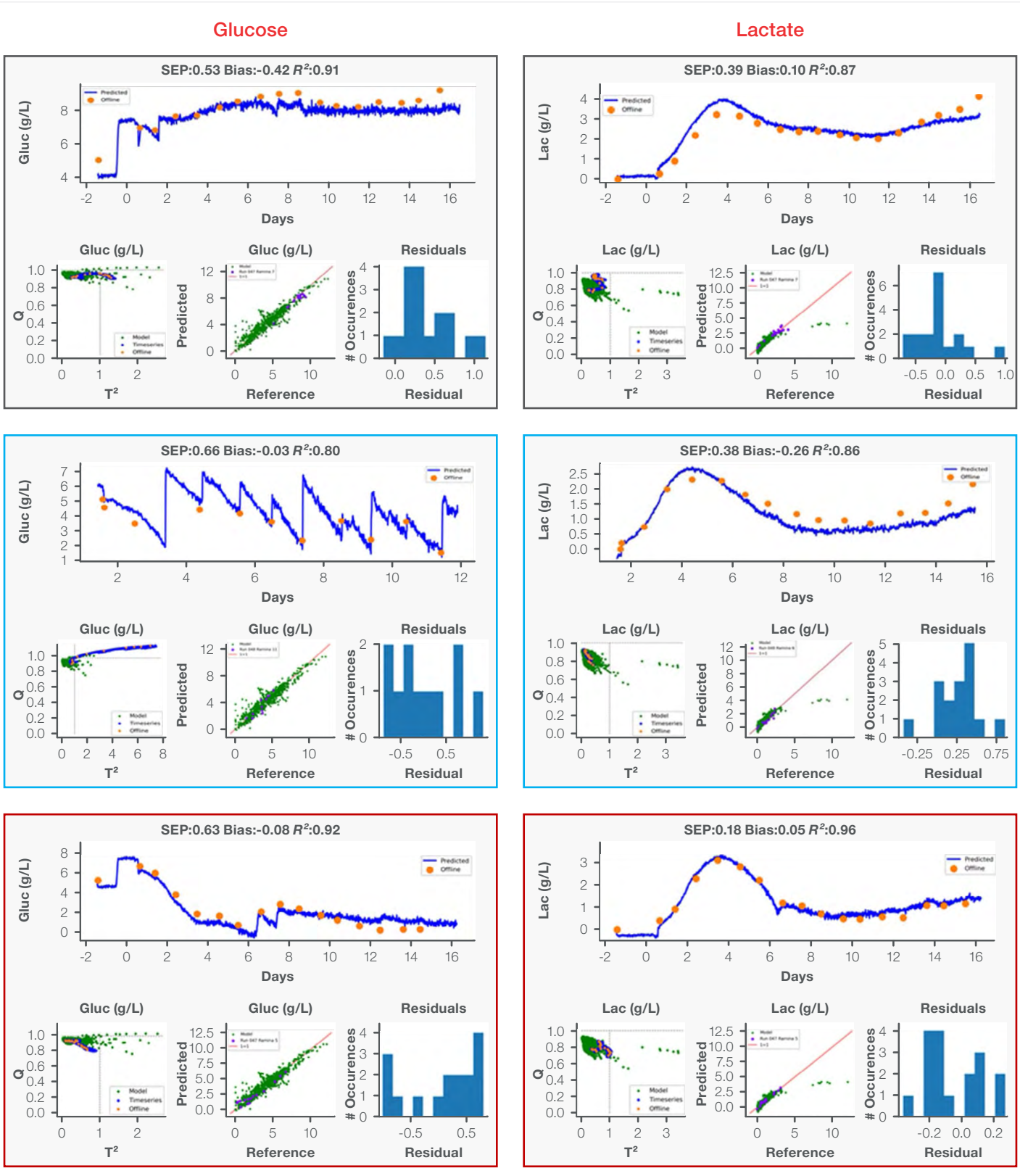


Figure 5. Results for full automated bioreactor runs under the feedback control of process Raman analyzer carried out using three different control logics highlighted in Table 2. Three different glucose controls were compared: Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch 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.

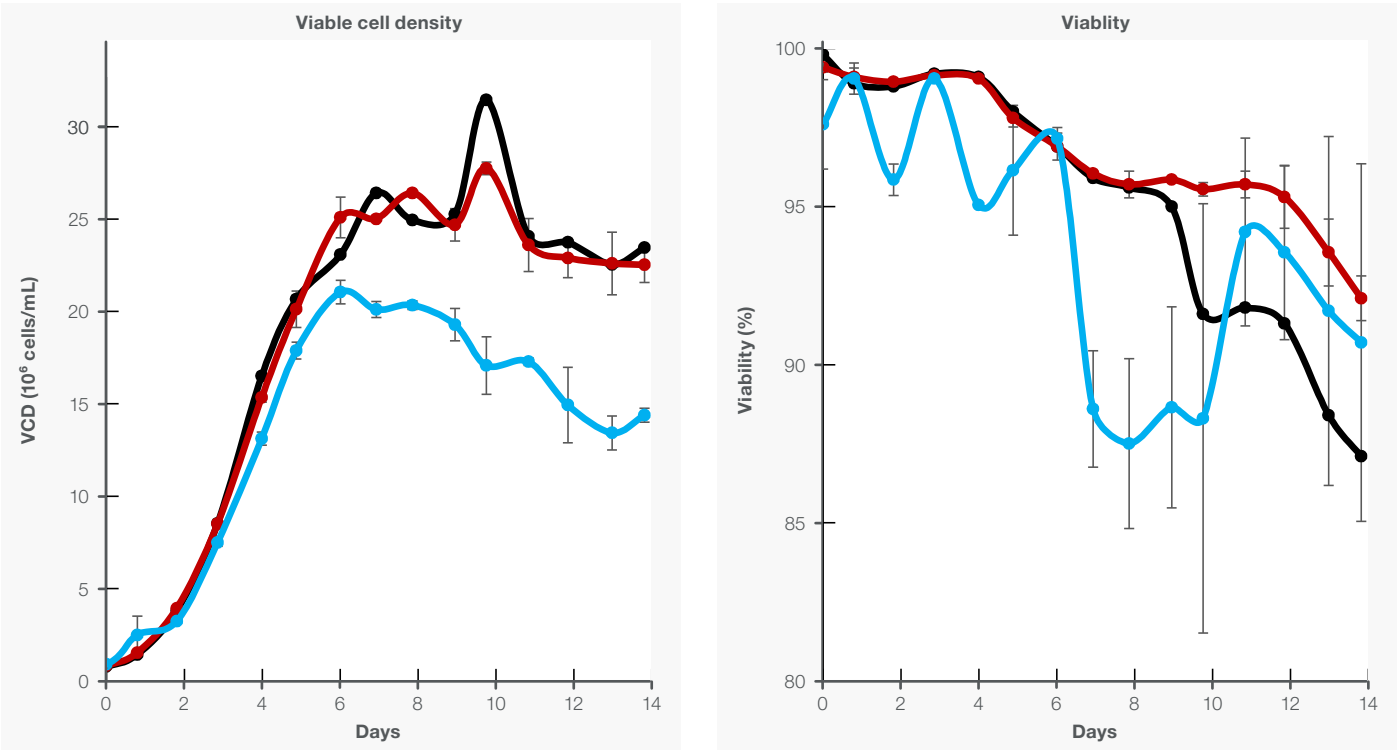


Figure 6. Showing viable cell density (VCD) and viability from reference measurement. Three different glucose controls were compared: Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate)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

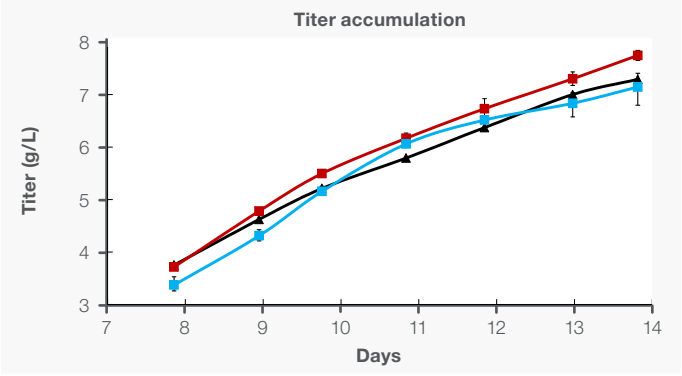


Figure 7. Showing enhancement in titer production by implementing advanced total carbon control through feedback control form process Raman. Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose +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

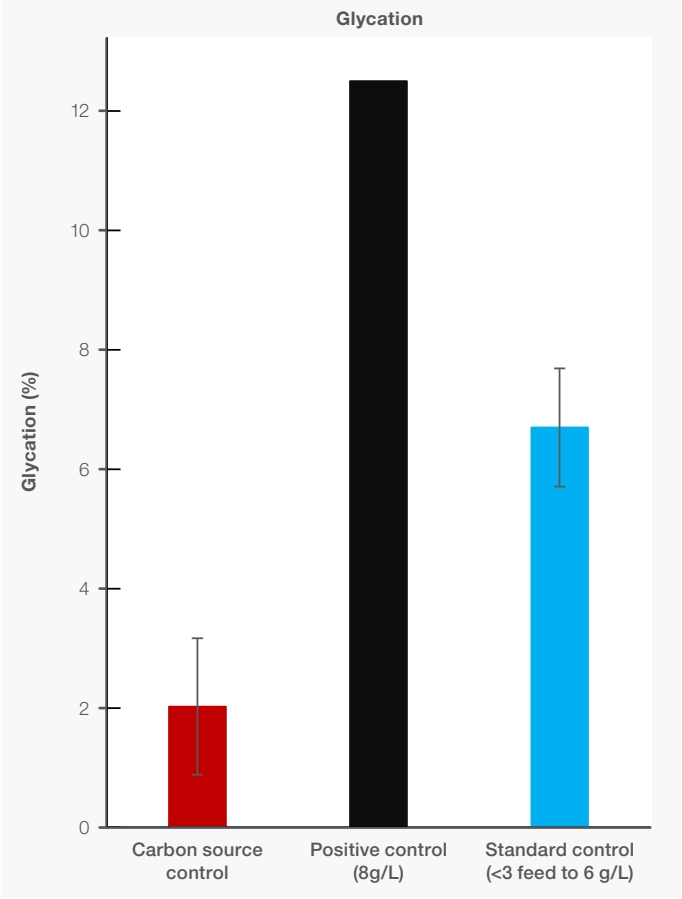
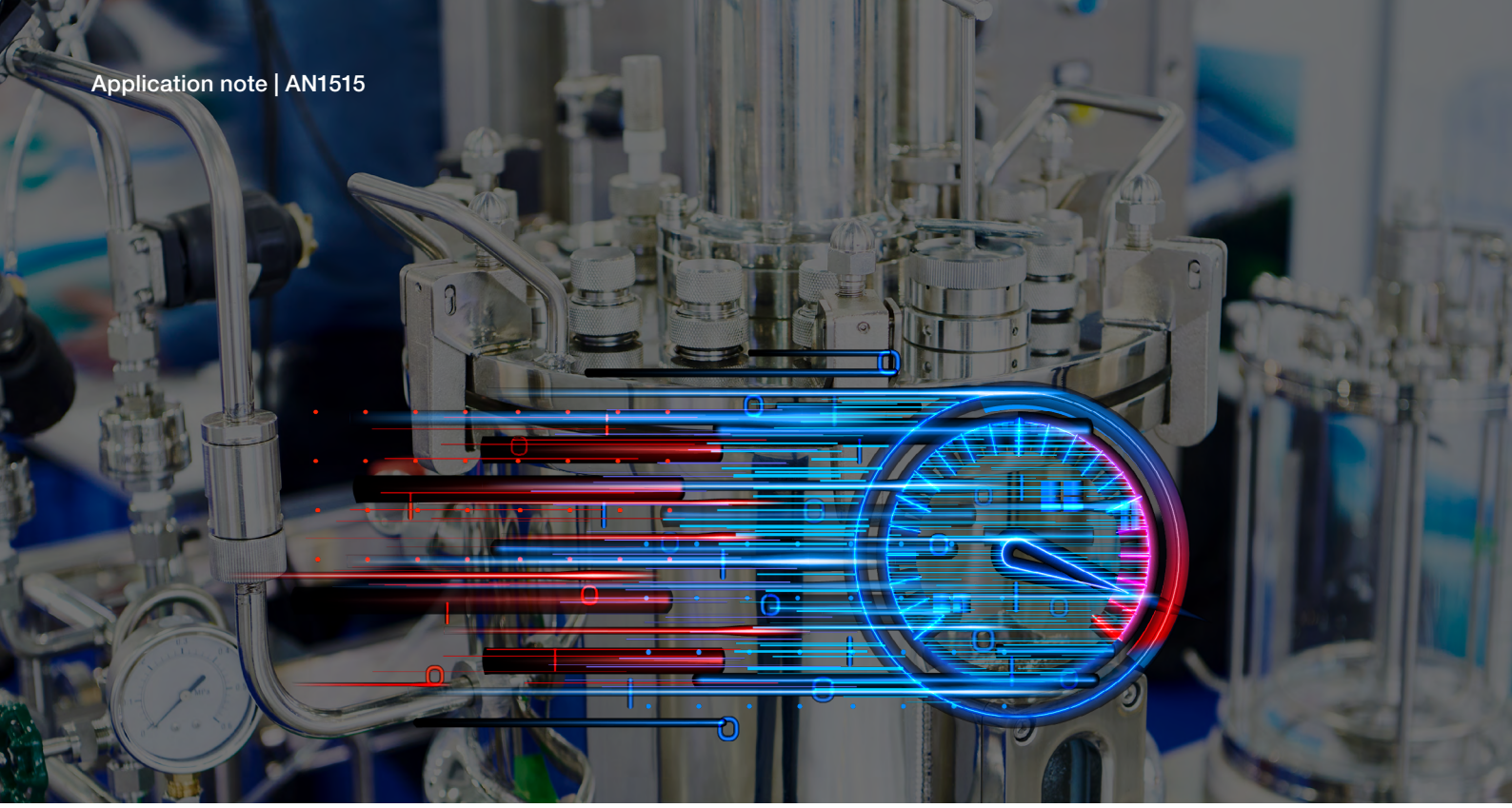


Figure 8. Significant reduction in glycation by maintaining the total carbon (glucose+ lactate) at 2 g/L. Black, automated continuous glucose control at 8 g/L, Blue, automated fed-batch standard glucose control 3 to 6 g/L, and Red, automated total carbon (glucose + lactate) control maintaining at 2 g/L.

References:

3. Abu-Absi, N. R.; Kenty, B. M.; Cuellar, M. E.; Borys, M. C.; Sakhamuri, S.; Strachan, D. J.; Hausladen, M. C.; Li, Z. J. Real Time Monitoring of Multiple Parameters in Mammalian Cell Culture Bioreactors Using an In-Line Raman Spectroscopy Probe. *Biotechnol. Bioeng.* **2011**, *108* (5), 1215–1221. https://doi.org/10.1002/bit.23023.
4. Zhou, M.; Crawford, Y.; Ng, D.; Tung, J.; Pynn, A. F. J.; Meier, A.; Yuk, I. H.; Vijayasankaran, N.; Leach, K.; Joly, J.; Snedecor, B.; Shen, A. Decreasing Lactate Level and Increasing Antibody Production in Chinese Hamster Ovary Cells (CHO) by Reducing the Expression of Lactate Dehydrogenase and Pyruvate Dehydrogenase Kinases. *J. Biotechnol.* 2011, *153* (1), 27–34. https://doi.org/10.1016/j.jbiotec.2011.03.003.
5. Villa, J.; Khadka, N.; Keck, K.; Zhang, L.; Zustiak, M. Process Raman as Platform Solution For Automated Glucose Feeding in Fed-Batch Bioreactors.
6. Villa, J.; Zustiak, M.; Ramirez, D.; Kruger, J.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Demonstrating Chemometric Model Transferability for 5 Mammalian Cell Lines and 5 Media Types Using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer to Monitor Upstream Bioprocesses.
7. Villa, J.; Zustiak, M.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Use of Lykos and TruBio Software Programs for Automated Feedback Control to Monitor and Maintain Glucose Concentrations in Real Time.



Application note | AN1515

Fully automated perfusion bioreactor under the multimodal feedback control using the MarqMetrix All-In-One Process Raman Analyzer

Authors

Juan Villa[1], Matthew Zustiak[1], David Ramirez[1], Lin Zhang[2], Nimesh Khadka[2]

[1]Thermo Fisher Scientific, St. Louis, Missouri USA
[2]Thermo Fisher Scientific, Tewksbury, Massachusetts USA

Industry/application:
Biopharma PAT / Automated Perfusion Bioreactor

Products used:
Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer, Thermo Scientific™ HyPerforma™ Glass Bioreactors (5 L), Glucose and Lactate Core Models, Gibco ExiCHO cell line, High-performance perfusion (HIP) CHO media

Goals:
Demonstrate the integration of process Raman as a single sensor for the multimodal feedback control of glucose feeding, addition of feed media, and cell bleeding in an automated perfusion run without any need of manual intervention.

Key analytes:
Glucose, Lacate, and Viable Cell Density (VCD)

Key benefits:

- Reduction of time and error achieved with no human intervention required
- Cost benefits attained by eliminating offline analytics
- Complete integration solution allows for simplified implementation
- Deeper process insight enables tighter process control and product uniformity

Figure 1. MarqMetrix All-In-One Process Raman Analyzer with 5 L DynaDrive Single-Use Bioreactor

Significance

In this study, we successfully demonstrated the integration of process Raman (Figure 1) as a single sensor for the multimodal feedback control of glucose feeding, addition of feed media, and cell bleeding in a perfusion run without any need of manual intervention. Such capabilities provide users with the potential of transforming biopharmaceutical manufacturing toward full automation, benefiting from tighter process control, reducing batch-to-batch variability, and ensuring product uniformity and quality. The work presented here also demonstrates the accuracy and reliability of real-time prediction of multiple critical process parameters from process Raman. This offers significant cost and time benefits to the user by eliminating the need for laboratory analytics, reducing the risk of contamination, enabling real time actionable decision-making, and mitigating human errors. Thus, this study establishes process Raman as a viable and reliable process analytical technology (PAT) for monitoring and automated control of bioreactor.

Introduction

Process Raman spectroscopy is an optical analytical technique that relies on the inelastic Raman scattering phenomenon.[1] Over the past few years, it has gained widespread adoption in the biopharmaceutical industry as a reliable process analytical technology for real-time monitoring and control of bioprocesses.[2] This technique offers numerous advantages over other spectroscopic methods, making it particularly well-suited for bioprocess applications. Some of the key advantages include its high molecular specificity, rapid analysis capabilities, minimal interference from water, nondestructive, in-line measurements, simplified spectral interpretation, and the elimination of sample preparation requirements.[3] All these features make process Raman ideally suited for monitoring and control of complex biological processes that occurs in aqueous medium.

A perfusion run is one class of bioreactor setup that enables users to achieve high cell density and sustained cell viability for an extended period.[4] It is widely used in biopharmaceutical and biotechnological processes to produce monoclonal antibodies, therapeutic proteins, cell and gene therapeutics, cultivated meat, and other products.[5] A typical perfusion run is shown in Figure 2A. When cells grow, the nutrients are consumed and must be replenished through the addition of fresh media to sustain growth. The used media not only contains metabolic byproducts and residual nutrients but also the soluble target product (*e.g.*, monoclonal antibodies) which is continuously removed from the bioreactor through the action of recirculatory and harvest pumping, and collected or diverted for further downstream processing. After separation of spent media via filtration, the residual media and cells are recirculated back to the bioreactor. The collected spent media can be processed through a series of downstream steps to purify and concentrate the target product. This progressive removal of spent media causes a reduction of reactor culture. The reduction is balanced by the addition of the equivalent mass of fresh feed media into the bioreactor through the feed pump. Thus, the total mass of cell culture during the whole the perfusion run is maintained at a constant level. Fresh feed media supplies the essential nutrients to sustain high cell growth. Throughout the perfusion run, the feed and harvest pump function continuously to maintain optimum nutrients that enable the development of high cell density and extended sustainability.

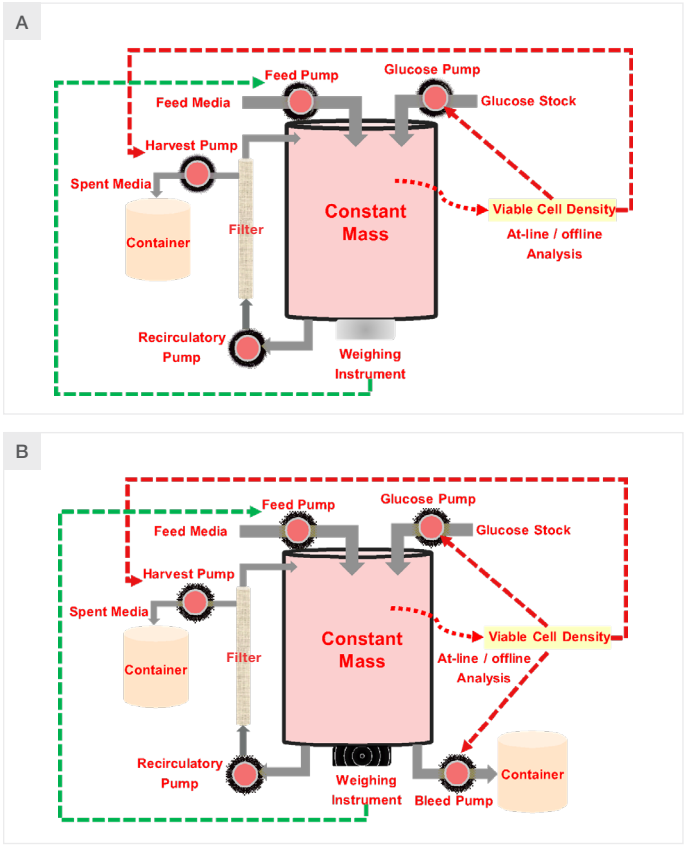


Figure 2. Different operation modes of the perfusion run. A normal perfusion run is shown in (A) while an additional version with bleed pump is shown in (B). The dotted red line shows dependence of glucose, bleed, and harvest pumps on viable cell density while green dotted line shows weight depended on feed pump.

The rate at which spent media is removed during a perfusion run is determined by the viable cell density (VCD). Currently, in practice, the analyst collects daily samples from the bioreactor and performs at-line or offline analysis to measure the VCD. Once the VCD is determined, the analyst follows an empirically defined perfusion protocol to calculate the required rate of flow on the harvest pump. This calculation considers factors such as the VCD, doubling time of cells, and feeding rate of cells (feed volume per cell per day). The calculated rate is then manually entered to control the speed of the harvest pump (Figure 2, red dotted arrow). As the mass of the bioreactor decreases due to the removal of spent media, the feed pump is activated through a feedback control loop of a weighing instrument to maintain a constant mass (Figure 2, green dotted arrow). Since the VCD changes every day during the run, the analyst must perform a VCD measurement each day throughout the duration of perfusion run before making any manual adjustment to the harvest pump.

Figure 2B illustrates a variation of the regular perfusion run setup. In addition to the components shown in Figure 2A, there is an additional bleed pump integrated into the system to remove cells and media from the bioreactor. This process, known as cell bleeding, enables the user to maintain the cell density at a specific threshold using a bleeding feedback control. In the current practice, analysts perform at-line or offline VCD measurements before manually performing cell bleeding.

Another important variable to maintain homeostasis in a perfusion run is the concentration of glucose, which serves as the main energy source for cells. Glucose is introduced into the bioreactor either continuously or in bolus mode using a glucose pump. In the current practice, analysts collect samples and measure the residual glucose levels and VCD. The VCD is then mathematically processed using an empirically defined glucose consumption rate per cell per day. By subtracting the residual glucose in the bioreactor, a new rate for the glucose pump is determined for each day. As a result, the rate of the glucose pump is also dependent on the measured VCD (as depicted in Figure 2 with a red dotted arrow).

There are several limitations of the current approach as highlighted below:

1. Cost, time, and error: The current practice requires dedicated resources throughout the entire duration of the run, which can last for weeks to months. This approach requires the need for sampling, laboratory analysis, and manual adjustment of the pumps. Sampling from an aseptic process increases the risk of contamination. Relying on laboratory data for decision-making adds time and operational costs. Additionally, frequent human intervention introduces the risk of errors in an already complex and expensive process.
2. Lack of real time control: The use of empirical values for cell doubling time and nutrient consumption rate per cell per day, assuming their constancy until the next measurement, can have detrimental consequences, potentially leading to the loss of the entire run. Cell doubling time and nutrient consumption rates depend on multiple factors and can vary significantly at different instances both within a run and between different runs. This approach can result in underfeeding when the true VCD exceeds the expected VCD, or overfeeding when the true VCD is below the expected VCD. Both scenarios are suboptimal for ensuring uniformity and product quality.
3. Lack of tight process control: Recent studies have shown that tightly controlling metabolite concentrations at predetermined levels can improve both the quantity and quality of the product. For example, maintaining a steady low glucose concentration may reduce unwanted glycation of monoclonal antibodies,[6] while maintaining constant amino acid concentrations throughout the run can enhance monoclonal antibody yield.[7] Achieving steady metabolite concentrations using the conventional approach is nearly impossible.

To overcome these limitations and transform biopharmaceutical manufacturing as highlighted in the process analytical technology (PAT) guidance by FDA,[8] we demonstrate an alternative approach of carrying out perfusion run with next generation automation through the integration of the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer as shown in Figure 3. As we demonstrated previously, in-line process Raman accurately predicted the concentration of glucose in the bioreactor.[9] These predictions were leveraged as feedback response to control glucose pump and maintain the desired concentration of glucose throughout the bioreactor run. Similarly, we have further demonstrated the use of process Raman based feedback control of bleed pump to maintain the viable cell density (VCD) in perfusion run.[10] In this proof-of-concept (PoC) study, we demonstrate the automated perfusion run using multimodal feedback controls for glucose feeding, supplementing feed media, and cell bleeding. Such automation of perfusion run by process Raman affords the user several advantages over the conventional approach as detailed in Table 1.

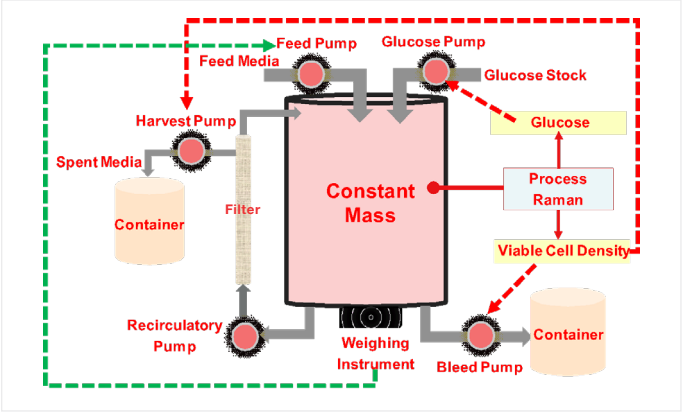


Figure 3. Demonstrating integration of process Raman for the feedback control for feeding glucose, feed (fresh) media, and carrying our cell bleeding.

Pump	Operation	Current practice	In-line Raman approach	Advantages of in-line Raman
Glucose pump	Feeds glucose (energy source)	Pull the sample and perform at-line measurement for VCD and residual glucose; manually adjust the pump to reach desired glucose concentration.	Directly measures glucose concentration in bioreactor and adjust the pump rate in real time as a feedback control automatically.	i. Eliminates cost of laboratory analytics ii. Reduces risk of contamination associated with sampling iii. Allows tighter and dynamic control of the glucose pump by using frequent data points iv. Allows to maintain glucose at target concentration to improve product quantity and quality.
Bleed pump	Removes cells and media from bioreactor	Pull the sample and perform at-line analytics to measure VCD and growth rate. Determine the volume that needs to be removed via bleed pump to reach desired VCD.	Directly measures VCD and provides dynamic and automated feedback control of the bleed pump.	Similar as above.
Harvest pump	Removes spent media	Carry out at-line VCD measurement after sampling and use the empirical feeding protocol to determine the rate of harvest pump.	Directly measures VCD which is multiplied by feed rate per cell per day to calculate harvest volume. The calculated harvest volume per day volume is used as real time feedback control to set the rate of the harvest pump.	Similar as above, and v. Unlike conventional approach that depends on empirical estimation of VCD, inline Raman allows a tighter and dynamic process control in real time through feedback control by directly measuring VCD. vi. Provides unprecedented “real time continuous process control” in contrast to “single point process control” in conventional approach
Feed Pump	Adds fresh media (vitamins, amino acids, and other nutrients) to the bioreactor	It is controlled by the feedback control of the reactor weight.	It is under weight control as conventional practice but also indirectly controlled by process Raman.	vii. Can be controlled more tightly as the feedback from process Raman are frequent, done in real time, and based on direct measurement.

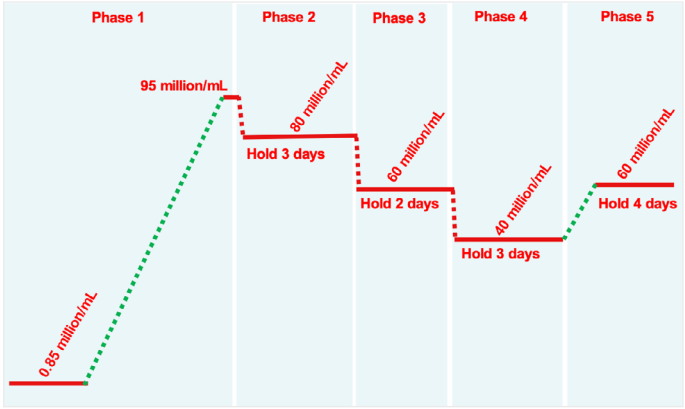
Table 1. Advantages of in-line process Raman for perfusion run.

Experimental details

Perfusion run set up. The perfusion run was carried out in Thermo Scientific™ HyPerforma™ Glass Bioreactors using a Gibco ExpiCHOs cell line expressing a mono-clonal antibody cultured in Gibco HIPCHO medium for both batch and perfusion phases. Several control logics were implemented for automated feedback controls as discussed below.

i. Glucose: The logic for automated feedback control for glucose was set to maintain the glucose concentration at 3 g/L for the entire run. The glucose concentration was continuously monitored using the MarqMetrix All-In-One Process Raman Analyzer. The real-time predicted glucose concentration from "glucose core model" was communicated to the Thermo Scientific™ TruBio™ Bioprocess Control Software using an OPC-UA platform, which in turn controlled the glucose pump. The initial glucose concentration was ~ 5 g/L. As the cell grew, the glucose was consumed, and the concentration dropped. When the predicted glucose concentration by process Raman was below 3 g/L, the feedback control for glucose pump was activated (Figure 3; red dotted arrow). The glucose was added to the bioreactor from the stock solution (450 g/L) to maintain the constant glucose concentration of ~ 3 g/L for the entire run.

ii. Viable Cell Density: The automated VCD feedback control was performed using real-time predictions from process Raman (Figure 3; dotted red arrow) in five phases as shown in Schematics 1 and Table 2. The initial inoculum of ExiCHOs cell line was 0.6 10^6/mL. The cells were allowed to grow until they reached the density of 95 10^6/mL (green dotted line) in phase 1. In this period, the bleed pump is inactive while the feed pump and the harvest pump were operating in continuous mode and adjusted daily to maintain an average cell specific feed rate while maintaining the constant mass of 2.3 kg. When the Raman model predicted the VCD of 95 x 10^6/mL, the feedback control was activated to control the VCD at 80 x 10^6/mL for 3 days as phase 2. After 3rd day of phase 2, phase 3 was initiated. In phase 3, the feedback logic was set to maintain the VCD at 60 x 10^6/mL for 2 days. In phase 4, the VCD was set to maintain at 40 x 10^6/mL for 3 days. Finally, in phase 5, the cells were set to maintain a VCD of 60 x 10^6/mL for 4 days. The control of the VCD was maintained by automated feedback control and synchronous action of the bleed pump, feed pump and harvest pump.



Schematics 1. VCD control logic for the entire perfusion run.

Phase	Density (10^6/mL)	Hold duration (days)
1	Cell growth 0.85 to 95	0
2	Cell bleed to 80	3
3	Cell bleed to 60	2
4	Cell bleed to 40	3
5	Cell growth to 60	4

Table 2. Density and duration of the run.

iii. Spent media: The predicted VCD by process Raman was used as the input response for automated feedback control for the harvest pump, as shown in Figure 3 (dotted red arrow). The feed rate was set to 60 pL/cell/day by controlling the spent media harvest pump flow rate.

iv. Feed media: The feed pump was under the feedback control of the weighing instrument as shown in Figure 3 (dotted green arrow). The decrease in mass due to removal of spent media activated the feed pump to add fresh feed media to the bioreactor in order to maintain the constant mass of 2.3 kg.

Chemometric models: The glucose and lactate PLS "core" models were developed as described previously.[11] The total cell density (TCD) and VCD PLS models were developed using similar strategy using the training Raman data collected only on perfusion runs. Table 3 summarizes the number of latent variables and spectral region selected and preprocessing methods used to develop glucose, lactate, and VCD model.

Analyte	Model type	No. of latent variables	Region selection cm^{-1}	Preprocessing
Glucose	PLS	5	1065-1232; 1595-1863; 2704-3078	Sav-Gol filter (1st Derivative; order = 2; Window width = 13) + SNV + Mean Center
Lactate	PLS	5	800 - 1750	Sav-Gol filter (1st Derivative; order = 2; Window width = 11) + L-1 Norm (Area = 1 for 1540-1750 cm^{-1}) + Mean Center
VCD	PLS	2	795-1936	Sav-Gol filter (1st Derivative; order = 2; Window width = 13) + SNV + Mean Center

Table 3. Density and duration of the run.

Results

1. Automated feedback control of glucose feeding: The glucose pump was under the feedback control of Raman for entire run. When the predicted glucose concentration by Raman dropped below 3 g/L, the glucose pump was turned on. Figure 4 shows the result of the automated feedback control of glucose feeding using in-line process Raman and glucose “core” model. The blue trace is the real-time prediction from process Raman while orange dots are the offline reference values. The root mean square error of prediction (RMSEP) for the entire run was 0.52 g/L which is within the tolerance error limit for this process ± 0.5 g/L. The low RMSEP for glucose prediction for entire run demonstrates the accuracy and the reliability of the process Raman for automated feedback control of glucose feeding, eliminating the need of offline sampling and manual intervention. The automated feedback control of glucose feeding was previously demonstrated but not as multimodal feedback control as shown in this study.

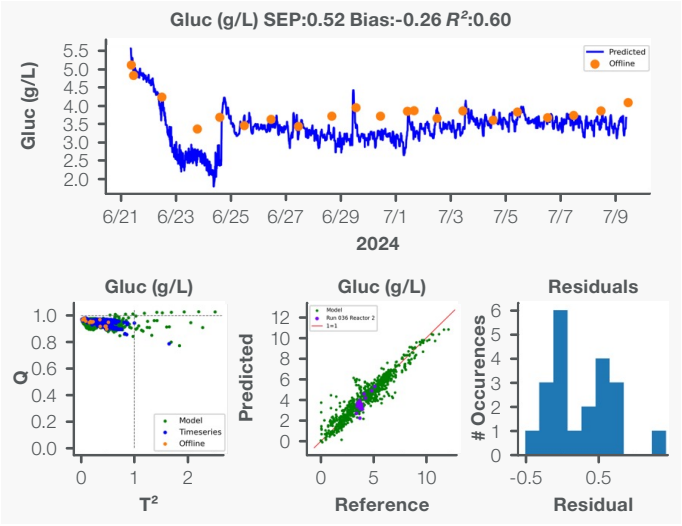


Figure 4. Automated feedback control of glucose feeding using in-line process Raman by maintaining glucose concentration at threshold of 3 g/L throughout the run. The blue trace are predictions form process Raman while orange dots are reference values.

The reduced Q residual and reduced Hotelling T^2 scores were used as criteria to gain statistical confidence on the predicted value. Only the prediction from those Raman spectra that had reduced Q residuals and reduced Hotelling T^2 scores less than 1 were considered reliable for making decisions and were communicated to the pump controller. The spectra that had either reduced Q residuals or reduced Hotelling T^2 scores greater than one were considered outlier and thus the predictions of these spectra were excluded as the feedback control. In this study > 99 % of the data points had reduced Q residuals or reduced Hotelling T^2 scores less than 1, indicating that the predictions from the test data were reliable with 95% confidence.

Automated feedback control of VCD: The results for the automated feedback control of the VCD are shown in Figure 5. The blue trace is the prediction form process Raman while the orange dots are reference measurements. The RMSEP for the VCD was 9.33 x 10^6/mL across the experimental concentration range of 0.63 x 10^6/mL to 95 x 10^6/mL. Like glucose, the test data for entire perfusion run falls within the VCD model space as shown in reduced Q vs reduced T^2 plot, providing statistical confidence to the predicted values.

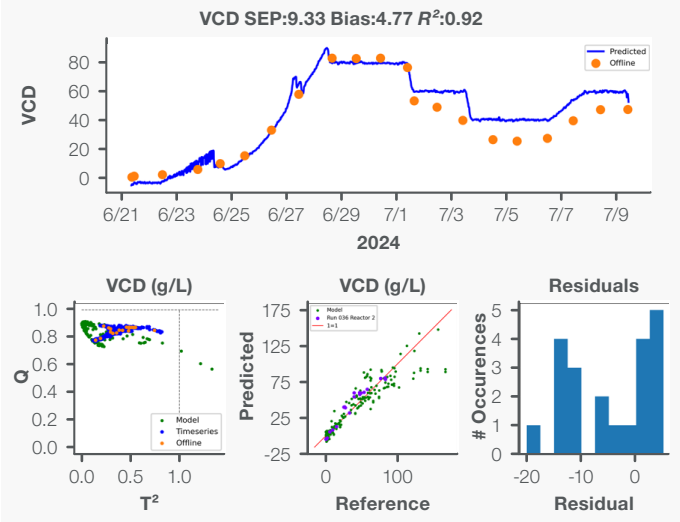


Figure 5. Automated feedback control of VCD. The blue trace are predictions form process Raman while orange dots are reference values.

The initial growth of cells from 0.63 to 95 x 10^6/mL in phase 1 took approximately 7 days. When the Raman prediction exceeded 95 x 10^6/mL, VCD control was enabled which activated the bleed pump to lower the VCD value to 80 x 10^6/mL which was accomplished in approximately 6 hours. In phase 3, the VCD value was further decreased to 60 x 10^6/mL that took about 7 hours of operation of the bleed pump. In phase 4, the VCD value was further lowered to 40 x 10^6/mL by action of the bleed pump operation for approximately 7 hours. Finally, for phase 5, the cells were allowed to grow for a day and the VCD value was maintained at 60 x 10^6/mL for 4 days. The transition time needed for the phase transition is shown in figure 6. All the VCD controls were performed solely based on real-time predictions from process Raman spectroscopy.

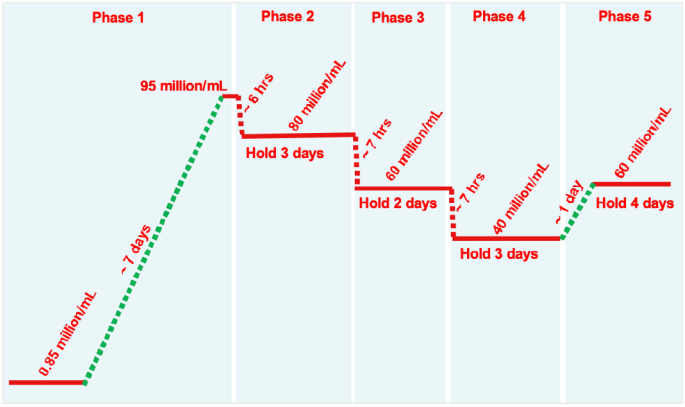


Figure 6. Time profile to reach appropriate VCD.

In the phase 1, the glucose, harvest, and feed pumps were active while after phase 1, the bleed pump was also turned on as shown in Figure 7. All four pumps were in direct or indirect automated feedback control of process Raman without any human intervention.

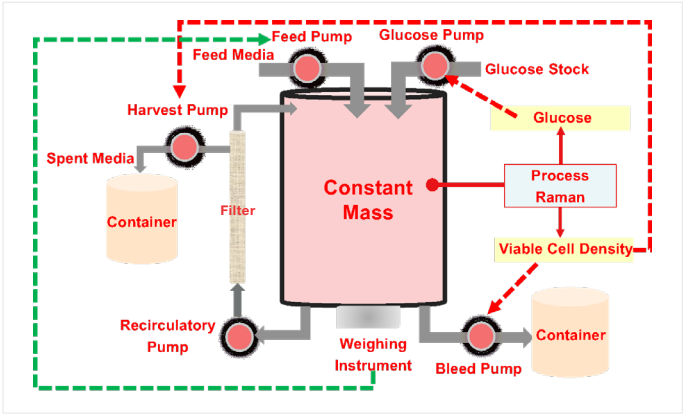


Figure 7. All four pumps were active after phase 1.

2. Performance indicator: In contrast to the conventional perfusion run, this study implemented a novel approach where the glucose pump, harvest pump, bleed pump, and feed pump (indirectly) were operated under the feedback control of the process Raman spectrometer. While we plan to conduct a direct comparison between regular and automated perfusion runs to evaluate the benefits on product quality and quantity, in this study we focused on monitoring the lactate profile. Lactate is a critical process parameter that provides insights into process performance. In a regular perfusion run, lactate is typically produced during the exponential growth phase (first few days), reaching a maximum concentration of approximately 1 to 2 g/L, and then remains constant or decreases for the duration of the run. Elevated lactate production can occur in cases where there is inadequate oxygen supply or inhibition of the citric acid cycle or electron transport chain in cells.[12] Therefore, lactate serves as an indicator of cell health in the bioreactor. In addition, high lactate concentrations are known to have an adverse impact on monoclonal antibody production.[13]

As shown in Figure 8, the lactate production profile in this proof-of-concept study follows the trend observed in regular perfusion runs. The lactate concentration reached a maximum of 2 g/L on day four and remained approximately 1.5 g/L for the remaining days. This result indicates that the multimodal automated feedback control demonstrated in this study provided an optimal condition for cell growth and expansion.

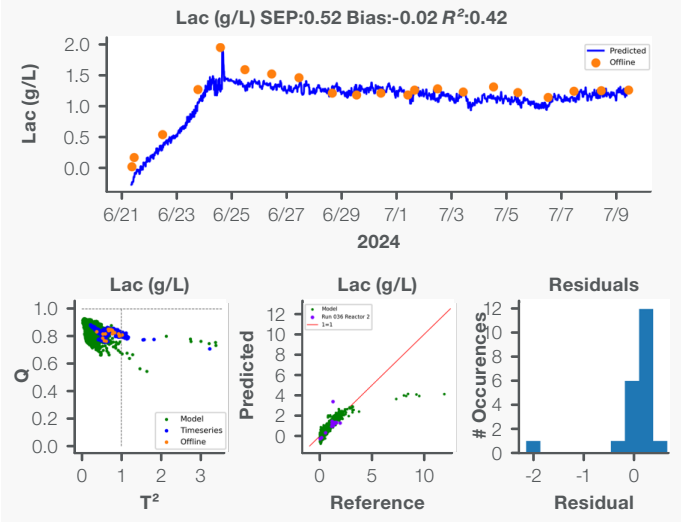


Figure 8. Real-time monitoring of lactate production for the entire perfusion run.

Conclusion

- ✓ In this study, we successfully demonstrated the fully automated feedback control of pumps for glucose feeding, feed media addition, and cell bleeding using in-line process Raman spectroscopy. This multimodal feedback control, facilitated by a single Raman sensor, offers unparalleled advantages in terms of cost, integration, operation, and maintenance compared to utilizing multiple instruments.
- ✓ The entire bioreactor run was operated without any human intervention, except for occasional sampling for reference measurements. The accuracy and reliability of in-line process Raman for automation, as demonstrated in this study, present significant opportunities for the next generation of biomanufacturing. This technology eliminates human errors, the need for sampling, and the risk of contamination, as well as the associated costs and time required for laboratory analysis.
- ✓ The high frequency of Raman data provided in near-real time offers a valuable advantage for bioreactor monitoring compared to other existing at-line, online, or offline technologies. The frequent Raman data allows for a better understanding of process variations over time and enables a tighter control strategy using a feedback loop. This ensures enhanced process control, reduced batch-to-batch variability, and improved product uniformity.

References

1. *Modern Raman Microscopy: Technique and Practice - Cambridge Scholars Publishing*. https://www.cambridgescholars.com/product/978-1-5275-6784-9 (accessed 2024-07-31).
2. Buckley, K.; Ryder, A. G. Applications of Raman Spectroscopy in Biopharmaceutical Manufacturing: A Short Review. *Appl. Spectrosc.* **2017**, 71 (6), 1085–1116. https://doi.org/10.1177/0003702817703270.
3. Esmonde-White, K. A.; Cuellar, M.; Lewis, I. R. The Role of Raman Spectroscopy in Biopharmaceuticals from Development to Manufacturing. *Anal. Bioanal. Chem.* **2022**, 414 (2), 969–991. https://doi.org/10.1007/s00216-021-03727-4.
4. *perfusion-terminology-white-paper.pdf*. https://assets.thermofisher.com/TFS-Assets/BPD/Reference-Materials/perfusion-terminology-white-paper.pdf (accessed 2024-08-03).
5. Matanguihan, C.; Wu, P. Upstream Continuous Processing: Recent Advances in Production of Biopharmaceuticals and Challenges in Manufacturing. *Curr. Opin. Biotechnol.* **2022**, 78, 102828. https://doi.org/10.1016/j.copbio.2022.102828.
6. Yuk, I. H.; Zhang, B.; Yang, Y.; Dutina, G.; Leach, K. D.; Vijayasankaran, N.; Shen, A. Y.; Andersen, D. C.; Snedecor, B. R.; Joly, J. C. Controlling Glycation of Recombinant Antibody in Fed-Batch Cell Cultures. *Biotechnol. Bioeng.* **2011**, 108 (11), 2600–2610. https://doi.org/10.1002/bit.23218.
7. Webster, T. A.; Hadley, B. C.; Dickson, M.; Hodgkins, J.; Olin, M.; Wolnick, N.; Armstrong, J.; Mason, C.; Downey, B. Automated Raman Feed-Back Control of Multiple Supplemental Feeds to Enable an Intensified High Inoculation Density Fed-Batch Platform Process. *Bioprocess Biosyst. Eng.* **2023**, 46 (10), 1457–1470. https://doi.org/10.1007/s00449-023-02912-2.
8. Research, C. for D. E. and. *PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance (accessed 2024-06-19).
9. Villa, J.; Zustiak, M.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Use of Lykos and TruBio Software Programs for Automated Feedback Control to Monitor and Maintain Glucose Concentrations in Real Time.
10. Villa, J.; Zustiak, M.; Martin, A.; Zhang, L.; Khadka, N. A Novel Strategy of Using Process Raman for Feedback Control of Viable Cell Density in Perfusion Cell Culture.
11. Villa, J.; Zustiak, M.; Zhang, L.; Martin, A.; Khadka, N. Reliable and Transferable Raman "Core" Models for Monitoring Bioreactor Using the MarqMetrix All-In-One Process Raman Analyzer.
12. Zagari, F.; Jordan, M.; Stettler, M.; Broly, H.; Wurm, F. M. Lactate Metabolism Shift in CHO Cell Culture: The Role of Mitochondrial Oxidative Activity. *New Biotechnol.* **2013**, 30 (2), 238–245. https://doi.org/10.1016/j.nbt.2012.05.021.
13. Zhou, M.; Crawford, Y.; Ng, D.; Tung, J.; Pynn, A. F. J.; Meier, A.; Yuk, I. H.; Vijayasankaran, N.; Leach, K.; Joly, J.; Snedecor, B.; Shen, A. Decreasing Lactate Level and Increasing Antibody Production in Chinese Hamster Ovary Cells (CHO) by Reducing the Expression of Lactate Dehydrogenase and Pyruvate Dehydrogenase Kinases. *J. Biotechnol.* **2011**, 153 (1), 27–34. https://doi.org/10.1016/j.jbiotec.2011.03.003.

Application note | AN1415

Process Raman as platform solution for automated glucose feeding in fed-batch bioreactors

Authors

Juan Villa[1], PhD, Bioprocess Scientist

Nimesh Khadka[2], PhD,
Senior Application Specialist

Kevin Keck[1], System Design Engineer

Lin Zhang[2], PhD, Manager of Algorithm and Chemometric Development

Matthew Zustiak[1], PhD, Director of Bioproduction Collaboration Center

[1]Thermo Fisher Scientific, St. Louis, Missouri USA
[2]Thermo Fisher Scientific, Tewksbury, Massachusetts USA

Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer and Thermo Scientific MarqMetrix Performance BallProbe Sampling Optic.

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

Premise and goals

Fed-batch bioreactors are widely used in the biopharmaceutical industry, from process development to manufacturing. Many drugs are currently produced using the fed-batch feeding strategy. The timing and amount of bolus glucose feeding in these processes are determined by manual sampling, offline analytics, and pre-established mathematical relationships.

In this study, we aimed to enhance the efficiency of fed-batch bioreactors by integrating in-line process Raman spectroscopy as a process analytical technology (PAT) tool. Leveraging the feedback control from the process Raman, we automated bolus glucose feeding without altering the existing workflow. The automated glucose feeding was performed once a day at a specified time to reach the defined concentration, mimicking the conventional manual fed-batch approach currently in practice.

Key analytes

Glucose feeding in fed-batch bioreactor

Key benefits

- Efficiency is improved by bringing automation to the widely used fed-batch bioreactor operation, with cost benefits achieved through tighter process control, elimination of the need for laboratory analytics, and improved product repeatability and quality.
- Automation increases the reliability of process control by reducing dependence on analysts and minimizing human errors.
- This approach enables the immediate integration of process Raman into already existing processes with monitoring and control capabilities.

Fed-batch bioreactors are widely used in the biopharmaceutical industry for manufacturing various drugs, including monoclonal antibodies.[1] In a fed-batch bioreactor, glucose is added as a carbon and energy source in defined amounts and at specific times, based on data from laboratory analytics. The fed glucose is consumed by growing cells. When the glucose level falls below a set threshold, the next batch of glucose is added. This fed-batch process continues until the end of the bioreactor run.

Although widely used, the conventional fed-batch strategy has several aspects that can be improved to enhance efficiency and reduce costs. Currently, analysts periodically monitor the bioreactor and perform laboratory analytics to make feeding decisions and to track the progress of the run. These manual processes are time-consuming and prone to human error, leading to inconsistencies in product quality and increased costs. For instance, unexpected rapid cell growth during periods when analysts are not present can result in a sudden depletion of glucose, causing batch failure. Similarly, accidental loading of high glucose concentrations during feeding can lead to the production of unwanted glycated products. To prevent such failures while maintaining all the benefits of the established fed-batch process, in-line Raman spectroscopy offers a viable solution. The analytical technique provides real-time measurements to aid automatic feedback, allowing for tighter process control.

Previously we and others have demonstrated process Raman as a reliable tool to maintain constant glucose concentration in a fully automated fashion throughout the bioreactor run.[2,3] In this study (see Figure 1), we showcase the human-free operation of fed-batch bioreactors by automating the bolus feeding of glucose using feedback control from the in-line Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer. The automation workflow mimicked the traditional fed-batch process by feeding glucose once a day at a specified time and dosing to a predefined concentration. To compare the performance of automated and manual fed-batch bioreactors, we evaluated the production of lactate profile, the total quantity of titer (product), and the quality attributes of the titer. Lactate is primarily produced during the incomplete oxidation of glucose and is often used as a marker for measuring oxygen availability, which is directly related to cell health.

Our automated approach enhances process efficiency by eliminating the need for laboratory analytics, resulting in significant cost benefits. Automation ensures product repeatability and quality by providing consistent and accurate feeding decisions in real time. Additionally, it reduces dependence on analysts and minimizes human errors, making process control more reliable. The integration of Raman technology into existing workflows offers advanced multimodal monitoring and control capabilities, paving the way for immediate adoption in biopharmaceutical manufacturing.

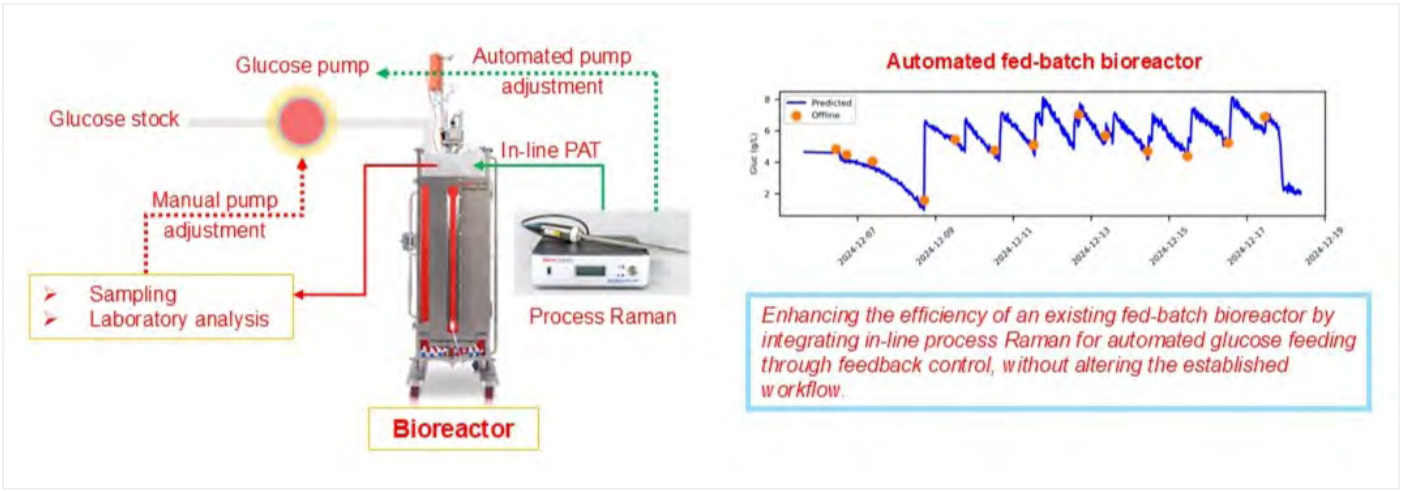


Figure 1. Showing how the manual glucose pump adjustment (red dotted line) workflow in a conventional bioreactor was replaced by automation using in-line process Raman (green dotted line).

Materials and methods

Cell culture

miCHO(TM) CHO-K1 cells (ATUM) were inoculated in Efficient Pro medium supplemented with 1.5 mg/L insulin and 1% anticlumping agent at a density of 0.75 million cells/mL in a 5L 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 a medium specialized for mammalian cells, in standard fed-batch bioreactors, in duplicate, using a Thermo Fisher Scientific platform process for 12 days. The cells were grown in chemically defined medium and fed daily starting from day 3 with a two-step feeding process using Efficient Pro feed 2 and Enhancer.

Raman data acquisition

Real-time Raman data was acquired using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer, integrated with the Thermo Scientific™ MarqMetrix™ Performance BallProbe™ Sampling Optic. The acquisition parameters were set to a power of 450 mW, an integration time of 3000 ms, and an average of 20 scans.

Chemometric model development

Chemometric models were developed using Raman training data collected from previous bioreactors with the same acquisition parameters. The partial least squares (PLS) algorithm was used to develop the glucose and lactate models.

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}; this peak is 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 biomolecules.

The selected spectral regions were preprocessed in the following order:

1. *Savitzky-Golay (Sav-Gol) filter (1st derivative, order = 2, window width = 13)*
2. *Standard Normal Variate (SNV)*
3. *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 optimum LVs—those that minimized the root mean square error of calibration and cross validation while maintaining their ratio close to 1—were selected.

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

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. Spectral regions and preprocessing used for model development.

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).

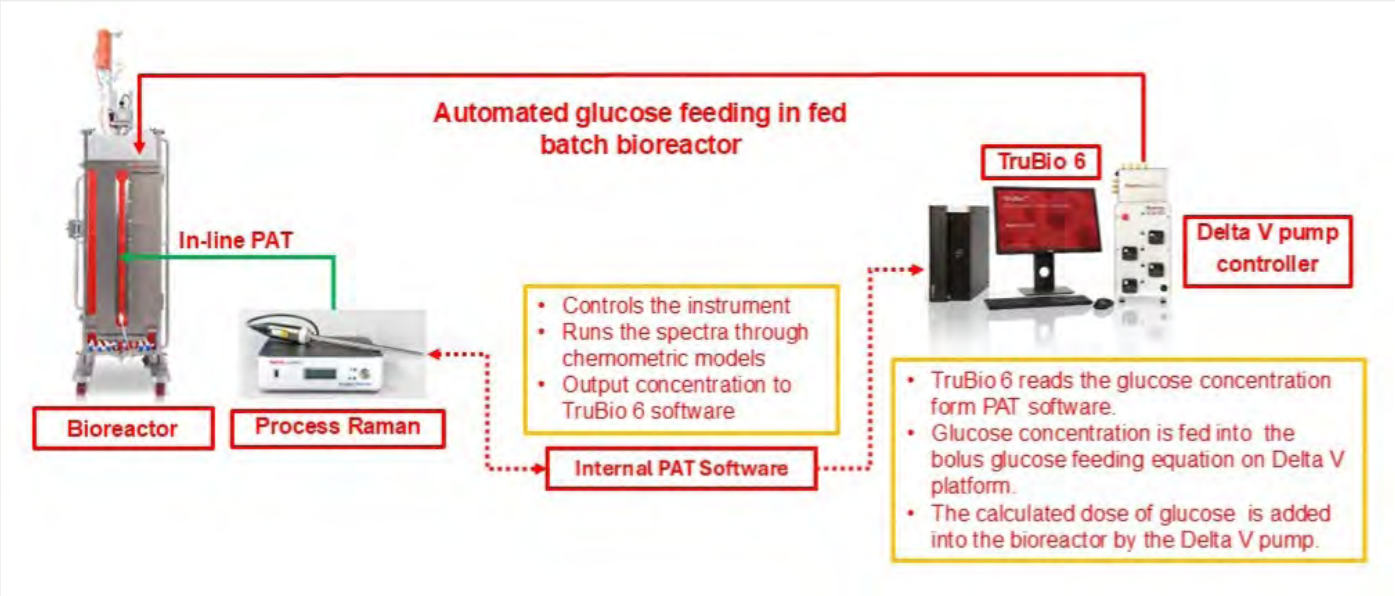


Figure 2. Schematics of workflow for automated feedback control for feeding glucose in a fed-batch bioreactor.

Control strategy

The internally developed PAT software was used to manage the process Raman and mathematically interpret the spectral information into concentration by running it through the chemometric models. The predicted glucose concentrations were communicated to the Thermo Scientific™ TruBio™ software, which in turn controlled the glucose feeding into the bioreactor via the Delta V pump. A simplified schematic is shown in Figure 2.

An automated bolus feeding control strategy was programmed into Finesse Solutions TruBio v 6.0.0 through the logic functions. Comparison statements were first utilized to feed into a logical statement to evaluate multiple criteria simultaneously. (All conditions must be satisfied before the action of turning on a peristaltic pump attached to a G3Lab Universal Controller is performed.)

Using a timer and a sine function, a periodic output was established where the sine function's value exceeded a defined threshold and remained above that threshold for a period of 30 minutes once in a 24-hour period. This can be achieved in many ways, however, the values chosen in this example can be seen in Equation 1. The input variable, x, is a timer that was selected to count in minutes and the constant inside the sine function was chosen such that the period was 24 hours. The constant in front of the sine function was selected to give sufficient precision in the duration above the threshold. In this case that threshold was 991,444 and was set as the first conditional statement.

$$f(x) = 1{,}000{,}000 \cdot \sin\left(\frac{720}{1{,}440} \cdot x\right)$$

Equation 1.

The second condition that needs to be met is for the glucose concentration to be less than a specified value, in this case 4 g/L. A communication protocol enables glucose concentration readings from probe measurements through the MarqMetrix All-In-One Process Raman Analyzer to be sent into TruBio through the PAT software.

Results and discussion

The models were developed in the SOLO software and exported in .xml format to run in the PAT software. The statistics for the developed glucose and lactate PLS model are summarized in Table 2. The models were also replicated in the Python platform, and the results are discussed below.
The details of the model are described below separately in the supplementary section.

Model parameters	Glucose model	Lactate model
Model Range	0-12 g/L	0-12 g/L
Number of Latent Variables	5	5
RMSEC	0.43 g/L	0.23 g/L
RMSECV	0.49 g/L	0.31 g/L
R^2 CV	0.94	0.92

Table 2. Model statistics for glucose and lactate.

The real-time control of glucose feeding in the fed-batch bioreactor is shown in Figure 3a. The control strategy was established at the start of the bioreactor run with the following logic:

1. Based on historical data, glucose concentration was sufficient for the first two days, so no feedback control was active.
2. From day 3 onward, when the predicted glucose concentration from the in-line Raman reached 4 g/L or below, bolus glucose feeding was triggered to achieve a final concentration of 7.5 g/L.
3. If the predicted glucose concentration was above 4 g/L, the predicted glucose concentration from the in-line Raman was fed into the feedback control logic at a specified time (9:00 AM) to initiate glucose feeding, aiming for a final concentration of 7.5 g/L.

Note, only one glucose feeding was performed per day to mimic the conventional fed-batch run.

In Figure 3a, the blue trace represents real-time glucose predictions from the in-line process Raman, averaged over a 10-minute window. The orange dots show pooled samples for offline analytics, used as reference values to calculate the root mean square error of predictions (RMSEP). The initial glucose concentration in the feed media was about 5 g/L. As the cells grew, glucose was consumed, dropping the concentration to 1.5 g/L on day 3, when the control loop was activated.

As demonstrated in Figure 3a, the fed-batch bioreactor run was fully automated and maintained glucose levels between 4 and 7.5 g/L (dotted red line), with an overall RMSEP of 0.45 g/L. Note, on some days the glucose level spiked above 7.5 g/L. This error is within the expected prediction error of the model as indicated by the RMSECV value of ~ 0.50 g/L. These errors can be minimized using appropriate statistical process control logic, although such process is beyond the scope of this work. Nonetheless, the results clearly demonstrate the feasibility of automating glucose feeding in a fed-batch bioreactor using feedback control from in-line process Raman. The green dotted line represents the end of the bioreactor run.

Additionally, lactate production was monitored in real time for the automated fed-batch bioreactor using in-line process Raman. The real-time prediction of lactate, averaged over 10 minute segments, is shown as a blue trace in Figure 3b. The orange dots represent samples pooled for offline analytics. The RMSEP of lactate prediction was 0.24 g/L. Although not shown here, the profile of lactate production in the fed-batch bioreactor with and without automated glucose feeding was similar, indicating no alteration in the process when the manual steps were replaced with the integration of in-line process Raman for automated glucose feeding.

Finally, *titer production* was compared using offline analytics for the fed-batch bioreactor with automated glucose feeding and the conventional approach. Titer production was approximately 8 g/L in both modalities. The *protein qualities* from both processes were also assessed using liquid chromatography coupled with mass spectrometry (LC-MS), and no significant differences were observed.

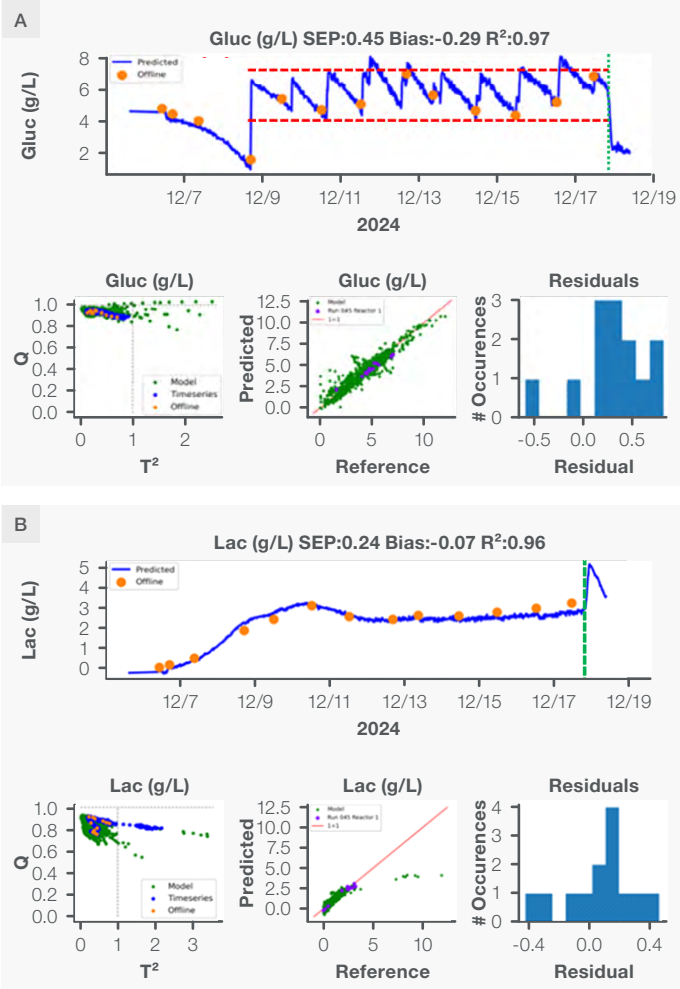


Figure 3. Real-time predictions (blue traces) of glucose (a) and lactate (b) using process Raman, and their correlation to offline reference values (orange dots). Figure (a) demonstrates the human-free glucose feeding for 14 days in a fed-batch bioreactor within the specific limit of 4 to 7.5 g/L (dotted red lines).

Conclusion

Process Raman is a reliable tool that provides a viable option to enhance efficiency in existing workflows by automating glucose feeding in conventional fed-batch bioreactors. The integration of in-line process Raman for automation is unlikely to disrupt established processes, as evidenced by the similar lactate production profile, equivalent titer production, and no significant differences in titer quality. Instead, it offers cost and time benefits, tighter process control, minimization of human errors, and assurance of process reproducibility. This study provides proof of concept, with the aim of encouraging bioprocess scientists and engineers to consider integrating process Raman into their existing fed-batch bioreactors to achieve greater efficiency through automation.

References

4. Yang, J.-D.; Lu, C.; Stasny, B.; Henley, J.; Guinto, W.; Gonzalez, C.; Gleason, J.; Fung, M.; Collopy, B.; Benjamino, M.; Gangi, J.; Hanson, M.; Ille, E. Fed-Batch Bioreactor Process Scale-up from 3-L to 2,500-L Scale for Monoclonal Antibody Production from Cell Culture. *Biotechnol. Bioeng.* **2007**, *98* (1), 141–154. https://doi.org/10.1002/bit.21413.
5. Villa, J.; Zustiak, M.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Use of Lykos and TruBio Software Programs for Automated Feedback Control to Monitor and Maintain Glucose Concentrations in Real Time.
6. Zhang, A.; Tsang, V. L.; Moore, B.; Shen, V.; Huang, Y.-M.; Kshirsagar, R.; Ryll, T. Advanced Process Monitoring and Feedback Control to Enhance Cell Culture Process Production and Robustness. *Biotechnol. Bioeng.* **2015**, *112* (12), 2495–2504. https://doi.org/10.1002/bit.25684.

Supplementary information

Details on PLS Raman models for monitoring bioreactor using Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer

Nimesh Khadka, PhD
Lin Zhang, PhD
Thermo Fisher Scientific, Tewksbury, Massachusetts USA

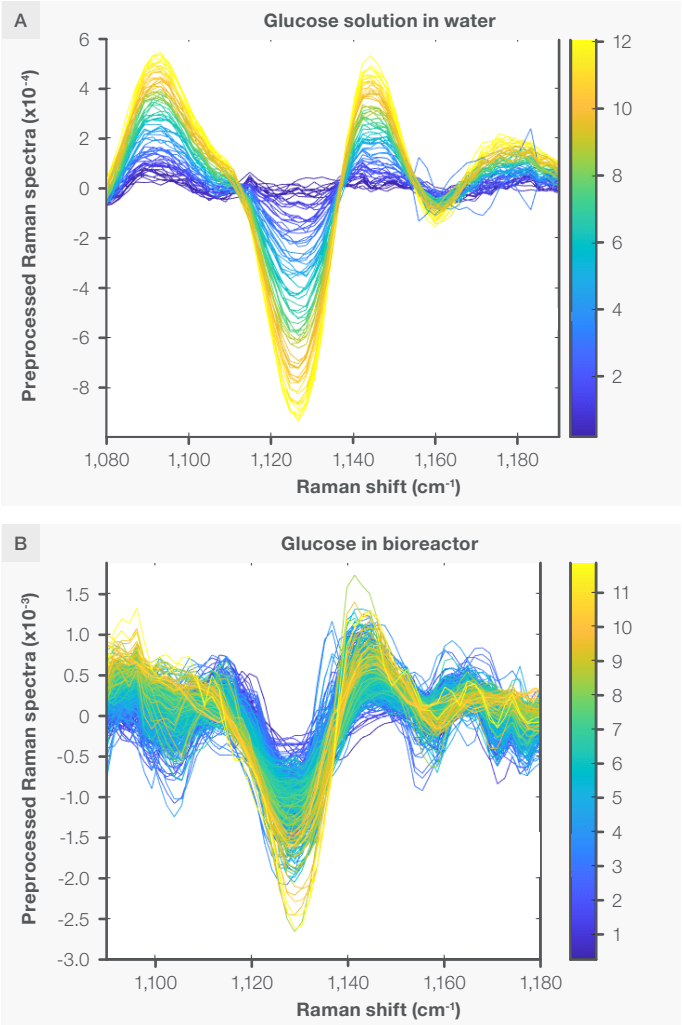


Figure S1. Characteristic Raman peaks of glucose at ~1125 cm^{-1} in aqueous solution (A) and bioreactor (B) shown as second derivative (negative peaks) spectra. The spectra are color coded by the concentration shown as the vertical bar.

Chemometric models development

Glucose PLS model

The PLS regression model for glucose was developed using a spectral region selection approach, focusing on the vibrational fingerprint of the glucose molecule. The Raman spectra of glucose in water and in the bioreactor, after applying the SavGol filter (second derivative, order = 2, window width = 13), are shown in Figures S1A and S1B. The negative peak at ~1125 cm^{-1} is attributed to the stretching vibrational modes of CO and CC and the in-plane bending of COH bonds (ν(CO), ν(CC), β(COH)) in the glucose molecule.

To normalize the spectra and enhance model robustness, three spectral regions were used: 1065-1232 cm^{-1}, 1595-1863 cm^{-1}, and 2704-3078 cm^{-1}. The 1065-1232 cm^{-1} region includes the characteristic Raman peak of glucose. The 1595-1863 cm^{-1} region includes the Raman peak of water due to the symmetrical bending of H-O-H bonds. Since the water concentration remains constant throughout the bioreactor run, this region serves as an internal reference for correcting path length differences (normalization) caused by factors like turbidity or slight variations in laser intensities. This normalization was achieved by applying SNV in the preprocessing step after baseline removal using the Savitzky-Golay filter (first derivative, order = 2, window width = 13). The normalization weight vector calculated from SNV was predominantly influenced by the spectral region corresponding to water.

The 2704-3078 cm^{-1} region includes Raman peaks mainly assigned to the symmetric and antisymmetric stretching vibration modes of various CH bonds, which are present in all biomolecules including glucose. Thus, this region provides information on total biomass in the bioreactor. Including these regions in the PLS glucose model ensures that the latent variables extract glucose-specific contributions from the overall biomass, thereby enhancing the accuracy and selectivity of the glucose PLS model.

The glucose PLS model was developed using five latent variables for the concentration range of 0 to 12 g/L, as shown in Figure S2B. The RMSECV did not improve after five latent variables, as shown in Figure S2C. The loadings for these latent variables containing glucose information are shown in Figure S2D.

Evaluation of the glucose model

i. **Specificity:** The specificity of the glucose core model was evaluated using variable importance in projection (VIP) scores. The VIP score plot shows the importance of each Raman shift in explaining glucose concentration. The VIP scores of the glucose core model are shown in Figure S2E, with the red dotted horizontal line representing the significance threshold (VIP score = 1). Raman shifts with scores higher than 1 are considered important for the model. The spectral region around ~1125 cm^{-1} has significantly higher scores, indicating specificity for glucose.

ii. **Accuracy/precision:** The glucose core model was developed with glucose concentrations of 0 to 12 g/L in the training dataset. Accuracy and precision were evaluated using RMSECV and CV bias. As listed in Table 2, the low RMSECV of 0.49 g/L and low CV bias of -0.013 g/L demonstrate high accuracy. The total measurement error is the square root of the sum of squared bias and precision errors.[1] Using RMSECV as total error and CV bias as bias error, the precision error was calculated to be 0.48 g/L. The model is statistically more accurate above glucose concentrations of 1.5 g/L (3*RMSECV) and can monitor or control glucose concentration with a tolerance limit of 0.5 g/L.

iii. **Linearity:** The R^2 for CV for the glucose core model is 0.94, demonstrating the linearity of spectral changes across the range of 0 to 12 g/L.

iv. **Q vs T^2 analysis:** The Q residual and Hotelling T^2 are used to evaluate the model. Q residual measures the residual after projecting data into the model space, while Hotelling T^2 measures sample variation within the model. Reduced Q residual and reduced Hotelling T^2, calculated by dividing Q residual and Hotelling T^2 by their corresponding confidence intervals, are used for normalized statistics. The reduced Q residual and reduced Hotelling T^2 distribution with 95% confidence interval for the core glucose model is shown in Figure S2F. Most data reside within the boundaries (dotted blue line) of reduced Q residual < 1 and reduced Hotelling T^2 < 1. Outliers are expected due to the variety in training data. When applying the model to new processes or instruments, predictions should be validated using orthogonal reference techniques if test samples have high values of reduced Q residual and reduced Hotelling T^2. Boundary limits should be determined by the user after a statistically significant process runs.

v. **Outlier analysis:** Cook's distance was calculated to identify outliers or influential data points. It measures the change in regression estimates when a particular observation is removed. A high Cook's distance indicates substantial impact on regression coefficients. Figure S2G shows all data have low Cook's distances, demonstrating no obvious outliers or influential data in the training set.

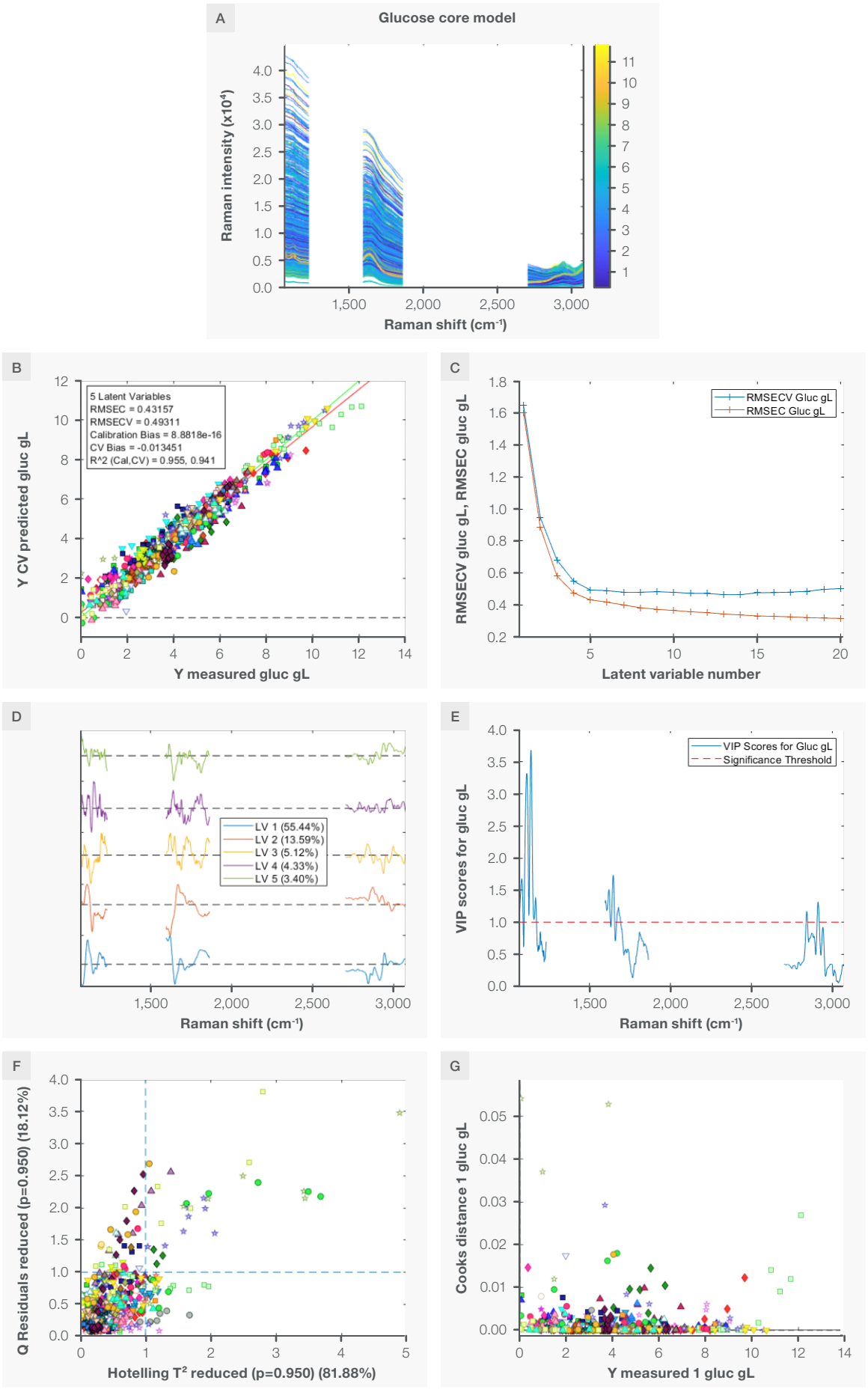


Figure S2. Glucose model: Plot A shows the region used to develop the model; plot B is the correlation plot between the measured and the prediction during cross validation; plot C shows RMSEC and RMSECV vs number of latent variables; plot D shows loading with percent variance captured; plot E shows VIP scores; plot F shows the reduced Q residual vs reduced Hotelling T^2 plot; and plot G is the Crook distance for all training samples.

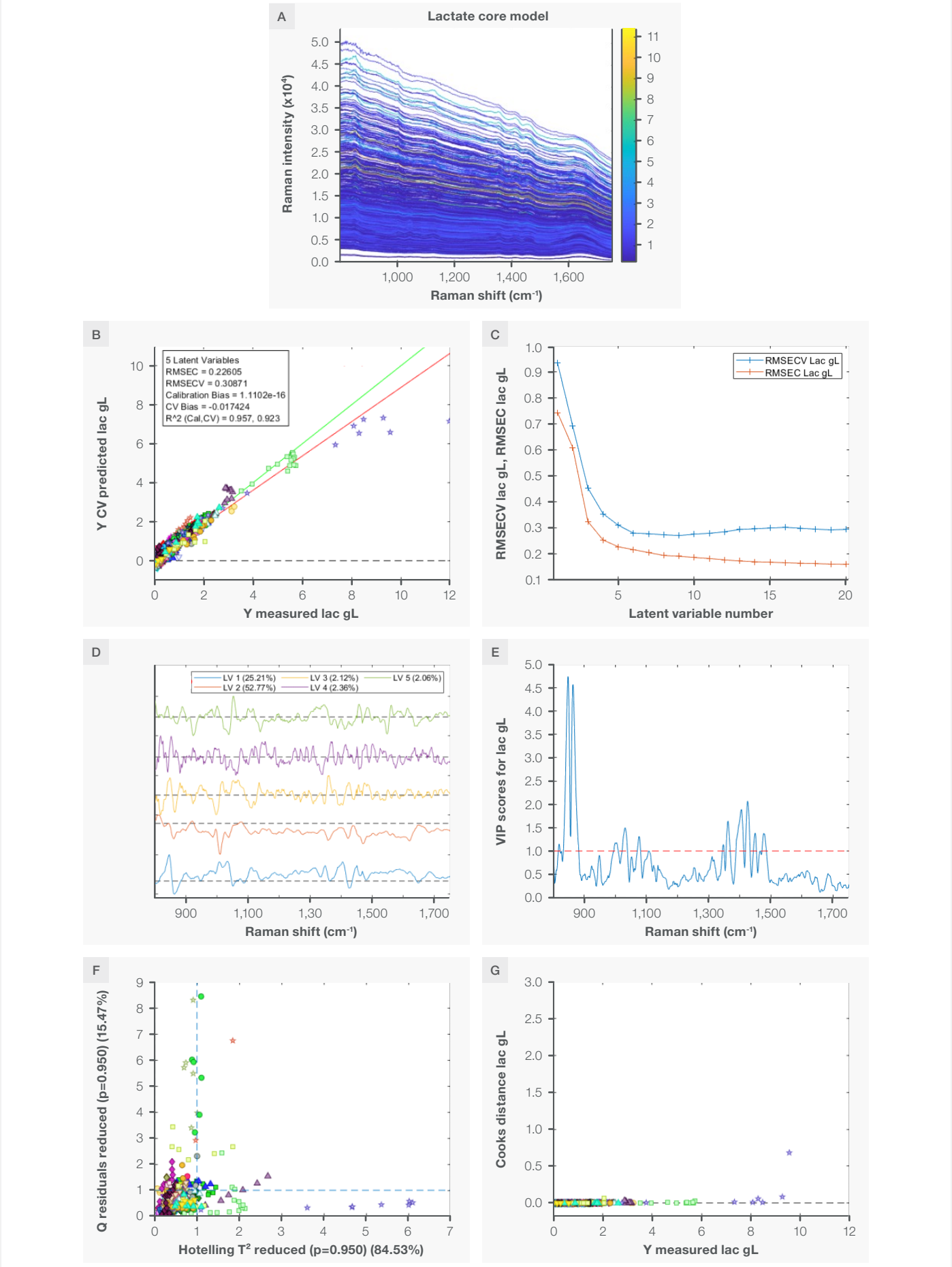
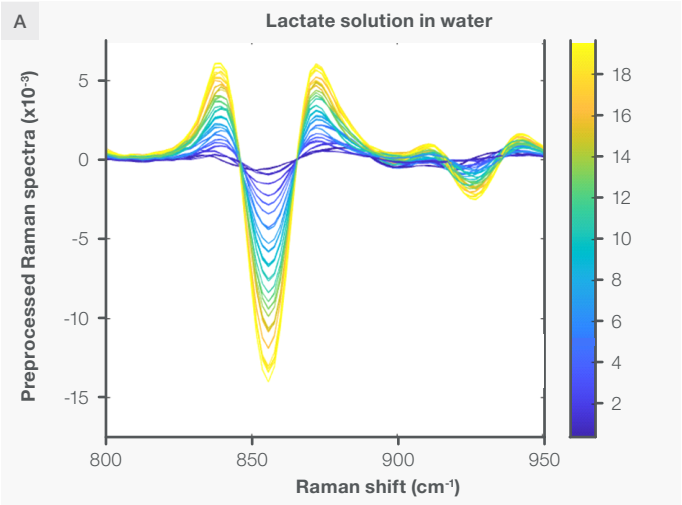


Figure S4. Lactate model: Plot A shows the region used to develop the model; plot B is the correlation plot between the measured and the prediction during cross validation; plot C shows RMSEC and RMSECV vs. number of latent variables; plot D shows loading with percent variance captured; plot E shows VIP scores; plot F shows the reduced Q residual vs. reduced Hotelling T^2 plot; and plot G is the Cook's distance for all training samples.

Lactate PLS model

The lactate model was developed using the same strategy as described above for the glucose model. Figure S3 shows the characteristic Raman peak for lactate at ~860 cm^{-1} in water (A) and in the bioreactor (B) after applying the SavGol filter (second derivative, order = 2, window width = 13). To ensure specificity for lactate, the model was developed using a single spectral region (800 to 1750 cm^{-1}) that includes the characteristic Raman peak for lactate and the water band for normalization, as explained above.

The lactate model was developed using five latent variables, as shown in Figure S4B. The choice of five latent variables was based on the predicted residual error sum of squares (PRESS) against the number of latent variables, as shown in Figure S4C. The loadings for the model are shown in Figure S4D.



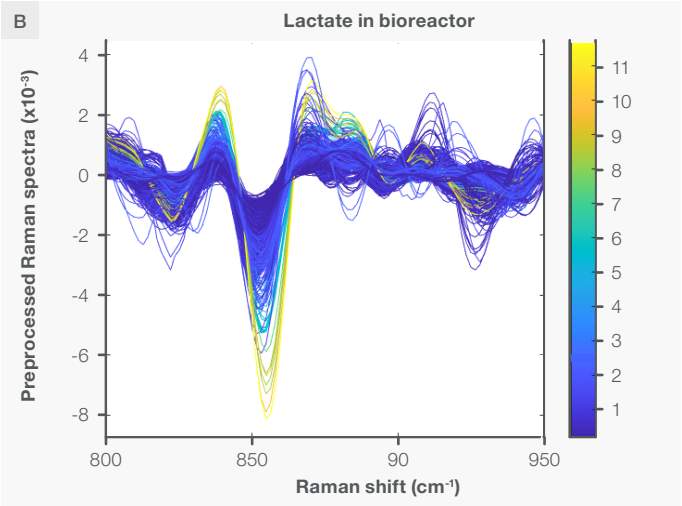


Figure S3. Characteristic Raman peaks of lactate at ~860 cm^{-1} in aqueous solution (A) and bioreactor (B) as second derivative (negative peaks) spectra. The spectra are color-coded by the concentration shown as the vertical bar.

Evaluation of lactate model

i. **Specificity:** The VIP score plot (Figure S4E) demonstrates that the region ~820 to 880 cm^{-1} is important in the lactate model. This region is associated with the stretching vibrational mode of the C-COO- bond of lactate. Thus, the VIP score plot confirms the model's specificity for lactate.

ii. **Accuracy/precision:** As explained above for the glucose model, the RMSECV of 0.31 g/L, CV bias of -0.0174 g/L, and precision error of 0.31 g/L for the concentration range of 0 to 12 g/L demonstrate that the lactate model has accuracy and precision within acceptable tolerance for typical bioreactor process monitoring.

iii. **Linearity:** The linearity of the lactate model is demonstrated by a CV R^2 of ~0.94 for the concentration range of 0 to 12 g/L. Considering the diverse bioprocess conditions in bioreactors where the training set was collected, a CV R^2 of ~0.94 represents a high correlation between spectral information and measured concentration.

iv. **Q vs. T^2 analysis:** The distribution of the training data in the Q vs. T^2 biplot related to 95% confidence boundaries is shown in Figure S4F. The 95% confidence boundaries of raw Q and T^2 values from the training dataset are used to normalize reduced Q residual and reduced Hotelling T^2 to 1, respectively. These boundaries are represented by the dotted blue line. Predictions from the model are trusted with high confidence if test samples fall within the 95% confidence boundaries. When a test sample generates reduced Q residual or reduced Hotelling T^2 significantly higher than 1, additional reference testing is recommended to validate the prediction.

v. **Outlier analysis:** The low Cook's distance for training data indicates that there were no outliers on the training set, as shown in Figure S4G.

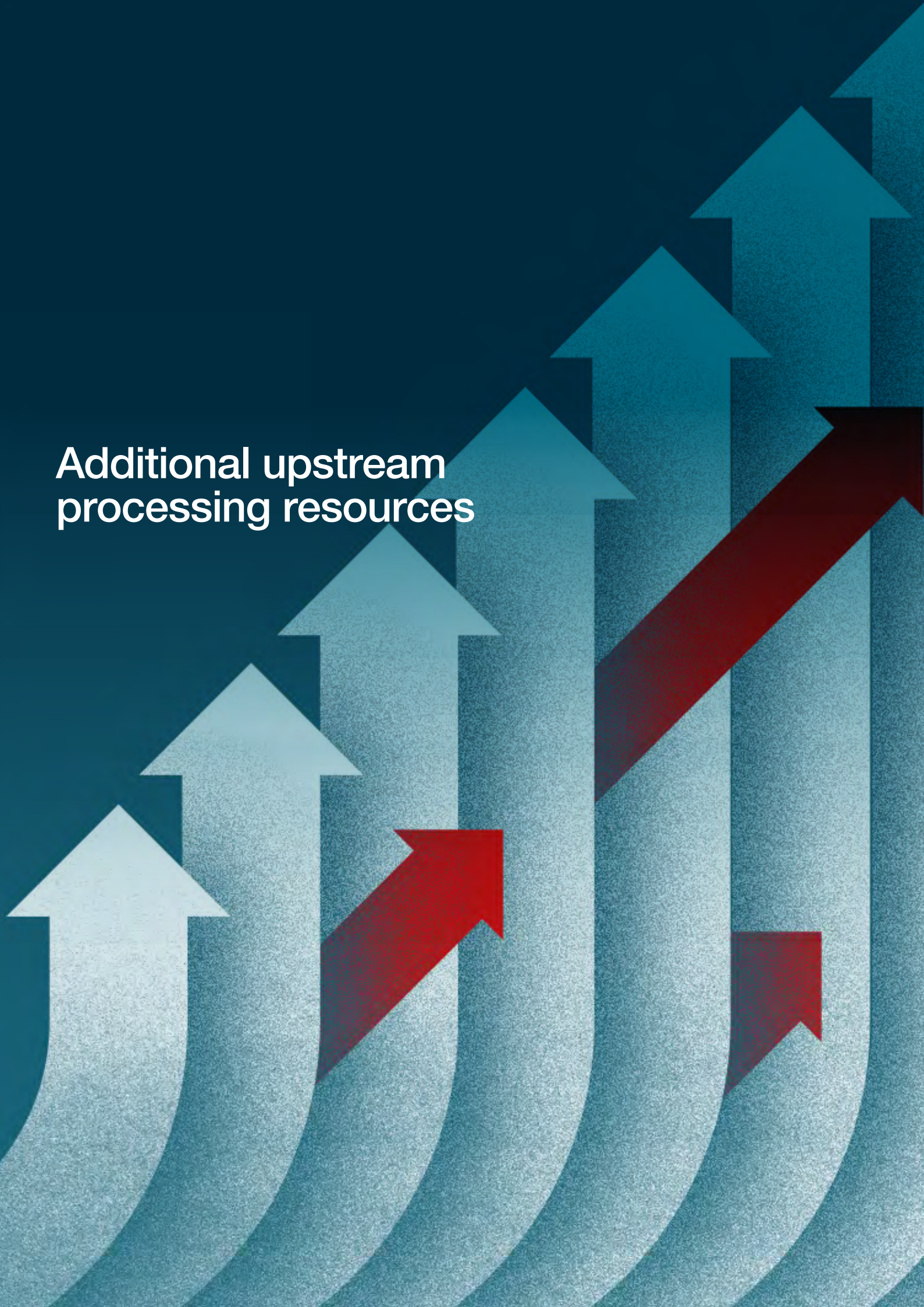
Performance of glucose and lactate model

The performance of glucose and lactate models were tested previously on five different cell lines / media, different scales of bioreactors, and also for the automated feedback control.[2,3] The average root mean square of prediction (RMSEP) for glucose was ~ 0.5 g/L while the average RMSEP for lactate was ~ 0.2 g/L. The low RMSEP demonstrates the accuracy, reliability, and transferability of the models across different processes and scales.

References

6. Bellon-Maurel, V.; Fernandez-Ahumada, E.; Palagos, B.; Roger, J.-M.; McBratney, A. Critical Review of Chemometric Indicators Commonly Used for Assessing the Quality of the Prediction of Soil Attributes by NIR Spectroscopy. *TrAC Trends Anal. Chem.* **2010**, *29* (9), 1073–1081. https://doi.org/10.1016/j.trac.2010.05.006.
7. Villa, J.; Zustiak, M.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Use of Lykos and TruBio Software Programs for Automated Feedback Control to Monitor and Maintain Glucose Concentrations in Real Time.
8. Villa, J.; Zustiak, M.; Ramirez, D.; Kruger, J.; Kuntz, D.; Zhang, L.; Khadka, N.; Broadbelt, K.; Woods, S. Demonstrating Chemometric Model Transferability for 5 Mammalian Cell Lines and 5 Media Types Using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer to Monitor Upstream Bioprocesses.

Additional upstream processing resources



Single-use technologies

Streamlining seed train scale-up through utilization of the turndown ratio of the DynaDrive S.U.B.

Keywords
DynaDrive S.U.B.,
single-use bioreactors,
scalability, turndown

Introduction

Upstream bioproduction has seen a substantial movement in the industry toward single-use systems. This has been driven primarily by the need to reduce contamination risk and cleaning requirements when compared to stainless steel systems, and to allow for faster changeover of equipment between batches. At the same time, bioprocessing manufacturing processes have matured significantly, and intensification of cell culture processes has pushed the limits of these legacy single-use systems. In recent years, Thermo Fisher Scientific has brought enhancements to the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) platform with 5:1 S.U.B. and enhanced S.U.B. options, which have allowed for higher turndown ratios and optimized mixing and gassing strategies for intensified processes. Now Thermo Fisher Scientific has launched a next-generation bioreactor for the biopharmaceutical industry with the Thermo Scientific™ DynaDrive™ S.U.B. (Figure 1).

Figure 1. DynaDrive S.U.B.s in 50 L, 500 L, 3,000 L, and 5,000 L sizes.

Building on our extensive experience and nearly two decades of end users' feedback, the DynaDrive S.U.B. employs a new agitator drive technology with carefully engineered hardware that enables exceptional performance. The DynaDrive S.U.B. allows for a higher turndown ratio of at least 10:1 and up to 20:1 in the larger sizes, with a reliable power input of up to 80 W/m^3, and scalability through each size.

The innovative impeller design enables a higher turndown ratio than previously seen in single-use systems, offering working volumes as low as 5 L in the 50 L S.U.B. These higher turndown ratios open a new paradigm of what is possible with seed trains, potentially eliminating multiple vessels and reducing logistical and operating costs dramatically while increasing the efficiency of the seed train through reduced connections and transfer losses.

S.U.B. seed train options

During seed train expansion, cell cultures are sequentially increased in volume and cell population to provide enough starting material to enter batch, fed-batch, or perfusion production unit operations. Small-scale cell cultures, usually in less than 1 L of working volume, are typically maintained in flasks on an orbital shaking platform. Shake flask culture requires careful handling within a biosafety cabinet in a separate, higher-classification cleanroom to limit the risk of contamination. Due to the heightened risk associated with these open-unit operations, it is desirable for bioprocess engineers to move the cell culture into a closed system as early as possible in the scale-up process. A commonly chosen closed system for expansion steps after shake flasks is the rocking-motion bioreactor. This single-use closed system employs a platform to mix cells and gases to maintain viable cultures. After expanding in the rocker, cultures are transferred to stirred-tank S.U.B.s for further expansion prior to inoculating the N-stage production vessel (Figure 2A).

Enhancements to our legacy S.U.B. systems, with the launch of the 5:1 and the enhanced S.U.B. systems, allowed for working volumes as low as 20% of the final working volume (e.g., 10 L in a 50 L vessel), which has allowed for scale-up of the seed train within the vessel and elimination of some seed train vessels (Figure 2B). With the innovative design features of the 50 L DynaDrive S.U.B., cell cultures in working volumes as low as 5 L can be grown in a stirred-tank vessel, allowing for scale-up processes to happen at even earlier stages. This extremely high turndown ratio eliminates the need for rockers and extra steps in the seed train process further on, with the ability to seed the 5,000 L DynaDrive S.U.B. at 20:1 directly from the 50 L scale. This essentially means that the seed train process can be limited to two reactors, with each having scale-up steps that take place within them. For example, the 5 L minimum working volume of the 50 L DynaDrive S.U.B. enables cell culture scale-up to transfer directly from shake flasks to the S.U.B. After the 5 L culture reaches a viable cell density suitable for expansion, additional culture medium can be added to the vessel as a second passage within the 50 L DynaDrive S.U.B. Once that culture reaches a viable cell density suitable for expansion, it can then be used to seed the 5,000 L DynaDrive S.U.B. at a 250–500 L working volume, which subsequently can be passaged within the reactor for inoculation of the production run (Figure 2C).

Alternatively, because of the flexibility of the DynaDrive S.U.B. systems, the 500 L DynaDrive S.U.B. can be used in the seed train in place of the 5,000 L DynaDrive S.U.B., increasing the required reactors to three, which allows for increased overall bioreactor production and throughput. In this scenario, the seed train would still utilize the 5 L minimum working volume of the 50 L DynaDrive S.U.B., but after reaching a viable cell density suitable for expansion, these cells would be used to seed the 500 L DynaDrive S.U.B. at a 25 L or 50 L volume. When ready, this would be expanded within the 500 L vessel and then used to inoculate the 5,000 L DynaDrive S.U.B. production run (Figure 2D).

Since every additional aseptic connection is a potential vector for contamination, the high turndown ratios of the DynaDrive S.U.B. systems enable risk reduction for bioprocess scale-up operations. Performing expansions in the same vessel saves time in setup and reduces the quantity of sterile connections needed for the overall seed train expansion.

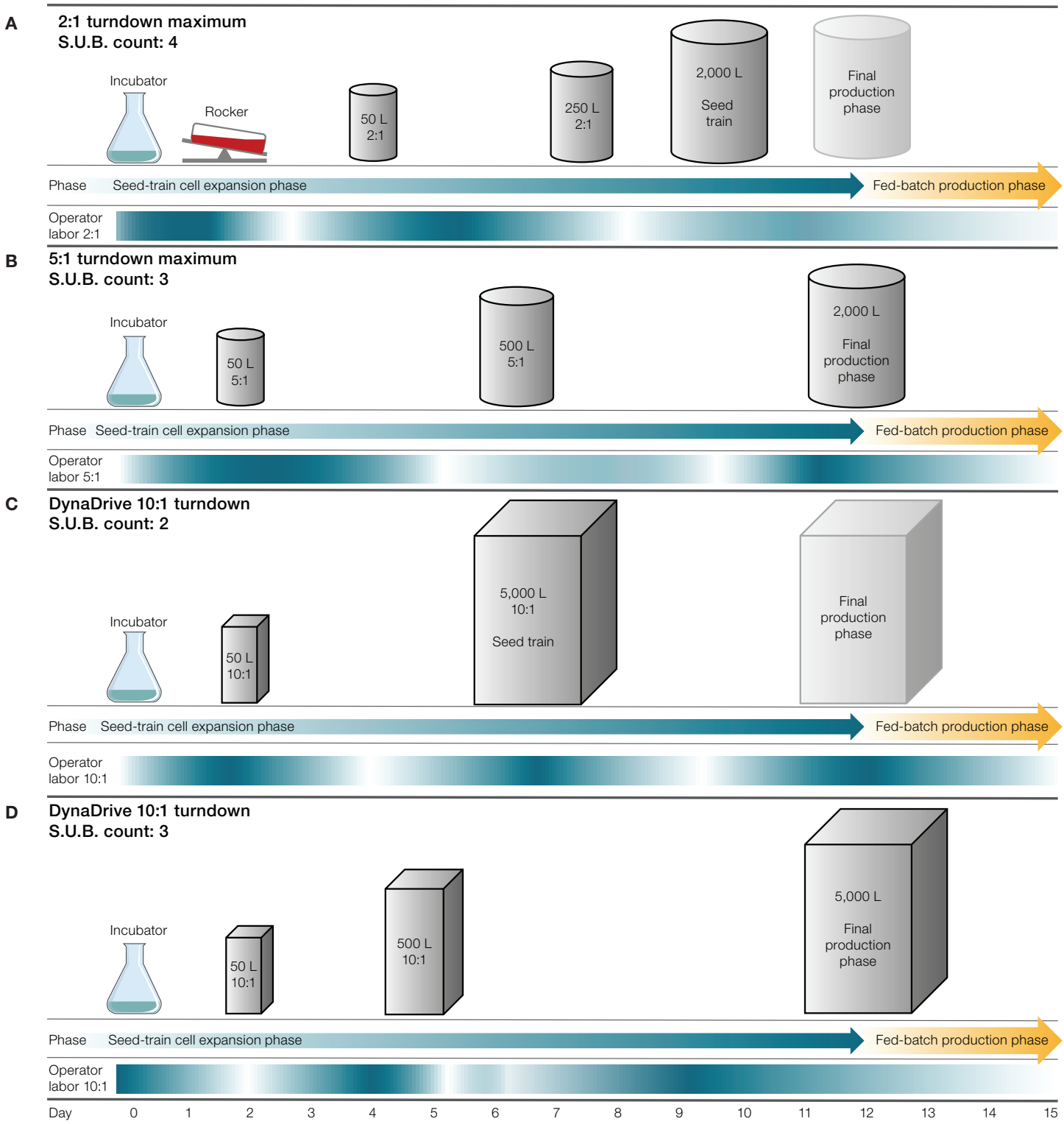


Figure 2. Improving facility efficiency with increased S.U.B. turndown ratios and careful logistical planning. Using bioreactors capable of high turndown ratios, such as the DynaDrive S.U.B.s, can enable a streamlined seed train for cell expansion. Benefits of high turndown ratios include increased facility space through reduction of bioprocess units, reduction of operator labor (e.g., setups, takedowns, and sterile or aseptic transfers), and reduction of logistical risks. **(A)** Example of a 2:1 turndown ratio operation that incorporates a rocking-motion bioreactor and stirred-tank S.U.B.s. **(B)** With the removal of at least one or more bioprocess units (e.g., rocker and S.U.B.), the cell expansion requires 20–25% less operator intervention and up to 50% fewer single-use Thermo Scientific™ BioProcess Containers, compared to a 2:1 turndown ratio operation. The high-intensity portions of operator labor (dark blue) of both the 5:1 and the DynaDrive S.U.B. seed trains are spaced at intervals of 2–4 days, reducing risk of operator error through fatigue or haste. **(C)** Using the 5,000 L DynaDrive S.U.B. at a turndown ratio of 10:1 as the N-1 bioreactor allows for a smaller footprint in the facility, less setup, and fewer sterile or aseptic transfers. **(D)** Alternatively, utilizing the 500 L DynaDrive S.U.B. in the seed train allows the 5,000 L DynaDrive S.U.B. to remain a dedicated production vessel. Maximum split ratio = 10; minimum cell density = 0.3 x 10^6 cells/mL.

Application and benefits

By utilizing DynaDrive S.U.B.s in seed trains, not only are there ideal turndown ratios of scaling within vessels, but the system has been optimized to provide ideal mixing and mass transfer, even at low turndown ratios, as shown in Table 1.

Also shown in Table 1 is a comparison of the monitoring capabilities of each system. In comparing the single-use closed system options for seed train expansion, stirred-tank S.U.B.s allow for better monitoring and control than rocker systems.

Due to design limitations of the rocker bioreactors, there are limited online process monitoring capabilities, whereas the 50 L DynaDrive S.U.B. can support temperature, pH, and DO probes as well as an additional probe at the 10:1 turndown ratio, with space for more probes at higher volumes (Table 1). This capability provides greater design space for bioprocess monitoring and enhanced process analytical technology (PAT) capabilities for quality by design (QbD) approaches.

Table 1. Highlighted performance comparisons and monitoring capabilities of the HyPerforma, enhanced HyPerforma, and DynaDrive S.U.B. systems.

S.U.B.	Rocker	HyPerforma		Enhanced HyPerforma		DynaDrive
Generation	Rocker	2:1	5:1	Enhanced fed-batch	Enhanced perfusion	DynaDrive
Vessel size	10 to 50 L	50 to 2,000 L		50 to 500 L		50 to 5,000 L
Scalable volumes	1 to 25 L	25 to 2,000 L	10 to 2,000 L	12.5 to 500 L	25 to 500 L	5 to 5,000 L
Turndown ratio	5:1	2:1	5:1	4:1	2:1	10:1 or 20:1
Sparge	Overlay only	Frit and drilled-hole sparger (DHS)		Enhanced DHS		
k_La at 20 W/m^3 with DHS only	No data	≤10 hr^{-1}		≤20 hr^{-1}	≤25 hr^{-1}	20–30 hr^{-1}
Max k_La	No data	≤15 hr^{-1}		30–40 hr^{-1}		>40 hr^{-1}
Max P/V	No data	40 W/m^3		100 W/m^3		80 W/m^3
Dissolved oxygen (DO) sensor	Must be single-use, limited options available	Standard or single-use available, multiple options				
pH sensor	Must be single-use, limited options available	Standard or single-use available, multiple options				
Temperature control	Electric, heat only	Jacket, temperature control unit (TCU)				
Additional sensor port options	None available	2 probe belts, no options below first probe belt	2 probe belts plus 1 DO and 1 pH option at low turndown ratio			Additional port options at low turndown ratio

Case study

Seed train evaluation using ExpiCHO-S cell line in 14-day fed-batch run

The following case study was done to evaluate the performance of the DynaDrive S.U.B.s across all scales from 50 to 5,000 L in a fed-batch process using CHO cells (Table 2), as well as to evaluate the performance of the cells during the seed train steps within these bioreactors.

Methods

Cells were expanded in shake flasks, the 50 L DynaDrive S.U.B., or both through the N-2 stage. The N-1 stage for each seed train was carried out at a 10:1 turndown ratio within the N-stage vessel. Fresh production medium was added to the S.U.B. after 3 days, resulting in the culture starting at the proper N-stage production volume and initial seed density. Operating conditions for both the N-1 and N-stage production vessels are described in Table 3. During the production run, daily bolus feeds of 2X concentrated EfficientFeed C+ AGT Supplement were added from days 3 to 13 through either a subsurface (5,000 L S.U.B.) or top feed (50 and 500 L S.U.B.) line. Following addition of EfficientFeed C+ AGT Supplement, glucose was supplemented in the same way on an as-needed basis after taking glucose measurements to bring the final glucose concentration to >4 g/L. Cell counts, viability, gases, nutrients, and metabolites were measured offline daily. All three DynaDrive S.U.B. volumes were tested in N-stage production runs. The 500 L and 5,000 L production runs came from the same seed train, with the 500 L N-1 being inoculated with cells from the 5,000 L N-1 stage reactor, prior to the start of that 5,000 L N-stage production run.

Table 2. Cell line evaluated in 50–5,000 L DynaDrive S.U.B.s.

Cell type	Gibco™ ExpiCHO-S™ Cells
Production medium	Gibco™ ExpiCHO™ Stable Production Medium
Feed supplement	Gibco™ EfficientFeed™ C+ AGT™ Supplement
Titer range	~3 g/L

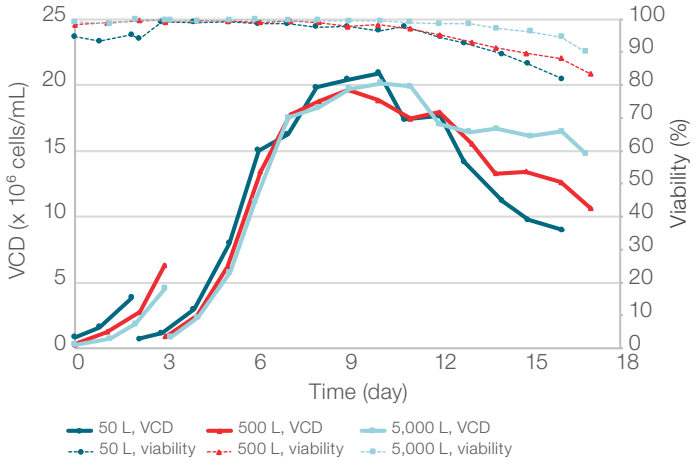
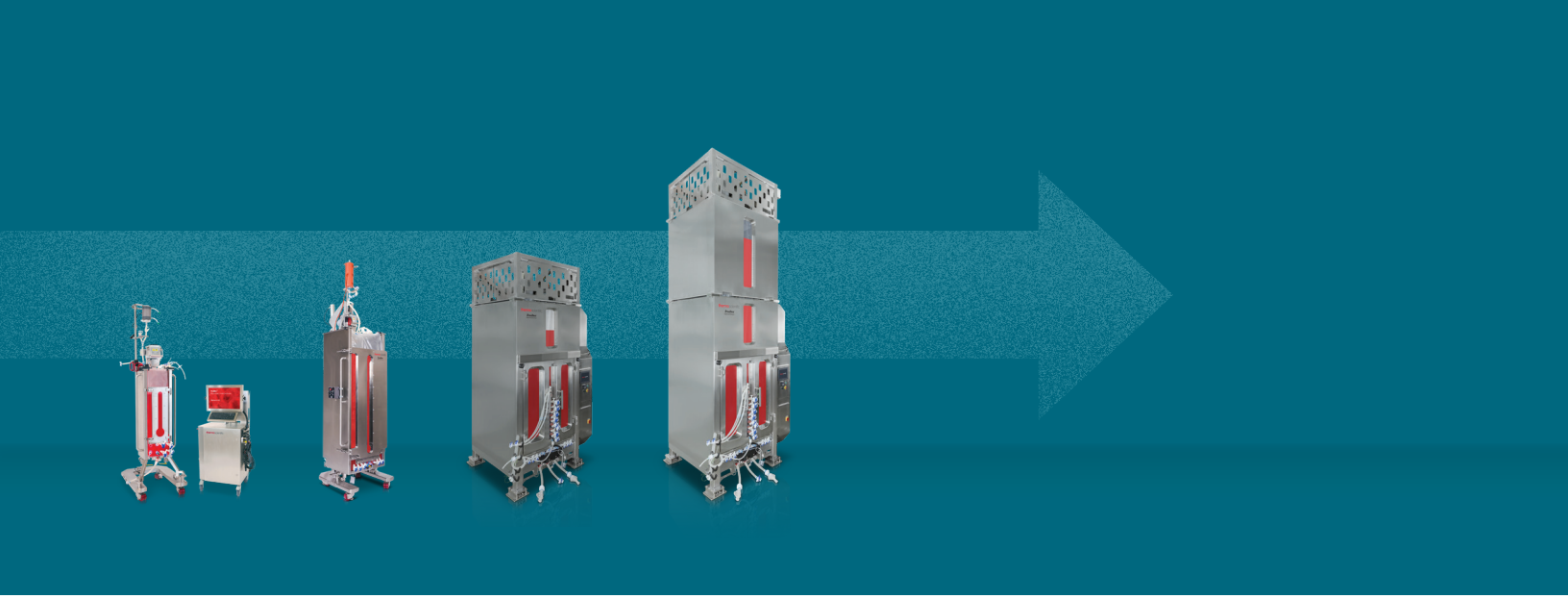


Figure 3. VCD and viability of ExpiCHO-S cell culture from N-1 through N-stage production culture in the 50 L, 500 L, and 5,000 L DynaDrive S.U.B.s. Each N-1 culture was seeded at a 10:1 volume. The low viability seen in the N-1 culture in the 50 L S.U.B. occurred in a prototype BPC with known effects on cell health at low working volumes. Production BPCs have been shown to have reduced impact on cell health and growth across multiple cell clones tested.

Table 3. Operating parameters for evaluation of ExpiCHO-S cells in the 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	5 L, 35 L*	50 L, 350 L*	500 L, 3,500 L*
Seeding density	0.3 x 10^6 cells/mL, 0.7 x 10^6 cells/mL*		
Temperature	37°C (N-1 and days 0–5), 34°C (days 5–14)		
pH	6.8–7.2		
pH control	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 through the macro DHS Base control: 1 N NaOH
Agitation	140 rpm, 120 rpm*	60 rpm	26 rpm for N-1 37 rpm (days 0–3) 33 rpm (days 3–14)
DO	40%		
Air crossflow/headspace (slpm)	1	6	10–20
DO cascade	Air supplemented with O_2 through DHS	Air supplemented with O_2 through DHS	Air supplied to both macro and micro DHS O_2 supplemented through micro DHS
Feeding strategy	Daily bolus of 1.05 L EfficientFeed C+ AGT Supplement and glucose (as needed)	Daily bolus of 10.5 L EfficientFeed C+ AGT Supplement and glucose (as needed)	Daily bolus of 105 L EfficientFeed C+ AGT Supplement and glucose (as needed)

* The N-1 culture conditions are listed first, followed by the N-stage culture conditions.



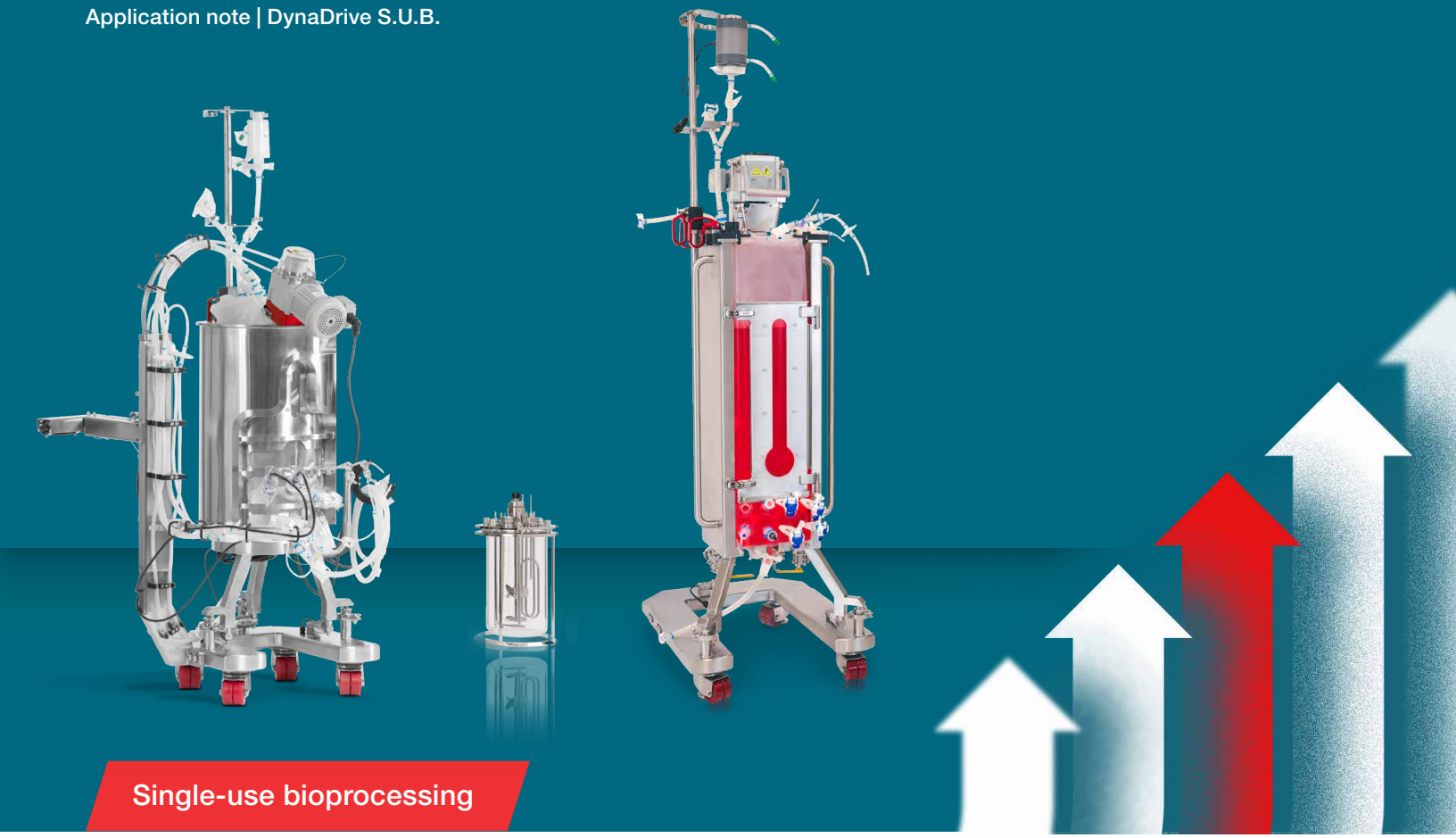
Results

Viable cell density (VCD) and viability (Figure 3) for the N-1 and production cultures show consistent growth profiles among the cultures, with similar cell density and viability trends at each step in the seed train. The viability at the end of the run was above 80% in all systems, which was within expectations.

Conclusion

Overall, the ability of the DynaDrive S.U.B. systems to operate at a 10:1 or 20:1 turndown ratio enables more efficient cleanroom utilization, reduces risk of contamination, and simplifies seed train expansion operations. This innovative product offering from Thermo Fisher Scientific enables consolidation of unit operations into fewer vessels and helps provide a more flexible manufacturing system for upstream bioprocessing.

Learn more at **thermofisher.com/dynadrive**



Single-use bioprocessing

Part 1: Intuitive bioprocess scale-up from bench scale to pilot scale

A comparative study of Thermo Scientific single-use bioreactors

Keywords
Single-use bioreactor, DynaDrive, CHO, fed-batch, scale-up, Efficient-Pro, bench scale, pilot scale

Introduction

The transition from bench scale to pilot scale is often viewed as a critical step in bioproduction process development, generally because of physical differences of the separate systems. The ability to generate scalable parameters is critical to enable confidence when progressing to clinical and production scales. An example of scaling up a CHO-based bioprocess from bench scale to pilot scale using the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) is outlined here, highlighting similarities and differences in control parameters and outcomes. In general, the culture carried out in the DynaDrive S.U.B. displayed key performance indicators that matched or exceeded those of the bench-scale bioreactor, including IgG titer, specific productivity, and cell density.

Methods

IgG-producing CHO-K1 cells were thawed and propagated following standard procedures to establish a suitable seed train. The reactors used in this study included the 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor, the Thermo Scientific™ 5:1 HyPerforma™ S.U.B. (50 L), and the DynaDrive™ S.U.B. (50 L). Cell expansion was performed in a series of shake flasks until a sufficient number of cells were generated to inoculate the bioreactors. Control parameters used for the n-stage operation of each bioreactor are displayed in Tables 1–3.

Table 1. Operation and control parameters.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
n-1 stage seed volume	—	10 L	5 L
Target initial/ final volume	1.7/2 L	36/50 L	36/50 L
Seed density ($x10^6$ cells/mL)	0.3	0.3	0.3
Temperature set point (°C)	37	37	37
Agitation (rpm)	350	183	105
Power input per volume (W/m^3)	100*	20	20
Tip speed (m/sec)	1.01	1.06	0.59
Impeller configuration	2 down-pumping pitched-blade impellers	1 down-pumping pitched-blade impeller	3 down-pumping pitched-blade impellers
Sparger configuration	Drilled pipe sparger 7 x 800 µm holes	Drilled-hole sparger (DHS) 360 x 178 µm pores	DHS 1,448 x 80 µm pores
Target glucose conc. (g/L)	3	3	3
Foam control			
High-threshold output	45	45	45
Foam alarm delay (sec)	60	60	60
Splash delay (sec)	5	5	5

* Approximate. Power number for the impeller configuration used has not been determined at time of publication.

Table 2. Dissolved oxygen (DO) control gassing strategy.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
DO set point (%)	40	40	40
DO PID			
Gain	0.07	0.10	0.10
Reset	200	200	200
O_2			
Controller output	15 → 100%	15 → 100%	15 → 100%
MFC scaling	0 → 0.5 slpm	0 → 5 slpm	0 → 3 slpm
N_2			
Controller output	0 → 40%	0 → 30%	0 → 30%
MFC scaling	0.15 → 0 slpm	1 → 0 slpm	1 → 0 slpm
Air			
Overlay	0.2 slpm	5 slpm	5 slpm
Sparge controller output	—	—	15 → 30 → 45%
MFC scaling	—	—	0 → 0.5 → 0 slpm

Table 3. pH control strategy.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
pH set point	7.15	7.15	7.15
pH PID			
Gain	0.04	0.04	0.04
Reset	200	200	200
pH deadband	Not enabled	Not enabled	Not enabled
CO_2			
Controller output	–100 → 0%	–100 → 0%	–100 → 0%
MFC scaling	0.08 → 0 slpm	2 → 0 slpm	2 → 0 slpm
Base	Not enabled	Not enabled	Not enabled

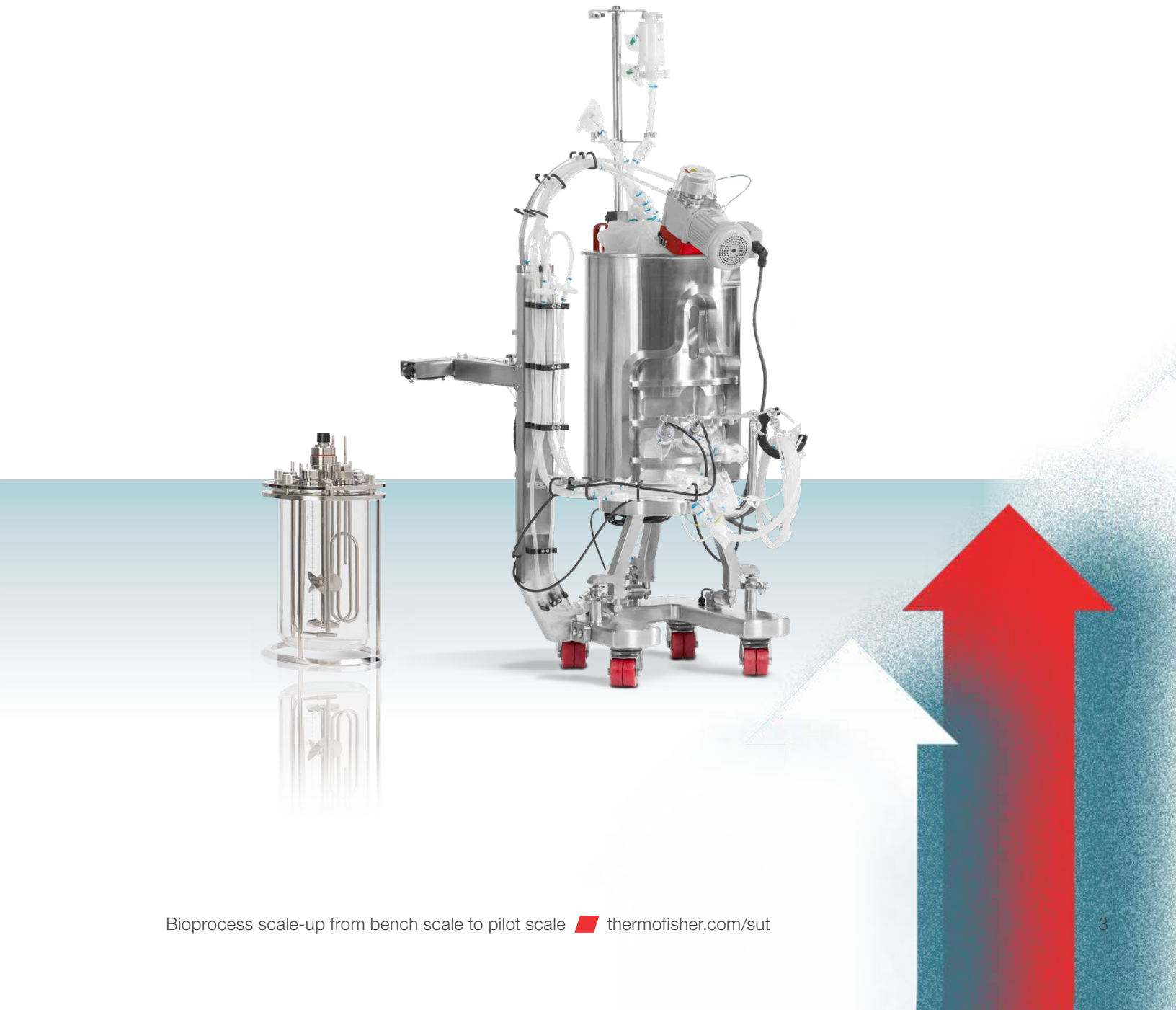
Although a power input per volume (P/V) of 20 W/m^3 was targeted for the 50 L S.U.B.s, the P/V was approximately 100 W/m^3 in the 3 L glass bioreactor culture. While the difference in P/V between scales is large, this is not a significant concern as P/V is frequently an impractical or unmeaningful scaling parameter at the benchtop scale. In this case, an agitation rate was selected for the 3 L glass bioreactor culture that provided for tip speed comparable to that of the 50 L HyPerforma S.U.B.

Gibco™ Efficient-Pro™ medium was used throughout the expansion and in the bioreactors for the n-stage production process. Gibco™ Efficient-Pro™ Feed 1 was used to supplement the production run cultures from day 3 onward, feeding 2.25% of the current vessel volume daily (see Equation 1). A 2 M glucose solution was also used to supplement the cultures, targeting a 3 g/L glucose concentration upon starting the fed-batch phase of the process. Gibco™ FoamAway™ Irradiated AOF Antifoaming Agent was used to control excess foam during operation via the use of the foam probe and pump triggered by Thermo Scientific™ TruBio™ automation software “Foam Hi Lim Out” as a remote set point for the pump.

Equation 1: Daily feed rate

$$\text{Feed rate}\left(\text{mL/min}\right) = (\text{Current volume (L)}) \left(\frac{1{,}000\ \text{mL}}{1\ \text{L}}\right)\left(\frac{0.0225}{\text{day}}\right)\left(\frac{1\ \text{day}}{1{,}440\ \text{min}}\right)$$

Reactors were sampled daily, and measurements were recorded for cell count, cell size, cell viability, metabolites, and protein titer.



Results

Culture growth was similar among all reactors, with peak viable cell densities (VCD) between 60.4 x 10^6 cells/mL and 72.0 x 10^6 cells/mL (Figure 1). Comparing cell viability, some discrepancies between scales were apparent (Figure 2). The 50 L HyPerforma and DynaDrive S.U.B. cultures had comparable cell viability profiles, with the viability of the 3 L glass bioreactor culture trending slightly higher throughout the latter half of the process. Protein production was also similar among the conditions tested, with peak protein titer reaching between 3.48 and 3.73 g/L (Figure 3). Productivity behavior is further elucidated by evaluating specific productivity (Q_P), which is shown in terms of pg/cell per day (Figure 4). Excluding a couple of minor deviations, the specific productivity lies within a similar range for each of the vessels during the fed-batch portion of the process, with the overall trend being similar across all conditions.

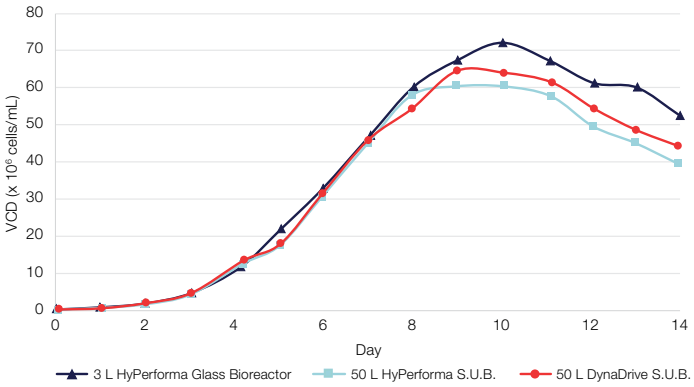


Figure 1. Viable cell density (VCD) profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

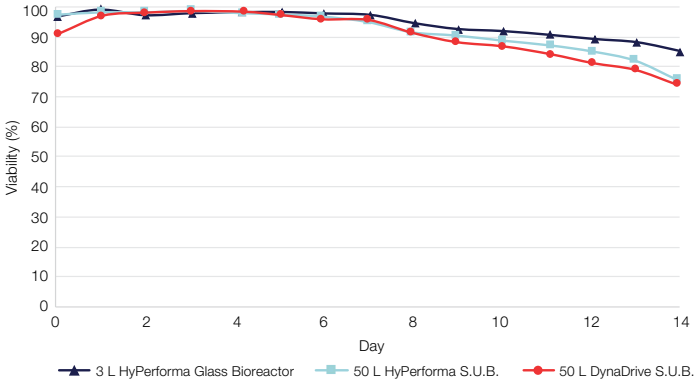


Figure 2. Viability profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

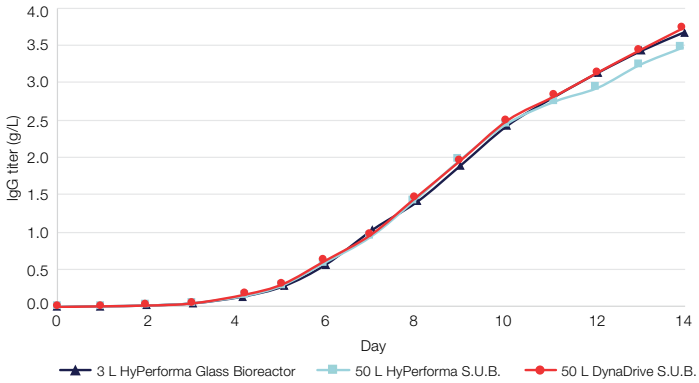


Figure 3. IgG titer profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

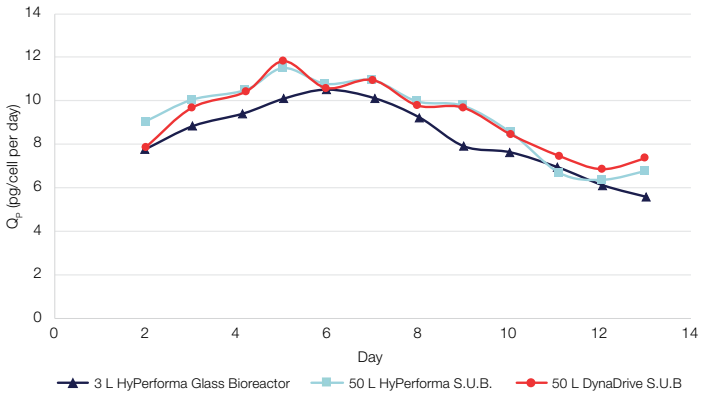


Figure 4. Specific productivity (Q_P, a 3-point moving average) profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B. Q_P is expressed in terms of picograms IgG per cell per day.

The lactate concentration in each reactor increased over the first few days, with a primary peak on day 4, after which the concentration dropped for 4–6 days before increasing again until process termination (Figure 5). While the lactate concentration in the 3 L culture was lower than that of both 50 L cultures during the primary peak, the lactate accumulation rate was higher in the vessel from day 10 and beyond, resulting in a higher final concentration. The relatively larger increase in lactate concentration in the 3 L glass bioreactor culture coincided with a drop in pCO_2 near the end of the process, falling as low as ~25 mmHg, whereas pCO_2 in both 50 L cultures was maintained at 40–80 mmHg from day 3 onward.

Scaled total gas flow rates (vessel volume per minute, VVM) show a lower O_2 sparge rate in the glass reactor and the DynaDrive S.U.B. compared to the HyPerforma S.U.B. (Figure 6). Nitrogen flows are also shown in the figure, and any sparged air flow rates were treated as 21% O_2 and 78% N_2. A direct comparison of the gassing requirements of the glass bioreactor and S.U.B.s is somewhat difficult due to the relatively high P/V in the glass reactor and varying rates of sparged N_2 in each condition. Even so, it can be seen from the similarity in scaled flow rates that the 50 L DynaDrive S.U.B. serves as a straightforward option when considering scalability from bench to pilot scale. Inspection of the CO_2 sparge rates (Figure 7) and referring to Figure 5 show that acceptable pCO_2 concentrations were maintained in the 50 L cultures even while the CO_2 flow rate was insignificant.

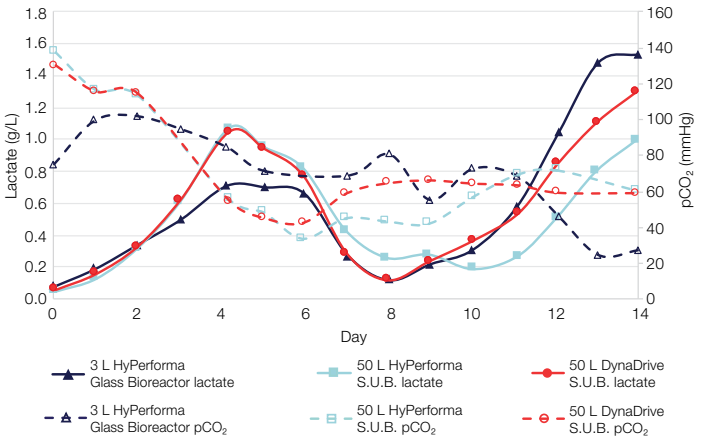


Figure 5. Lactate and pCO_2 profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

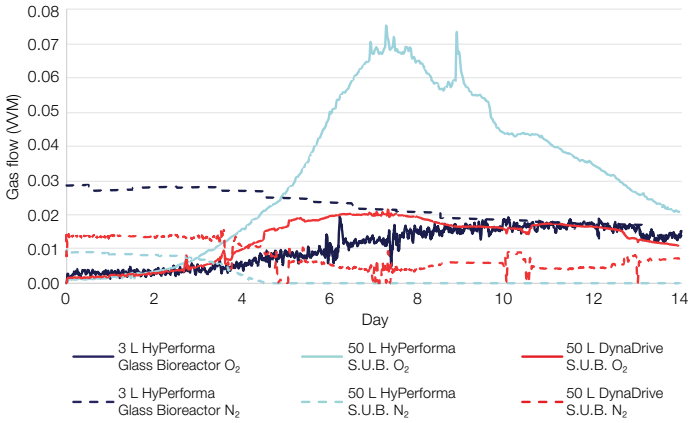


Figure 6. Oxygen and nitrogen gas flow through the drilled-hole sparger for each vessel in terms of vessel volume per minute.

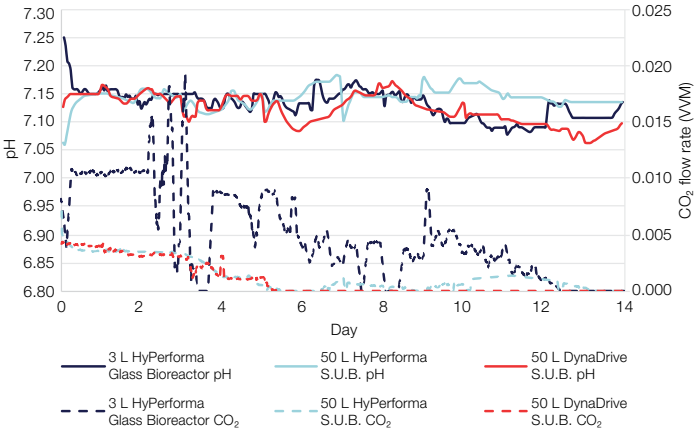
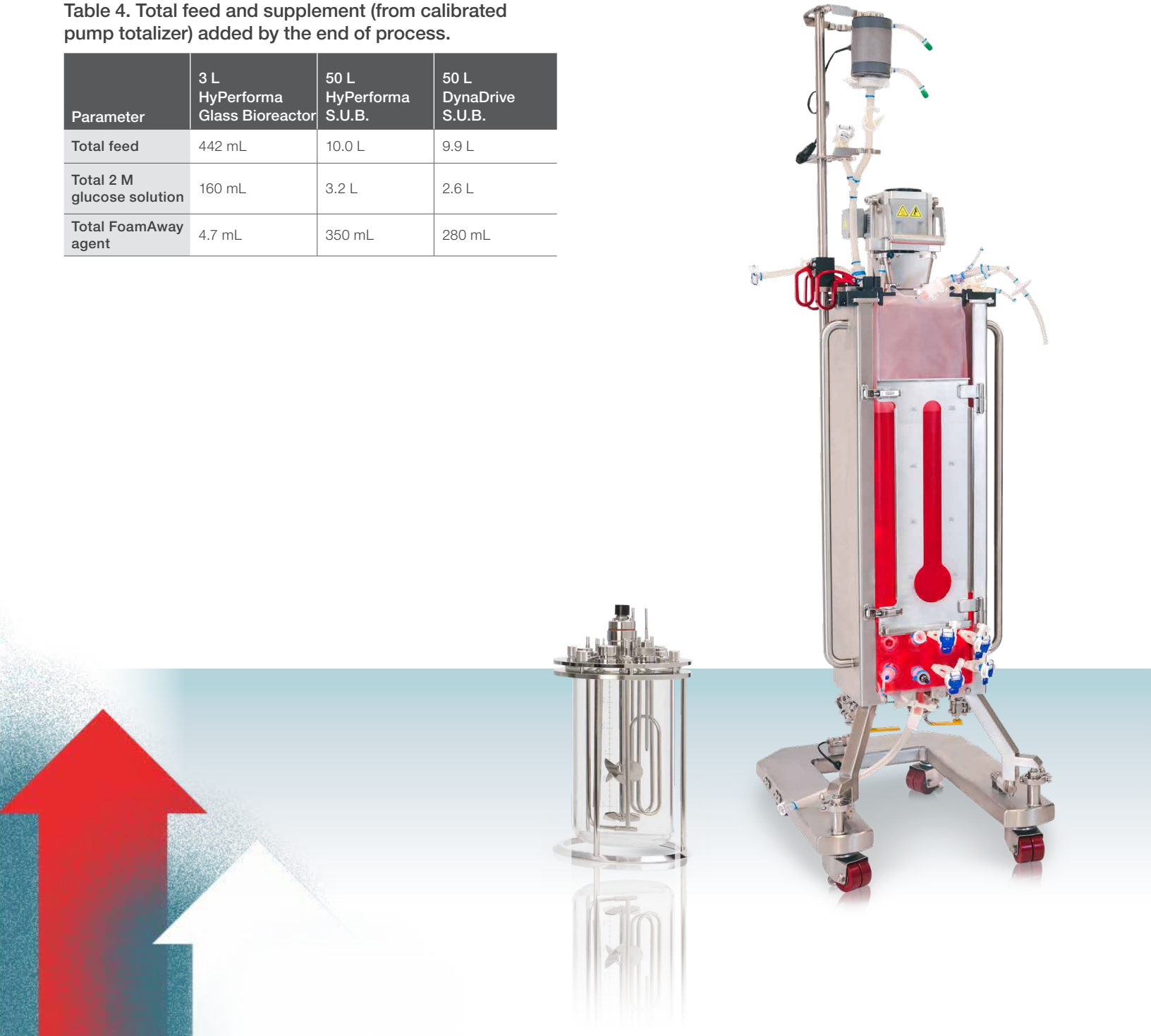


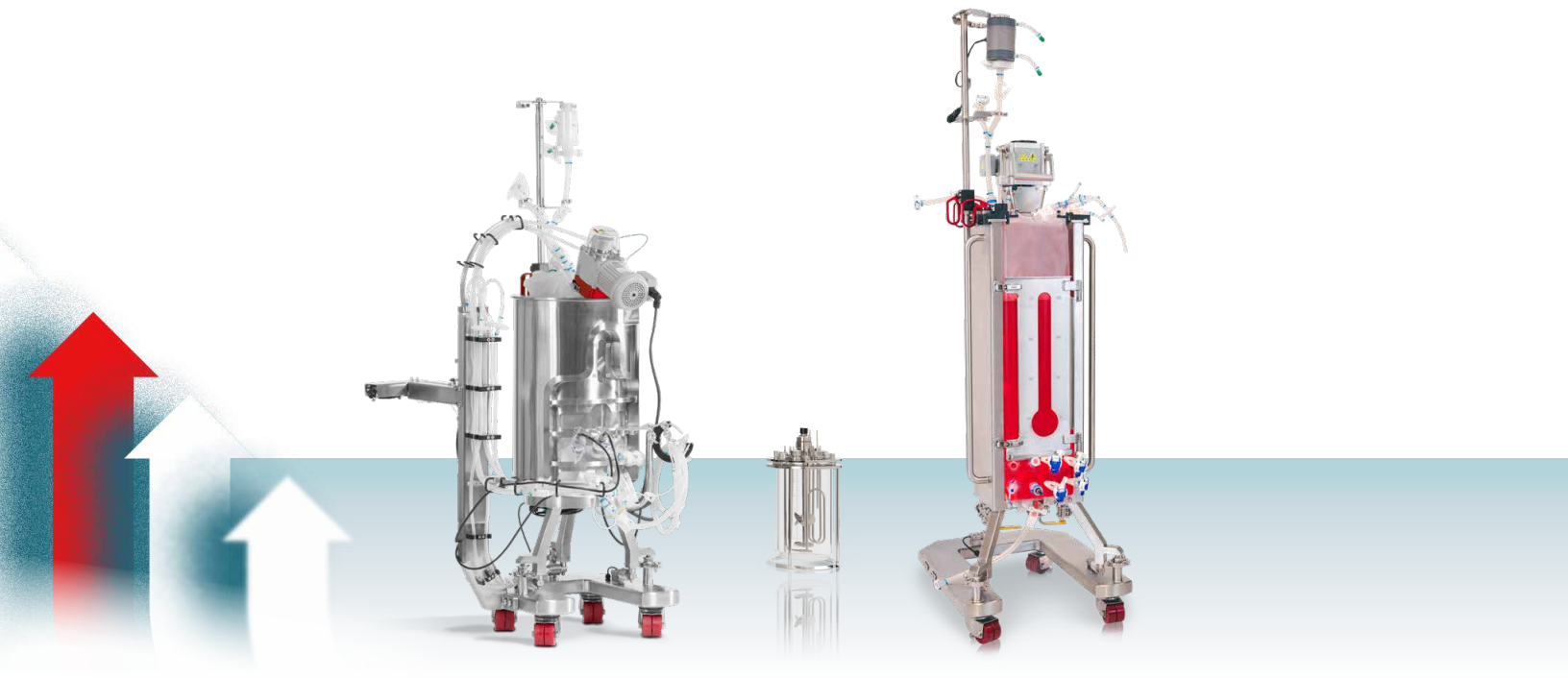
Figure 7. pH profile and carbon dioxide gas flow through the drilled-hole sparger in terms of vessel volume per minute.

Totals for feeds and supplements at the time of run termination are provided in Table 4. Antifoam requirements are significantly lower in the 3 L culture compared to the 50 L cultures due to much smaller sparged gas flow rates, larger pore sizes in the drilled-hole sparger, and the foam sensor's proximity to the liquid level in the smaller culture.

Table 4. Total feed and supplement (from calibrated pump totalizer) added by the end of process.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
Total feed	442 mL	10.0 L	9.9 L
Total 2 M glucose solution	160 mL	3.2 L	2.6 L
Total FoamAway agent	4.7 mL	350 mL	280 mL





Discussion and conclusion

Considerations for scale-up of cell culture processes frequently center around agitation and gassing strategies. The control parameters used in this study were found to produce comparable results when scaling from benchtop glass bioreactors to Thermo Scientific™ S.U.B.s at the 50 L scale. Cell growth profiles, metabolism, and productivity were maintained in similar ranges in each of the bioreactors. While an aggressive CHO-K1 clone was used, with VCD reaching 65 x 10^6 cells/mL in the DynaDrive S.U.B., the cell cultures were easily maintained within the design constraints of the reactors, with mixing and gassing demands being met in the glass bioreactor and the S.U.B.s without difficulty. The final protein titer at the time of harvest was 3.48 g/L in the glass bioreactor and 3.73 g/L in the DynaDrive S.U.B., with productivity being maintained during the duration of the culture.

Author
Jace Parkinson, Engineer II, Systems Design, Thermo Fisher Scientific, Logan, UT

Ordering information

Product	Cat. No.
Efficient-Pro AGT Medium	A5322303
Efficient-Pro AGT Feed 1	A5209102
FoamAway Irradiated AOF Antifoaming Agent	A1036902
HyPerforma G3 Lab Controller	F100-2695-001
HyPerforma G3 Lite Controller	F100-2701-001
HyPerforma G3 Pro Controller	F100-2961-001
HyPerforma Glass Bioreactor (3 L)	F100-2680-002
HyPerforma 5:1 Single-Use Bioreactor (50 L)	SUB0050.9500
Bioprocess Container for HyPerforma 5:1 S.U.B. (50 L)	SH31073.01
DynaDrive Single-Use Bioreactor (50 L)	DDB0050.1011
DynaDrive Bioprocess Container (50 L)	SH31192.01

Single-use technologies

Scale-up evaluation of the DynaDrive S.U.B.s

Part 1: ExpiCHO-S Cells and CHO-S Cells (cGMP banked)

Authors: Jordan Cobia, Product Manager; Ben Madsen, R&D Manager

Keywords
Single-use bioreactor, DynaDrive S.U.B., scalability, fed-batch

Introduction

As a molecule approaches commercial launch and more is known about the potential market demand, companies are often faced with the decision to scale up or scale out their manufacturing processes. Generally, when scale-up production vessels of more than 2,000 L are required, this decision also involves moving from single-use bioreactors (S.U.B.s) to traditional stainless-steel systems.

Additionally, more recently developed process intensification methods have allowed manufacturers increased product output, pushing titers past 10 g/L in some cases. These output achievements require increased production efficiency and input, pushing many bioreactor systems past their limits. S.U.B. quality requirements, robustness, and functional performance can all become constraints, especially at scales up to 2,000 L. For example, as oxygen transfer rate (OTR) becomes a limiting factor, most traditional S.U.B.s rely primarily on increased sparging flow to increase oxygen mass transfer.

Maintaining a dissolved oxygen (DO) target in high-demand cell cultures can be increasingly difficult due to limitations in the amount of mixing power that can be distributed effectively through the drivetrain of traditional S.U.B.s. Sparging through a micro-sparger has become a widely used strategy to improve OTR in traditional S.U.B.s and typically requires a secondary sparger to facilitate removal or stripping of dissolved CO_2 (measured as the partial pressure of CO_2, or pCO_2). Some cell lines, however, are sensitive to the higher shear produced by micro-sparging, and process scale-up cannot depend on this method alone to ensure sufficient O_2 delivery or CO_2 removal.

A next generation of S.U.B., the Thermo Scientific™ DynaDrive™ S.U.B., with vastly improved mixing and mass transfer performance, is now enabling scale-up to 5,000 L and process intensification. Previous limits are no longer a burden for the DynaDrive S.U.B., and it continues to leverage known and acknowledged benefits of legacy units. DynaDrive S.U.B.s are multifunction reactors for a range of applications, including intermediate-scale production of preclinical, clinical, and commercial material, as well as perfusion for both production and N-1 seed processes. Additionally, each DynaDrive S.U.B. includes features that are improved over legacy and alternative S.U.B. options:

- Each system is equipped with a Thermo Scientific™ BioProcess Container (BPC) load assist device, reducing handling and setup time, increasing safety, and providing consistent BPC loading. BPC loading can be accomplished in less time at the 50 L and 500 L scales compared to legacy S.U.B.s, and in less than 45 minutes at the 3,000 L and 5,000 L scales.
- Best-in-class enhanced drilled-hole sparger (DHS) provides repeatable and reliable performance that users of S.U.B.s have embraced due to its linear scale-up benefits.
- Revolutionary drivetrain design with multiple impellers allows increased power input and efficiency while offering reduced shear rates.
- Cuboid design contributes to better BPC fit and increased baffled-like mixing efficiency, and allows more productive use of facility footprint.
- 10:1 or better turndown ratio reduces facility requirements and investment costs while increasing flexibility in seed train applications and all aspects of scale-up.
- Continuous mixing during harvest and minimal hold-up volume (<1%) after drain.
- Improved exhaust system for the 3,000 L and 5,000 L S.U.B.s, allowing for increased gas flow rates and utmost reliability typically required for production-scale cultures.

These major design changes have enabled a power-to-volume (P/V) ratio of up to 80 W/m^3 in all sizes, t_{95} mixing times of less than 60 sec, and k_La performance of at least 40 hr^{-1} at all scales (Table 1).

Additionally, the DynaDrive S.U.B. allows for process scale-up and transfer from legacy S.U.B.s, offering benefits of consistent BPC film, assurance of supply, robust quality controls, BPC integrity, and industry-leading BPC customization options. End users can continue using previously qualified traditional and single-use sensing options as well as inlet and exhaust filters and other peripheral components integrated through high-strength porting and line sets.

Goal

The goal of this study was to evaluate the performance of the DynaDrive S.U.B. across 50 L–5,000 L scales using two different cell lines (Table 2) together with previously developed processes specific to those cell lines for manufacturing up to a 2,000 L scale. These experiments were designed to demonstrate that the DynaDrive S.U.B. could be successfully implemented for use with multiple cell lines across scales with standard scale-up criteria. Both cell lines were subjected to a 14-day fed-batch run at full working volume for each scale.

Table 1. Comparison of DynaDrive S.U.B. capabilities.

Parameter	50 L S.U.B.	500 L S.U.B.	5,000 L S.U.B.
Maximum volume	50 L	500 L	5,000 L
Turndown ratio	10:1	20:1	20:1
k_La	>50 hr^{-1}	>50 hr^{-1}	40 hr^{-1}
t_{95} mixing times	<30 sec	<40 sec	<60 sec
Maximum P/V ratio	80 W/m^3	80 W/m^3	80 W/m^3

Table 2. Cell lines evaluated in 50 L–5,000 L DynaDrive S.U.B.s.

Parameter	Cell line 1	Cell line 2
Cell type	Gibco™ ExpiCHO-S™ Cells	Gibco™ CHO-S™ Cells (cGMP banked; part of the Gibco™ Freedom™ CHO-S™ Kit)
Production medium	Gibco™ ExpiCHO™ Stable Production Medium (SPM)	Gibco™ Dynamis™ Medium
Feed supplement	Gibco™ EfficientFeed™ C+ AGT™ Supplement	
Titer range	Medium: ~3 g/L	Low: ~1 g/L
Cell line characteristics	Platform cell line	Legacy cell line
Case study	1	2

Case study 1

Scale evaluation using ExpiCHO-S Cells in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks or pilot-scale S.U.B.s through the N-2 stage. The N-1 stage for each seed train was performed at a 10:1 turndown ratio. Fresh production medium was added to the S.U.B. after 3 days, resulting in the culture starting at proper N-stage production volume and initial seed density. Operating conditions are described in Table 3. Daily bolus feeds of 2X concentrated EfficientFeed C+ AGT Supplement were added from day 3 to 13 via either a subsurface (5,000 L S.U.B.) or top feed line (50 L and 500 L S.U.B.s). Glucose was supplemented in the same manner on an as-needed basis after taking a glucose measurement following addition of EfficientFeed C+ AGT Supplement to bring the final glucose concentration to >4 g/L. Cell counts, viability, dissolved gases, nutrients, and metabolites were measured offline daily. Titer samples were filtered and frozen daily starting on day 6 for batch testing at the culmination of the run.

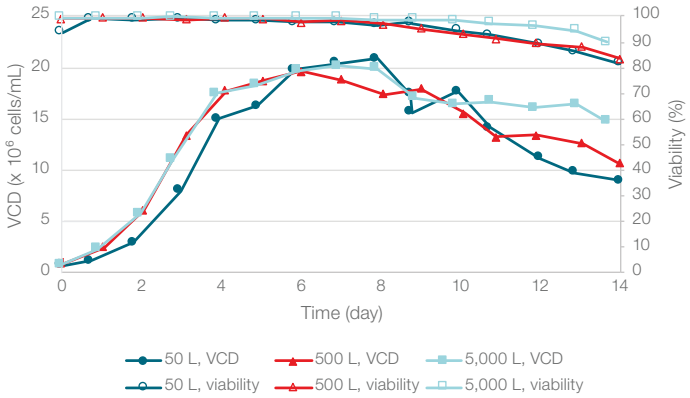


Figure 1. VCD and viability comparison of ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

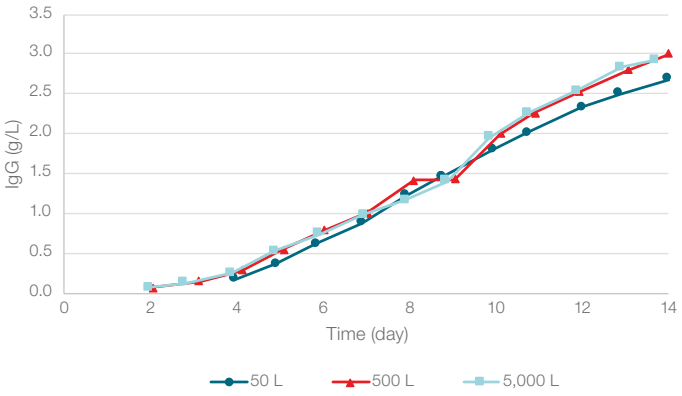


Figure 2. Titer results for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

Table 3. Operating parameters for evaluation of ExpiCHO-S Cells in 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	35 L	350 L	3,500 L
Seeding density	0.7 x 10^6 cells/mL		
Temperature	37°C (days 0–5) 34°C (days 5–14)		
pH	6.8–7.2		
pH control	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 through macro DHS Base control: 1 N NaOH
Agitation	120 rpm	60 rpm	26 rpm (days 0–3) 33 rpm (days 3–14)
DO	40%		
Air headspace	1 slpm	6 slpm	10–20 slpm
DO cascade	Air supplemented with O_2 through DHS	Air supplemented with O_2 through DHS	Air supplied to both macro and micro DHS; O_2 supplemented through micro DHS
Feeding strategy	Daily bolus of 1.05 L EfficientFeed C+ AGT Supplement, and glucose (as needed)	Daily bolus of 10.5 L EfficientFeed C+ AGT Supplement, and glucose (as needed)	Daily bolus of 105 L EfficientFeed C+ AGT Supplement, and glucose (as needed)

Results

Viable cell density (VCD) and viability for the cultures show consistent growth profiles among the cultures, with similar cell density and viability trends (Figure 1). Peak VCDs were similar at ~20 x 10^6 cells/mL, and the end-of-run viability was above 80% in all systems. IgG concentration measured 2.7–3.0 g/L on day 14 and is within ranges observed historically (Figure 2). While CO_2 levels in the 5,000 L culture were within expected conditions, levels in the 50 L and 500 L cultures were slightly lower than anticipated, indicating possible over-stripping of CO_2 (Figure 3).

Metabolite data collected offline indicated healthy cultures with maintained glucose and low levels of metabolic byproducts, including lactate and ammonium, staying within ranges observed historically (Figure 4). Minimum glucose concentrations were maintained according to the culture protocol by feeding with EfficientFeed C+ AGT Supplement daily, and a glucose solution when needed.

The gas flow rates were controlled based on culture oxygen demand (Figure 5). For the 50 L and 500 L cultures, DO was maintained by first sparging air, then supplementing with O_2 through the single DHS. For the 5,000 L culture, DO was maintained by sparging air through the macro DHS at a constant flow rate and sparging an air–O_2 mix through the micro DHS. Using these strategies, DO was maintained at 40 ± 5% with no major fluctuations during the duration of the run. Importantly, gas flow requirements for the cultures remained very low at all scales, never reaching past 0.035 vessel volumes per minute (VVM) for either the 50 L or 500 L S.U.B. For the 5,000 L S.U.B., gas flow requirements were below 0.01 VVM for the micro DHS with a constant 0.005 VVM for the macro DHS. These low gas flow rates in conjunction with the relatively low power inputs used at low rpm represent only about 30% of the available performance capacity of the system. The sparge strategy of the 5,000 L S.U.B. was adjusted on days 3–6 to successfully keep pCO_2 levels below 80 mm Hg for the balance of the cell culture run.

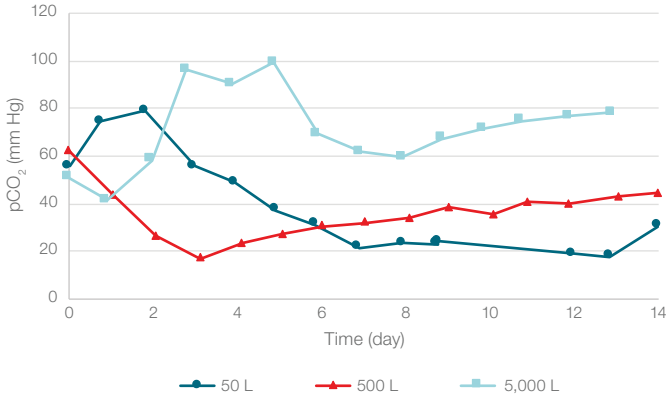


Figure 3. pCO_2 profile measurements for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

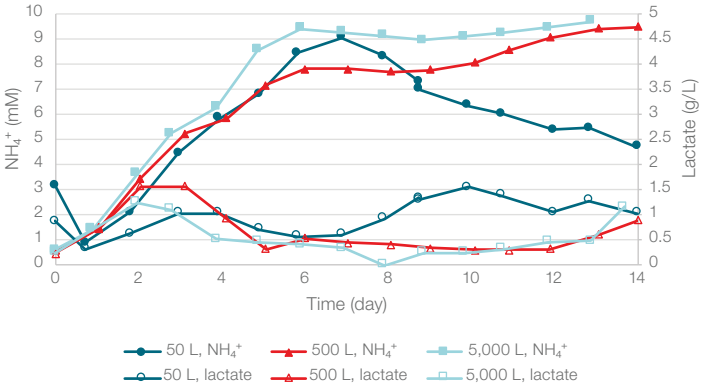


Figure 4. Lactate and NH_4^+ profiles for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

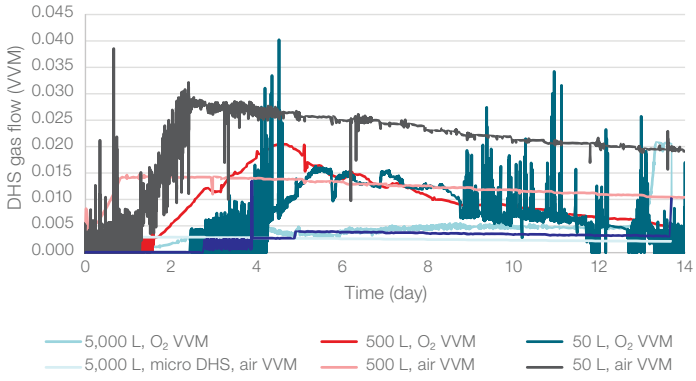


Figure 5. DHS gas flow rates for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

Case study 2

Scale evaluation using CHO-S Cells (cGMP banked) in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks or pilot-scale S.U.B.s until seeding into the production vessels at either a 10:1 or 20:1 turndown ratio. The 50 L and 500 L S.U.B.s were brought to full volume with fresh production medium after 1–2 days, while the 5,000 L S.U.B. was filled to a 5:1 turndown ratio after 3 days and then full production volume after another day of growth. Operating conditions for each bioreactor are described in Table 4. A 2X concentration of EfficientFeed C+ AGT Supplement was added continuously from day 3 to 11 through either a subsurface (5,000 L S.U.B.) or top feed line (50 L and 500 L S.U.B.s). Glucose was supplemented in a continuous drip as needed depending on culture demands, to maintain glucose concentrations of 1–3 g/L. Cell counts, viability, dissolved gases, metabolites, and nutrients were measured offline daily. Titer samples were filtered and frozen daily starting on day 6. Titer samples were batch tested at the end of the run.

Results

VCD and viability for the cultures show similar growth profiles among the cultures, with similar peak VCD and viability trends (Figure 6). The 5,000 L culture exhibited slightly slower growth and lower peak VCD compared with the 500 L culture (VCD of 23 x 10^6 and 26 x 10^6 cells/mL, respectively) but was still within expected ranges. Productivity throughout the run was within expected ranges (Figure 7). The 50 L culture exhibited the lowest harvest titer, likely due to low pCO_2 in the latter half of the run.

Metabolites and gas flow demands were similar in concentration and magnitude compared to the ExpiCHO-S cell cultures. Glucose was maintained in the S.U.B.s at around 1–5 g/L, while lactate and ammonium concentrations were within ranges observed historically (data not shown).

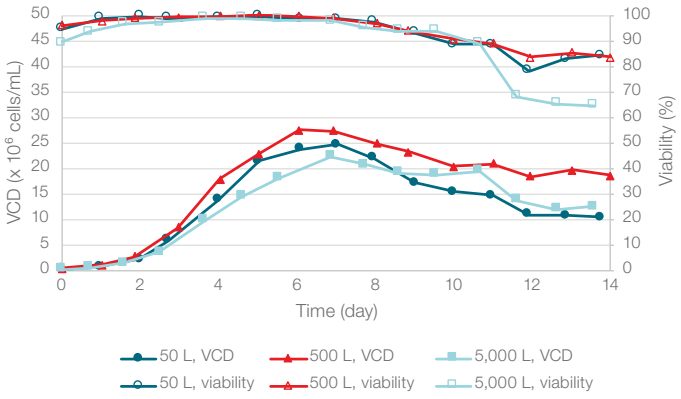


Figure 6. VCD and viability comparison of CHO-S Cells in the 3 DynaDrive S.U.B.s.

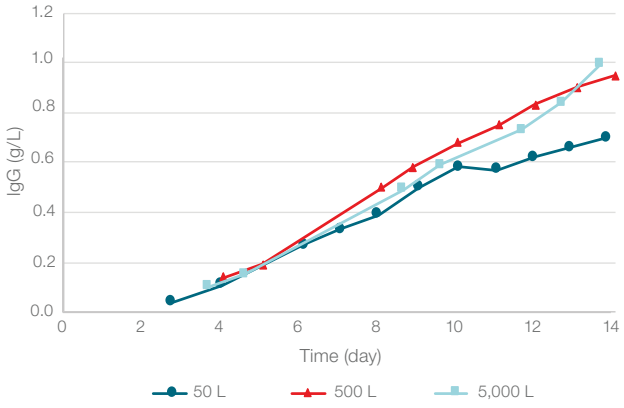


Figure 7. Titer results for CHO-S Cells in the 3 DynaDrive S.U.B.s.

Table 4. Operating parameters for evaluation of CHO-S Cells in 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	42.5 L	425 L	4,250 L
Seeding density		0.3 x 10^6 cells/mL	
Temperature		37°C	
pH		6.8–7.2	
pH control	Acid control: sparged CO_2 Base control: not applicable	Acid control: sparged CO_2 Base control: not applicable	Acid control: sparged CO_2 through macro DHS Base control: not applicable
Agitation	120 rpm	68 rpm	31–41 rpm
DO		30%	
Air headspace	3 slpm	6 slpm	10–20 slpm
DO cascade	N_2 and O_2 through the DHS	N_2 and O_2 through the DHS	N_2 and O_2 through the micro DHS
Feeding strategy	7.5 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed	75 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed	750 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed

Discussion

As cell cultures are scaled to large-volume S.U.B.s, several factors can become more difficult to manage and control. Often, decisions related to culture parameters and operation thresholds must be balanced to provide the culture with the best opportunity for success. The DynaDrive S.U.B. assists with ensuring culture success due to specifically designed aspects of the S.U.B., including the geometrically scalable agitator drivetrain and the uniquely linear sparge performance of the DHS, which both directly benefit mass transfer and mixing abilities.

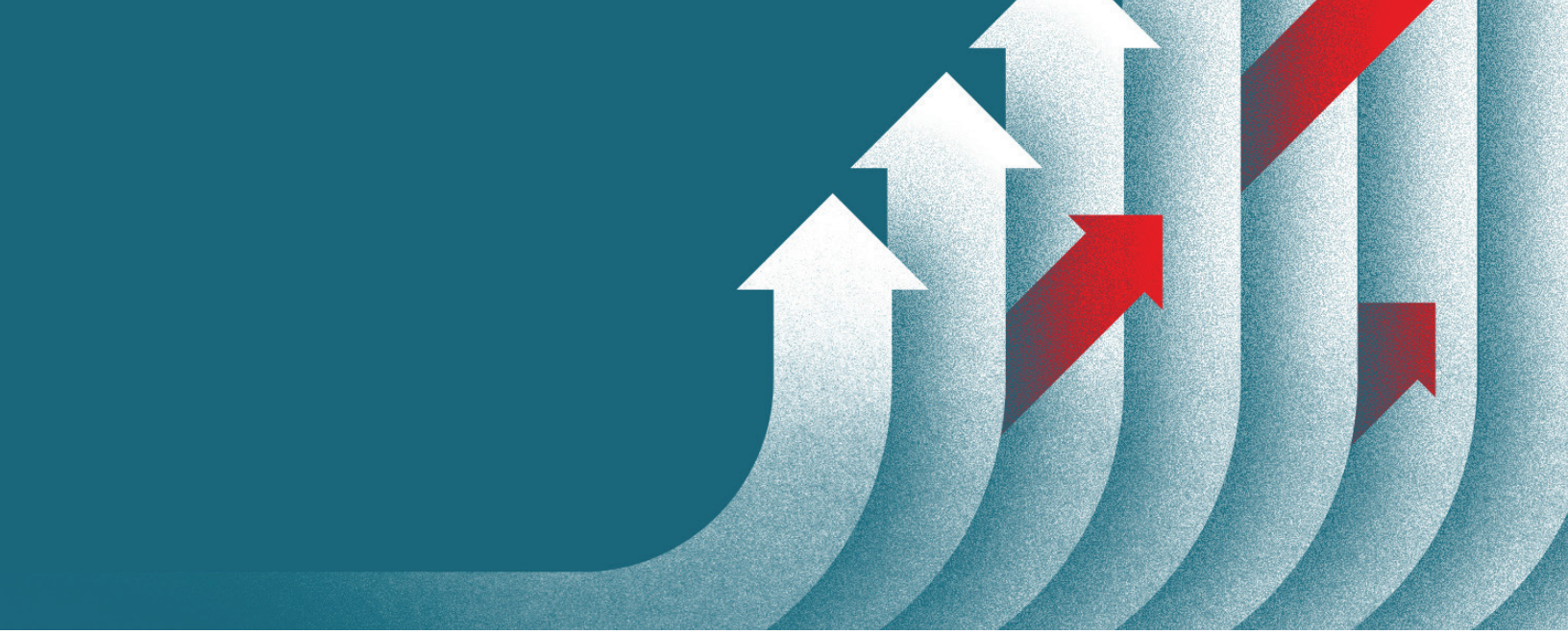
The agitator drivetrain of the DynaDrive S.U.B. with multiple impellers at optimal locations allows for optimal and scalable power input. The cultures performed in these case studies were operated at a power input of approximately 20 W/m^3, far below the maximum recommended 80 W/m^3. Mass transfer in this design is more proportional to the power input of a system due to the mixer's ability to both disperse and retain sparged gas. Therefore, if a culture were to demand more mass transfer, increased mixer speeds above those tested here would be an option for improved performance.

The gas flow rates observed in these studies were far below the maximum rated gas flow rates for each system. As with power input for each system, mass transfer is shown to be linearly proportional and scalable to gas flow rate in the DynaDrive S.U.B.s. For the 50 L and 500 L ExpiCHO-S cell cultures, total gas flow rate through the DHS was never higher than 0.035 VVM, less than 25% of the maximum 0.15 VVM limit. For the 5,000 L ExpiCHO-S cell culture, total gas flow rate through the micro DHS reached only 0.007 VVM (30 slpm) for most of the culture. Additionally, the 5,000 L culture used a minimal macro DHS flow rate of only 15 slpm of air to maintain CO_2 stripping and to provide additional O_2 mass transfer. These chosen gas flow rates resulted in easily maintained O_2 mass transfer to support cultures of light to moderate demand with cell densities at 20–30 x 10^6 cells/mL. More demanding clones are expected to require higher gas flow rates or agitation but still remain within design limits of the DynaDrive S.U.B.s. It is important to note that the DHSs were designed with specific pore quantities and sizes to provide these high levels of mass transfer at low gas flow rates, removing the need for other sparger types such as the legacy sintered sparger.

One of the biggest concerns when scaling to larger S.U.B. sizes is the ability to control dissolved CO_2 concentrations within ideal physiological ranges (60–100 mm Hg). While spargers in smaller S.U.B.s typically provide sufficient CO_2 stripping capability due to their often oversized bubbles, shorter liquid column heights, and favorable overlay surface-to-volume ratios, spargers for larger S.U.B.s can be limited in providing sufficient CO_2 stripping while maintaining required O_2 mass transfer at reasonable gas flow rates. The 5,000 L DynaDrive S.U.B. is equipped with 3 separate DHSs to provide optimal gassing to drive mass transfer for both gases of interest. The two larger macro DHSs provide an amount of O_2 mass transfer while also providing more CO_2 stripping capability due to the larger bubbles created, while the single micro DHS provides higher O_2 mass transfer due to the smaller bubbles created. Balancing these spargers in tandem with agitation in these cultures allowed pCO_2 to remain within desired ranges for each culture, thus providing a very generous operating design window in anticipation of future process intensification that may be requested by the end user.

Culture conditions in a S.U.B. are highly variable and must be balanced with multiple inputs and outputs. Through experience and tracking online and offline readings, setpoints can be balanced for gas flow rates, agitation, pH, DO, and feed flow rates. Generally speaking, metabolite buildup such as lactate and respired CO_2 lead to acidic conditions in the reactor that require the addition of base to balance the culture pH. Additionally, especially in larger vessels, buildup of pCO_2 can be detrimental to cell health. The S.U.B.s in this study were able to maintain pCO_2 and pH conditions through the employed gassing strategies, leading to pCO_2 levels maintained within physiological conditions. The 50 L and 500 L cultures actually showed a propensity to exhibit too much CO_2 stripping. While the pH in each culture was balanced sufficiently, more optimal gassing and pH control strategies could be employed in the future to provide more optimal growth and production conditions.

Finally, each reactor was seeded at a low working volume: 10:1 turndown ratio in the 50 L S.U.B. and 20:1 turndown ratio in the 500 L and 5,000 L S.U.B.s. This has been shown to reduce the complexity of seed train requirements by eliminating intermediate vessels, consumables, and operation space. While using a low turndown ratio in a production vessel such as the 5,000 L DynaDrive S.U.B. is not ideal for some commercial applications seeking to maximize daily productivity, this feature allows for flexibility in manufacturing spaces not previously available.



Conclusions

The DynaDrive S.U.B.s were able to support both clones in this study and provide controlled conditions to achieve target VCDs and titers while maintaining high viability. This was achieved by choosing simple scale-up parameters among the S.U.B. sizes, including consistent power input and gas flow rates. The low gas flow rates and agitation rates tested provided sufficient mass transfer to maintain DO setpoints while maintaining pCO_2 levels at or below maximum limits. Additionally, the ability of each S.U.B. to be seeded at low volume allowed reduction in seed train complexity by using the S.U.B. at the N-1 culture stage. Fewer manipulations, media preparations, and fluid transfers, and less overall consumption of resources have the potential to noticeably mitigate risk, reduce waste, and lower operating costs.

Overall, the different scale-up processes demonstrated in these studies show the versatility of the DynaDrive S.U.B. to maintain culture setpoints compared to historical processes with simple scale-up criteria. The modified drivetrain and sparging, when compared to legacy S.U.B. products, did not have adverse effects on the cell cultures, thus enabling a much larger design space for process development and production than previously available. Additionally, the DynaDrive S.U.B. provides consistent, scalable performance from 50 L to 5,000 L. With this demonstrated consistency, the DynaDrive S.U.B. will enable users to scale up their process with minimal process changes to meet demand for commercial therapeutics.

Learn more at **thermofisher.com/dynadrive**

Application note

Raman spectroscopy

MarqMetrix All-In-One Process Raman Analyzer – Chemometric model transferability across instruments

Authors

Mike Bates, Nimesh Khadka, David Kuntz, Lin Zhang, Sue Woods

Summary

- The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer represents a breakthrough in process analytical technology (PAT) by providing in-line, real-time and actionable monitoring of multiple analytes in complex systems.
- This paper demonstrates the successful transferability of one chemometric model across 10 different MarqMetrix All-In-One Process Raman Analyzers while maintaining an average prediction error of 0.21 g/L, 0.16 g/L and 0.3 g/L for glucose, glutamine, and lactate, respectively in a mixture.
- Measurement accuracy and precision is maintained when applying a chemometric model across multiple instruments, ensuring users do not have to spend time or resources to re-build a model for a new MarqMetrix All-In-One Process Raman Analyzer or new autoclavable probe.

Introduction

The MarqMetrix All-In-One Process Raman Analyzer is a Raman spectroscopy instrument designed to offer rapid, robust, scalable, and reliable identification, quantification, and characterization of molecules during any phase of R&D process development. It is an “all-in-one” instrument utilizing easily exchangeable, autoclavable probes to meet the various analytical needs for, but not limited to, upstream bioprocess monitoring, or characterization of fill and finish products.

Chemometric analysis allows users to develop a data analysis model to monitor the concentration of multiple analytes from their analyzer. There is a significant investment made from a time and resource perspective to build an accurate and robust chemometric model. As a result, it is imperative for a chemometric model to be used between instruments to fully leverage the value of this investment. Once a chemometric model is developed, it can be used with any MarqMetrix All-In-One Process Raman Analyzer

to monitor multiple reactors in parallel with high fidelity of model accuracy. The measurement accuracy and precision maintained when transferring chemometric models across different system hardware ensures that customers do not have to re-build a model when using a new instrument or new autoclavable probe.

Experimental set up

To demonstrate the transferability of a complex chemometric model, we evaluated three relevant analytes: glucose, glutamine and lactate in Gibco™ DMEM growth media. All three analytes occur in bioreactors in the g/L concentration ranges with glucose occurring in the range of 0-12 g/L, glutamine 0-2.5 g/L and lactate 0-20 g/L. A chemometric model was developed by collecting spectra from a set of calibration standards using one MarqMetrix All-In-One Process Raman Analyzer. Within the relevant concentration ranges for each analyte, 24 samples with randomized concentrations were selected using the uniform design method[2]. The model was then applied to spectra from a different set of 8 validation samples, measured using 10 unique MarqMetrix All-In-One Process Raman Analyzers. The acquisition parameters were optimized to maximize the signal-to-noise ratio of the spectral features corresponding to the analyte concentrations. Optimized parameters were 15 second integration time, 450 mW laser power and 10 replicate on-board signal averaging with automatic dark correction. These acquisition parameters resulted in a sample collection period of about 5 minutes. The model was able to predict the concentration of all three analytes in the 8-sample validation set with a high degree of accuracy and precision.

Each MarqMetrix All-In-One Process Raman Analyzer evaluated in this study included a unique hardware set comprised of spectrometer box, fiber optic cables and Thermo Scientific™ MarqMetrix™ Bioreactor BallProbe™ Sampling Optic tip. Average prediction error for each analyte was 0.21 g/L for glucose, 0.16 g/L for glutamine and 0.3 g/L for lactate. These results demonstrate the excellent transferability of this complex chemometric model across numerous MarqMetrix All-In-One Process Raman Analyzers.

Model analysis

A single PLS model was built using Eigenvector Solo software to predict all three analytes in each mixture. The PLS calibration model was built using 24 calibration samples with 3 replicates per sample. All calibration spectra were collected using one MarqMetrix All-In-One Process Raman Analyzer. The Raman fingerprint region between 870-3096 cm^{-1} was used to build the model. A Savitsky-Golay smoothing filter was applied to remove the random noise and improve the signal to noise ratio. Next, baselines were corrected followed by scattering correction and normalization. In addition, all data were mean-centered before model building. The calibration model was built using the cross-validation strategy of leave-one-sample-out. The 3 replicates for the same sample were carried together in this process. After these crucial pre-processing and cross-validation steps were performed, the resulting optimized model with 4 latent variables was selected. Results of the calibration and cross-validation of the model are shown in Table 1.

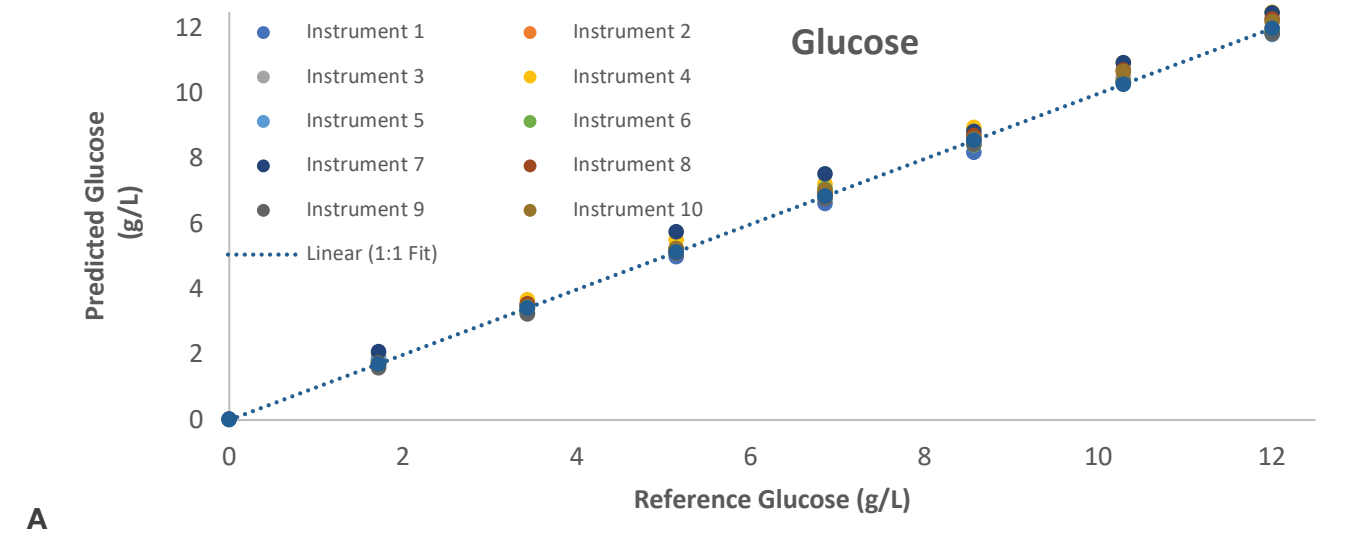
Model parameter	Analyte		
	Glucose	Glutamine	Lactate
RMSEC (g/L)	0.079	0.075	0.146
RMSECV (g/L)	0.095	0.088	0.176
Bias (g/L)	4.79E-05	-1.94E-05	-2.50E-05
CV Bias (g/L)	-2.25E-04	-6.45E-04	-4.42E-03
R^2 Calibration	0.9995	0.9902	0.9994
R^2 Cross-Validation	0.9993	0.9864	0.9991

Table 1. Calibration and cross-validation results for Chemometric model.

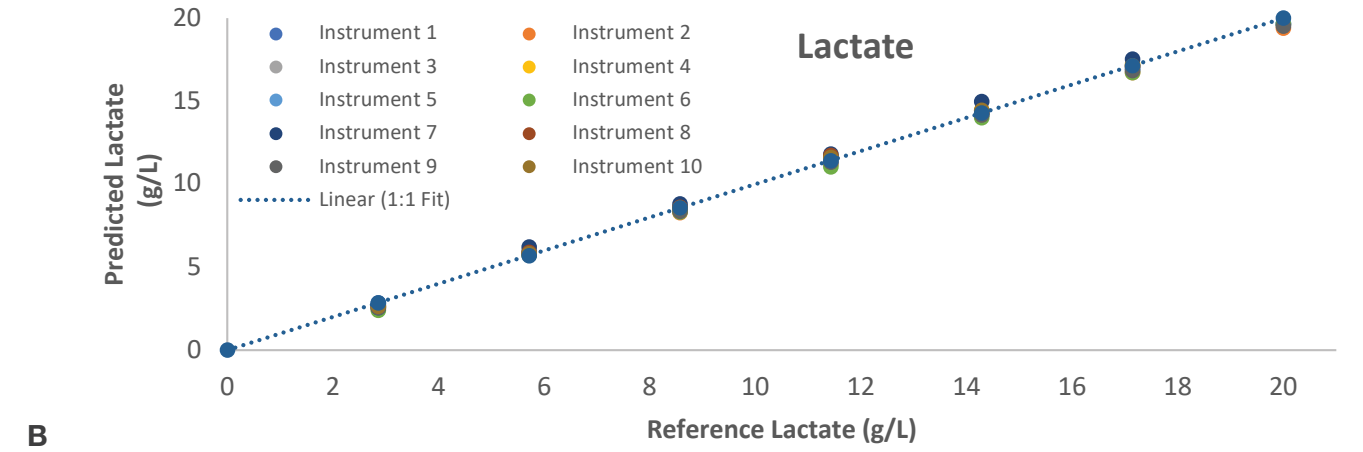
Spectra for the 8-sample validation set were subsequently collected on 10 different MarqMetrix All-In-One Process Raman Analyzers. The chemometric model was applied to the validation-set spectra for each MarqMetrix All-In-One Process Raman Analyzer to predict the analyte concentrations. The results discussed below show that high degree of accuracy and precision were maintained for the prediction of all three analytes across all 10 MarqMetrix All-In-One Process Raman Analyzers.

Results

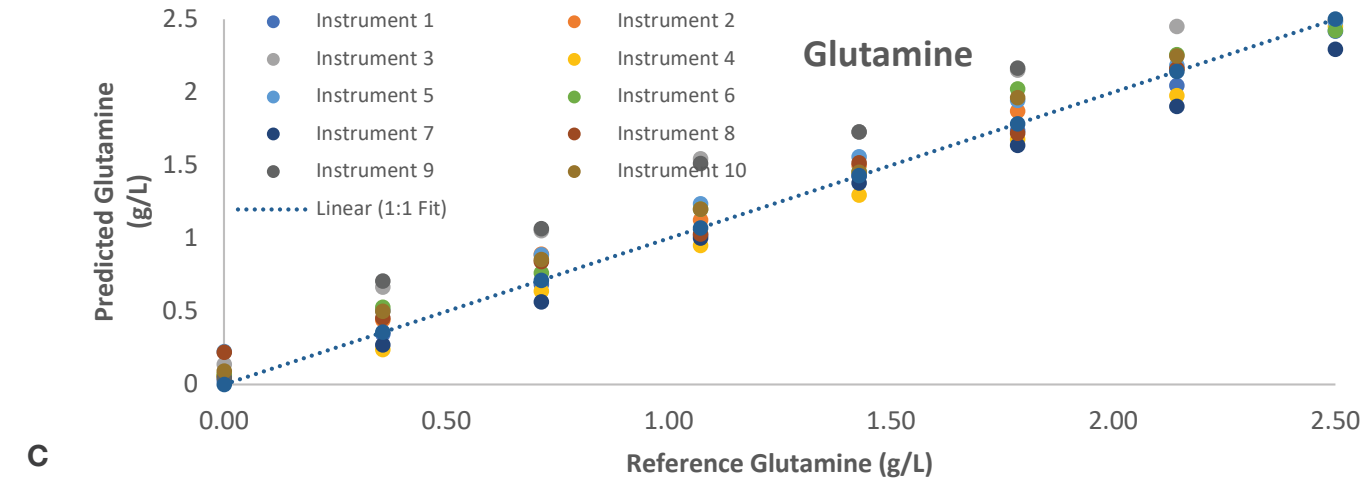
The development of robust chemometric models requires a significant investment of time and resources. To ensure that this investment provides long term value for our customers, we have demonstrated excellent transferability of chemometric models across numerous MarqMetrix All-In-One Process Raman Analyzers. The correlation plot in Figure 1 shows the predicted vs. reference values for glucose (Fig.1a), lactate (Fig.1b) and glutamine (Fig.1c). Each plot contains an overlay of the predicted values for all 10 MarqMetrix All-In-One Process Raman Analyzers. The precision of the chemometric model across 10 MarqMetrix All-In-One Process Raman Analyzers provides customers with consistent results. Furthermore, the accuracy of the model can be seen from the results of Table 2.



A



B



C

Figure 1. Prediction correlation plot across 10 MarqMetrix All-In-One Process Raman Analyzers.

Hardware	Average prediction error (g/L)		
	Glucose	Glutamine	Lactate
Instrument 1	0.18	0.05	0.20
Instrument 2	0.11	0.13	0.40
Instrument 3	0.12	0.15	0.21
Instrument 4	0.18	0.14	0.26
Instrument 5	0.18	0.08	0.40
Instrument 6	0.40	0.11	0.25
Instrument 7	0.13	0.38	0.32
Instrument 8	0.21	0.11	0.26
Instrument 9	0.47	0.15	0.44
Instrument 10	0.09	0.34	0.23
Average error (across 10 systems)	0.21	0.16	0.30

Table 2. Prediction error (RMSEP) calculated from Chemometric modeling.

Table 2 shows the average prediction error for each analyte on each MarqMetrix All-In-One Process Raman Analyzer and the average error for each analyte across all 10 MarqMetrix All-In-One Process Raman Analyzers. All parameters demonstrate a high degree of measurement accuracy with <0.5 g/L prediction error. Furthermore, in some cases, the prediction error is <0.1 g/L. This level of accuracy is in line with other published results for chemometric modeling[3] and is on par with other relevant measurement techniques for bioreactor monitoring.

With this accuracy and precision, controlling glucose concentrations within a bioreactor in the typical 2-4 g/L range can be readily achieved. Furthermore, through continuous monitoring and feedback utilization, even tighter control is possible leading to improved process and product consistency. Chemometric models developed using MarqMetrix All-In-One Process Raman Analyzer have demonstrated exceptional performance for multi-analyte monitoring in full-scale bioreactor studies. (Real time metabolite monitoring using the MarqMetrix All-In-One Process Raman Analyzer and the Thermo Scientific™ 500L HyPerforma™ Dynadrive™ SingleUse Bioreactor (S.U.B.) **MarqMetrix All-In-One Process Raman Analyzer Real time App note**

Conclusion

Customers will benefit from generating chemometric models which are transferrable between multiple MarqMetrix All-In-One Process Raman Analyzers. The ability of the MarqMetrix All-In-One Process Raman Analyzer to utilize the same chemometric model across multiple units provides users with the confidence of measurement accuracy and precision. Advanced signal processing and model optimization may be employed to further increase the level of prediction performance. This example simply provides a benchmarking reference for the development of chemometric models using MarqMetrix All-In-One Process Raman Analyzers.

References

1. FDA. Guidance for Industry: PAT—a Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, via FDA Website. 2004.. https://www.fda.gov/media/71012/download. Accessed October 17th 2022.
2. Zhang, Lin, et al. “Uniform design applied to nonlinear multivariate calibration by ANN.” Analytica Chimica Acta 370.1 (1998): 65-77.
3. Buckley, Kevin, and Alan G. Ryder. “Applications of Raman spectroscopy in biopharmaceutical manufacturing: a short review.” Applied spectroscopy 71.6 (2017): 1085-1116

Application note

Raman spectroscopy

MarqMetrix All-In-One Process Raman Analyzer

Introduction

Manufacturing processes can be extremely complex, especially biopharmaceutical manufacturing. With processes that rely heavily on living organisms to generate products of interest, such as those within the Biopharma industry, complete understanding of the process is essential to success, because the more you know about the process, the more control you can exercise. The primary problem process analytical instruments and technologies have sought to alleviate has always been a lack of visibility into the underlying reactions. What you see, and also understand, you can control and optimize.

Subtle variations in the environment can have a huge impact on yield and quality. The consequences can range from failed batches and inefficient use of resources to products that don't meet quality specifications, and limited data to help make improvements over time. Ultimately, patient safety and stakeholder interests are at risk.

We are dedicated to making instruments that help our customers gain greater control of their processes that involve chemistry. One of the most powerful and advantageous analytical technologies that we use is Raman spectroscopy. It's extremely accurate and versatile, generates very rapid results, covers a multitude of functions, and is non-destructive to whatever sample or substance is being analyzed.

Until recently, Raman Spectroscopy required a well-trained technician to operate, and demanded complex, bulky, and expensive equipment that also made it at times unsuitable for in-line or field use.

We have introduced the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer, a compact, easy-to-use, reliable, and affordable system that leverages the power of Raman spectroscopy to measure key variables in a process so that they may be controlled. The MarqMetrix All-In-One Process Raman Analyzer also makes Raman technology more accessible to non-experts through its

simplified user interface. Since it is also small, it is uniquely qualified to solve a common problem faced by those who use chemistry to make things, i.e., portability and ease of use whether in the field or in the process development space.

What is Raman spectroscopy?

The underlying technology, Raman spectroscopy, is not new; it has been around since the 1950s. What Raman instruments have lacked until now is the size, reliability, ease of use, and affordability that enables manufacturers to integrate Raman spectroscopy into their production processes where it can be instrumental in improving efficiency and quality. Before MarqMetrix All-In-One Process Raman Analyzer, integrating Raman technology into a manufacturing process was problematic. There were challenges with the hardware; it was difficult to use and required a scientist on staff to operate and maintain it. Reliability and cost were also issues. Better hardware was needed, as well as a simplified, more user-friendly interface. These features and advantages are now realized in MarqMetrix All-In-One Process Raman Analyzer, but what exactly is Raman spectroscopy all about?

Non-destructive analysis and process monitoring

The technology begins with a laser that is directed at a substance or sample through a fiberoptic cable with a probe at the end. The energy from the laser light causes covalently bonded molecules in the substance to vibrate and the light from the laser may scatter elastically (the same laser energy is released as what caused the molecule to vibrate) or inelastically (some of the laser energy is absorbed by the molecule and a lesser amount of energy is released than what caused the molecule to vibrate). Some of this inelastically scattered light makes its way back into a detector within the MarqMetrix All-In-One Process Raman Analyzer. The detector collects and interprets the light scattered from the sample to generate a "picture" called a Raman spectrum. What makes this technology powerful is that the Raman spectrum of a molecule is unique, and for that reason it's sometimes referred to as its molecular fingerprint. Just as fingerprints may be used to identify people, we use the Raman spectrum to identify a given substance because the fingerprint can tell us not only qualitatively what something is, but also quantitatively how much there is of it- one may determine both the identity and concentration of a given analyte of interest. The non-destructive nature of Raman spectroscopy provides a tremendous value-add. This is very important because it can be integrated directly into a production line to measure and analyze on a continuous basis, serving as a process monitor. This is what is called in-line or online measurement. Raman technology is also fast, and most substances can be measured in a matter of seconds or less.

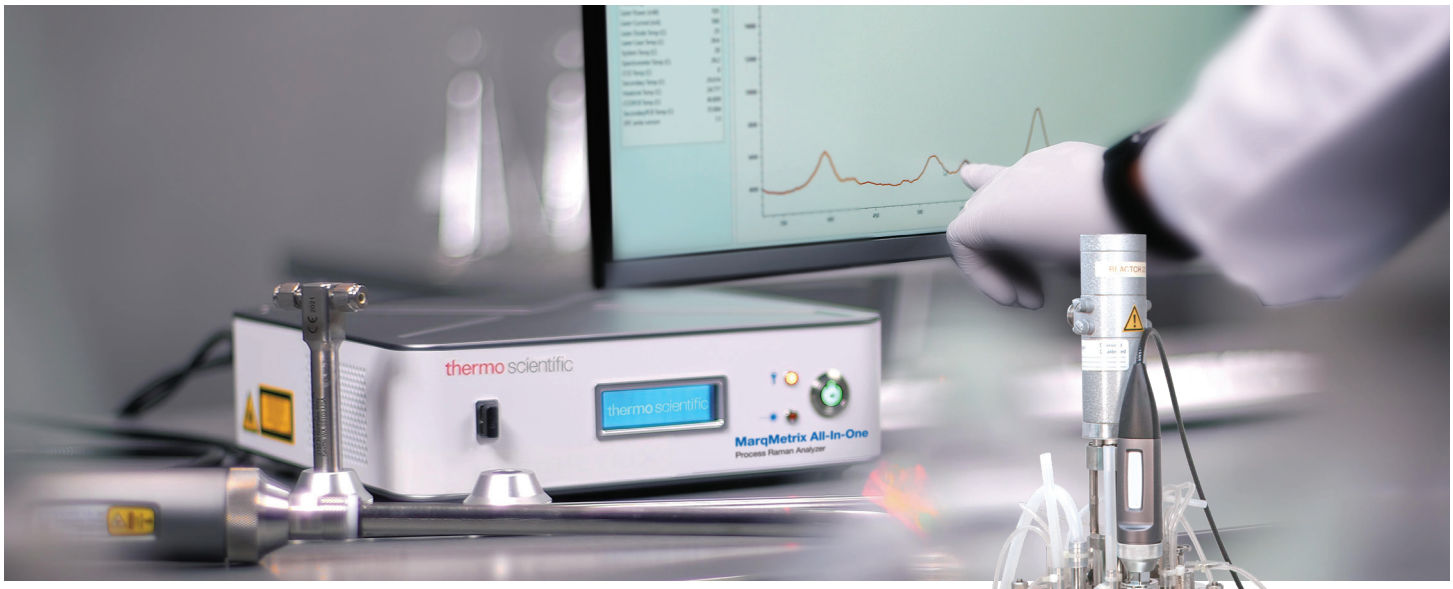
Versatile, precise, providing a wealth of information

Additionally, the Raman spectrum provides us with a great deal of information about the substance being tested; every one of the peaks in the Raman spectrum tells us something unique about what we're testing, providing us with many opportunities to identify what we're looking for. This is extremely important when measuring in a bioreactor, for example, containing many molecules of different types.

The next advantage is that there's a direct and linear relationship between the concentration of a given substance and the intensity of the peaks in the spectrum. This means that building quantitative models is easier. With a relatively small number of samples, we can build a model that accurately predicts concentration across our range of detection. We can also measure substances in all forms, whether they're solid, liquid, gas, powder, or slurry. A final advantage is that unlike other forms of spectroscopy, water does not distort Raman measurements; therefore, we can see clearly what's happening in the aqueous solution like that found inside of a bioreactor. Competitively, MarqMetrix All-In-One Process Raman Analyzer is better suited for inline and online measurements in aqueous-based solutions than competing technologies, but perhaps its greatest advantage is that it is non-destructive.

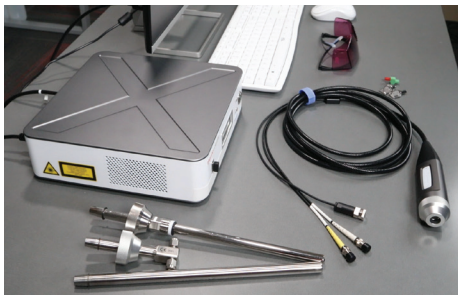
Process control

There are Raman applications that span the entire biopharma manufacturing process. Verifying the integrity of raw materials is the first step where MarqMetrix All-In-One Process Raman Analyzer can help ensure that the process gets off to a good start. In practice, we've observed notable variations in the chemical composition of growth media, variations significant enough to have an impact on yields and cycle times. It's important to be able to detect changes in the material in real time. For example, customers want to know what's happening in the bioreactor so that they can make adjustments if necessary. This is true regardless of whether or not manufacturing is done via batch or continuous flow, and MarqMetrix All-In-One Process Raman Analyzer works in either scenario. Do the cells have the right amount of glucose? Are too many secondary metabolites building up? Are the cells beginning to produce the product of interest? How much product has been produced, and does it have the right characteristics? MarqMetrix All-In-One Process Raman Analyzer enables answers to all of these questions, and because the unit can interface with third-party control systems, adjustments can be made in near real-time to optimize conditions. Finally, when capturing finished product downstream, manufacturers need to know exactly when and how much protein is coming out of a purification column, for example. These are just a few examples of how MarqMetrix All-In-One Process Raman Analyzer helps achieve higher efficiency and quality in chemistry-dependent manufacturing.



Robust yet simple operation

MarqMetrix All-In-One Process Raman Analyzer is easy to use. A technician with no prior Raman experience can typically begin taking measurements within 15 minutes of removing the instrument from the box. Facilities across the country can use MarqMetrix All-In-One Process Raman Analyzer to take hundreds of measurements daily without the need for any scientific staff to maintain and calibrate the instrument.



MarqMetrix All-In-One Process Raman Analyzer is compact. With a footprint less than one square foot and three inches tall, MarqMetrix All-In-One Process Raman Analyzer is sized to be placed at or near the point of measurement and with no moving parts other than its cooling fan, MarqMetrix All-In-One Process Raman Analyzer is designed to be reliable and stable. Up time exceeds 99% and calibrations performed in the factory remain accurate for years. By being easy to use and reliable, MarqMetrix All-In-One Process Raman Analyzer is also less costly to install and operate over time, with a very low cost of ownership.

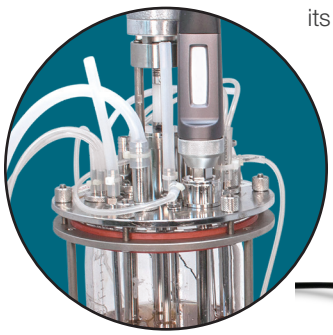
Software built to simplify chemometrics

Maximize the power of the MarqMetrix All-In-One Process Raman Analyzer with Thermo Scientific™ Lykos™ PAT Software, a remote control and monitoring software designed to simplify the complexities of chemometrics. Lykos PAT Software facilitates data acquisition and analysis by displaying, storing, and exporting bioprocess monitoring data in real-time, enabling remote control and monitoring of the MarqMetrix All-In-One Process Analyzer and providing an intuitive workflow to streamline processes for nonRaman spectroscopists and Raman experts alike.

A wide range of easily-swappable probes are available

The MarqMetrix All-In-One Process Raman Analyzer can be adapted to almost any R&D production or process system. It features a Thermo Scientific™ MarqMetrix™ Fiber Head and Thermo Scientific™ MarqMetrix™ BallProbe™ Sampling Optic that fits any need due to a wide array of easily interchangeable probes. Swapping the probe is easy; simply unscrew the fastener, remove the probe, swap in another, and tighten it down again. Our tapered fitting allows for easy indexing, requires no alignment, and there's no need to recalibrate the system.

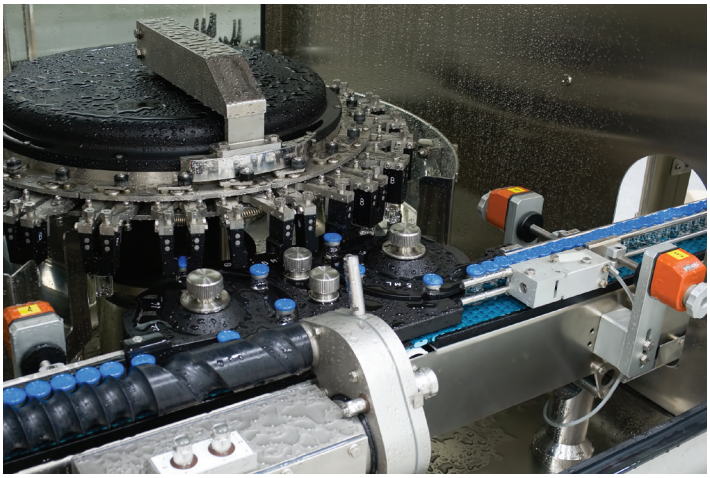
The MarqMetrix All-In-One Process Raman Analyzer lineup of standard fiber probes allows measurement of compounds in any form, e.g., solids, liquids, gas, slurries, pastes, and gels. It can measure by contact or immersion with its performance half inch ball probe and/or standard ball probes which range in size from 1/2" down to 1/8". For higher temperatures and harsh chemicals, there are process ball probes that are gold sealed and temperature safe

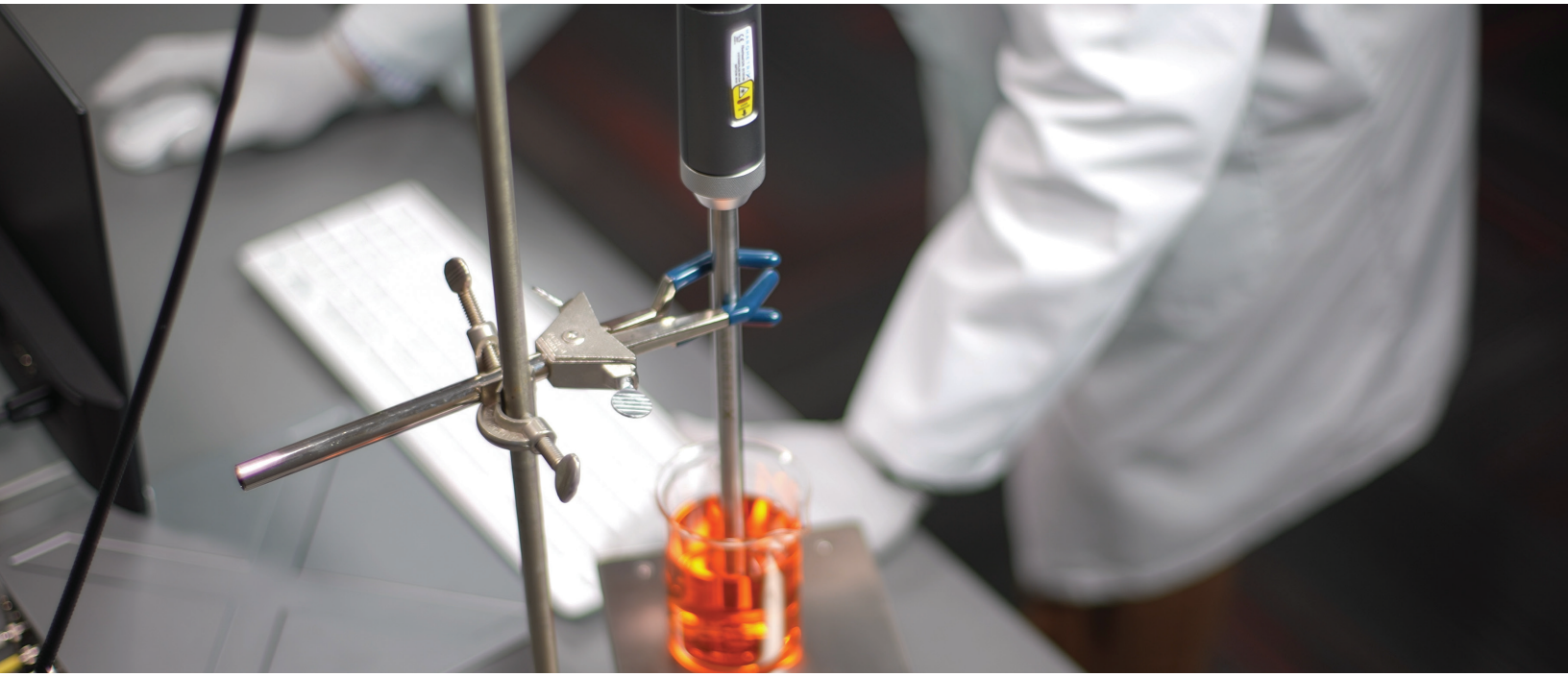


to 350 degrees Celsius. MarqMetrix BallProbe Sampling Optics are also available in bioreactor-specific versions with a seal and nut to allow for immersion in a reaction vessel, be it a dedicated stainless-steel bioreactor or a single-use bioreactor. These Thermo Scientific™ MarqMetrix™ Bioreactor BallProbe™ Sampling Optics are autoclavable, either while attached to the bioreactor, or on their own when cleaning and sterilizing the ball probe separately.

Integration into a high-pressure flow system is simplified with patented flow cell technology operable at 2500 psi. Custom probes are also available.

At Thermo Fisher, we're realizing the full potential of Raman technology by building better instruments, and we're all about making Raman spectroscopy more accessible to help our customers control chemistry. We're opening up new applications for Raman and increasing the returns on existing applications-returns that come in the form of faster cycle times, higher yields, and better quality.





Learn more at **thermofisher.com/marqmetrixaio**

Single-use bioreactor solution

The **DynaDrive Bioreactor Platform** is a high-performance, single-use bioreactor system designed for optimized mixing, mass transfer, and scalability in biopharmaceutical production.

Learn more at **thermofisher.com/dynadrive**

Process Raman analyzer

The **MarqMetrix All-In-One Process Raman Analyzer** is a compact, powerful spectrometer for nondestructive real-time analysis of critical process parameters directly in-line with processes. It can be used for direct process monitoring and control in upstream and downstream bioprocessing.

Learn more at **thermofisher.com/marqmetrixaio**