Application note

A Novel Strategy of Using Process Raman for Feedback Control of Viable Cell Density in Perfusion Cell Culture

Authors

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Introduction

Bioreactors have become essential tools in bioprocessing by enhancing and improving the production of biotechnological products across a wide range of industries.[1] These reactors provide controlled and optimum cell culture settings, making them popular in biopharmaceuticals, food production, and other industries. Bioreactor runs are often categorized into three modes: batch, fed-batch, and perfusion. Batch bioreactors involve the addition of a fixed volume of culture medium with cells, allowing the cells to grow and produce desired products over a specific period. In a fed-batch run, the bioreactors are constantly replenished with nutrients or media throughout the culture phase to promote cell growth and productivity. In a perfusion run mode, the cells are retained inside the bioreactor while spent media, waste products, and the product of interest are continuously removed and fresh media are added, ensuring a stable environment for enhanced cell density and sustained production.[2]

Perfusion bioreactors are gaining popularity in biopharmaceutical and cultivated meat industries because of their potential to boost yield. They can be integrated into continuous bioprocessing operations, enabling consistent product yield and quality. The extended run times of perfusion processes further amplify the productivity benefits.[3] A critical process parameter (CPP) for perfusion bioreactors is viable cell density (VCD), since VCD directly affects the production of monoclonal antibodies, viral vectors, tissue engineering, and grown meat.[4] Cell bleeding—the removal of cell biomass along with media—helps maintain cell health and a steady-state culture environment by controlling cell density. VCD is often the deterministic factor for initiating cell bleeding, emphasizing the significance of monitoring and managing VCD during perfusion runs.

Process Raman is a process analytical technology (PAT) solution for monitoring VCD and other CPPs in various bioreactors.[5–7] However, to the best of our knowledge, to date there has been no demonstration of feedback control of cell bleeding in a perfusion bioreactor using process Raman. In this study, we have presented *a novel approach to initiating and controlling cell bleeding in a perfusion run based on real-time prediction of VCD using process Raman*. We have previously demonstrated the automated feedback control of glucose feeding using process Raman.[8] With these results, we show possibilities of implementing process Raman as a single PAT sensor for automated feedback control of bioreactors by monitoring multiple CPPs in a single scan. This capability helps optimize and control the process and improves the uniformity and quality of the products.

Experimental Details

1. VCD Chemometric Model Development

The training data for VCD chemometric modeling were collected from 10 different perfusion runs, which included 3 L and 5 L glass bioreactors, as well as a 500 L Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.). These bioreactor runs lasted approximately 7–15 days. The data was collected in "in-line" mode using the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer integrated with a bioreactor immersible probe (Figure 1).

The signal acquisition parameters were optimized and set to a power of 450 mW, exposure time of 3000 ms, and 20 averages for all runs. Throughout the bioreactor run, samples were aseptically pooled and sent for offline reference value analysis. The timestamp for pooled samples was recorded for data analysis.

Before constructing the chemometric models, the quality of the spectra was visually assessed. Raman spectra were plotted, and any spectra with an intensity exceeding 50,000 counts in the spectral range of 800 to 3250 cm^{-1} were excluded from the training set. The data were preprocessed by removing the baseline using the Savitzky-Golay filter (1st derivative, polynomial order=2, and window width=13) and then normalized using standard normal variate (SNV). All the data were mean-centered before developing the partial least square (PLS) regression models. The PLS regression models were developed using only the selected region. A leave-one-out (leave-a-run-out) cross-validation strategy was employed for internal validation to prevent overfitting of the calibration model. The model was built and cross-validated using different numbers of latent variables, and the root mean square error of calibration and cross-validation (RMSEC and RMSECV) was calculated for each model. Finally, the minimum number of latent variables for the PLS model was selected such that adding more latent variables did not significantly improve the RMSECV.

Figure 1. A. Small footprint Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer with an immersible probe. B. 5 L bioreactor with All-In-One Process Raman Analyzer placed vertically C. 500 L Thermo Scientific DynaDrive Single-Use Bioreactor (S.U.B.).

2. Perfusion Cell Bleeding and Process Control

The perfusion run was performed in a 5 L bioreactor with the ExpiCHO cell line and Gibco Efficient Pro medium as the beginning and feed media. The experimental setup is depicted in Figure 2. The initial inoculum for the start of the perfusion bioreactor was 0.6 million cells/mL. Raman spectra were acquired using the MarqMetrix All-In-One Process Raman Analyzer. The predicted VCD results obtained from process Raman were transmitted to the TruBio system, which served as the feedback control for the bleed pump.

During the perfusion run, the feed media and glucose were continuously supplied to the bioreactor through a feed pump at the same rate as the removal of spent media by the harvest pump. The harvest media, made up of cells, media, and other components of the bioreactor, was removed from the bioreactor and passed through a filter. The filter was used to separate cells from spent media; spent media was removed to a container while the cells were returned back to the bioreactor. The volume of spent media removed was then replenished with fresh feed media. The exchange volume per day was determined following standard perfusion protocol.

The exchange of harvest/spent media and feed media was regulated by feedback from a weighing sensor, ensuring that the overall mass of the bioreactor stayed constant at 2.5 kg throughout the entire run. The cells were allowed to grow for 7 days under a feedback control of the weighing sensor.

On the seventh day, cell bleeding was initiated using the automated feedback control by Raman. The bleed pump facilitated the removal of cells and harvest media, while an equivalent mass of feed media was added through the feed pump to maintain a total bioreactor mass of 2.5 kg. In the TruBio system, a threshold logic for VCD was set at 40 million cells/mL. Whenever the predicted VCD value by process Raman exceeded 40 million cells/mL, the bleed pump was activated to initiate cell bleeding. Conversely, the bleed pump remained inactive when the predicted VCD was at or below 40 million cells/mL. Still, the harvest and feed pumps continued to operate, maintaining the total bioreactor mass at 2.5 kg.

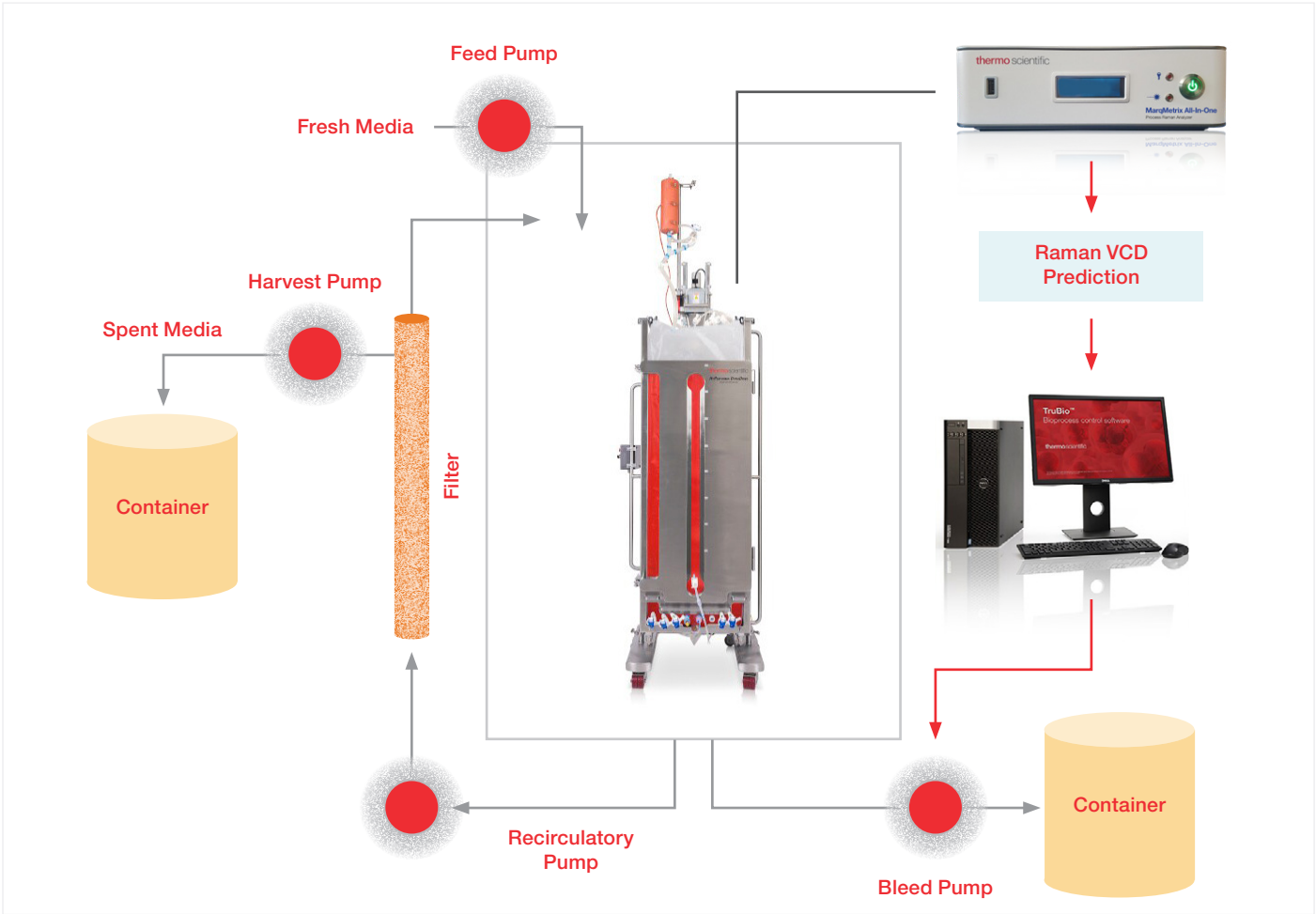

Figure 2. Experimental set up for the perfusion run. The Raman-based VCD prediction is communicated to TruBio system as feedback for controlling the cell bleeding through bleed pump. Throughout the run, the weight of the reactor is maintained at approximately constant weight by maintaining the in-flow volume through feed pump equal to the out-flow volume through the combination of harvest pump and bleed pump.

Results and Discussion

The results of the VCD prediction by the process Raman using a two latent variable PLS model and its implementation to control cell bleeding in the perfusion run are shown in Figure 3. In Figure 3A, the real-time VCD prediction from process Raman is depicted as a blue trace, while orange dots represent the offline reference measurements on different days. The red dotted line indicates the timepoint on day 7 when the cell bleeding logic was triggered. At this point, the Raman-based prediction of VCD was approximately 80 million cells/mL. The bleed pump was activated since the threshold was set to 40 million cells/mL. It took approximately 6 hours to bleed the cells at a rate of 6.25 mL/min. The depleted mass was replenished with feed media, maintaining a total bioreactor mass of 2.5 kg. After 6 hours, the VCD reached an equilibrium density close to the threshold of 40 million cells/mL, which was maintained for the following four days using feedback control based on a prediction of VCD from process Raman, as highlighted by the green rectangle.

Figure 3B shows the reduced Q vs. reduced Hotelling T^2 plot for the training data in the calibration model and the test data acquired during this perfusion run. The reduced Q vs reduced Hotelling T^2 scores for the test data (blue and orange dots) are below 1 and fall within the model space defined by the training data (green dots). This indicates that the VCD predictions from the model can be trusted with high confidence. Figure 3C shows the correlation plot, while Figure 3D displays the residual analysis. The root mean square error of prediction (RMSEP) was approximately 6 million cells/mL, and the R^2 value was 0.97. The low RMSEP within the concentration range of 0 to 70 million cells/mL and the high R^2 for prediction (indicating the goodness of fit of the model to the test data) clearly demonstrate that process Raman is capable of accurately predicting VCD, which can be reliably used as feedback to control the cell bleeding process in the perfusion bioreactor.

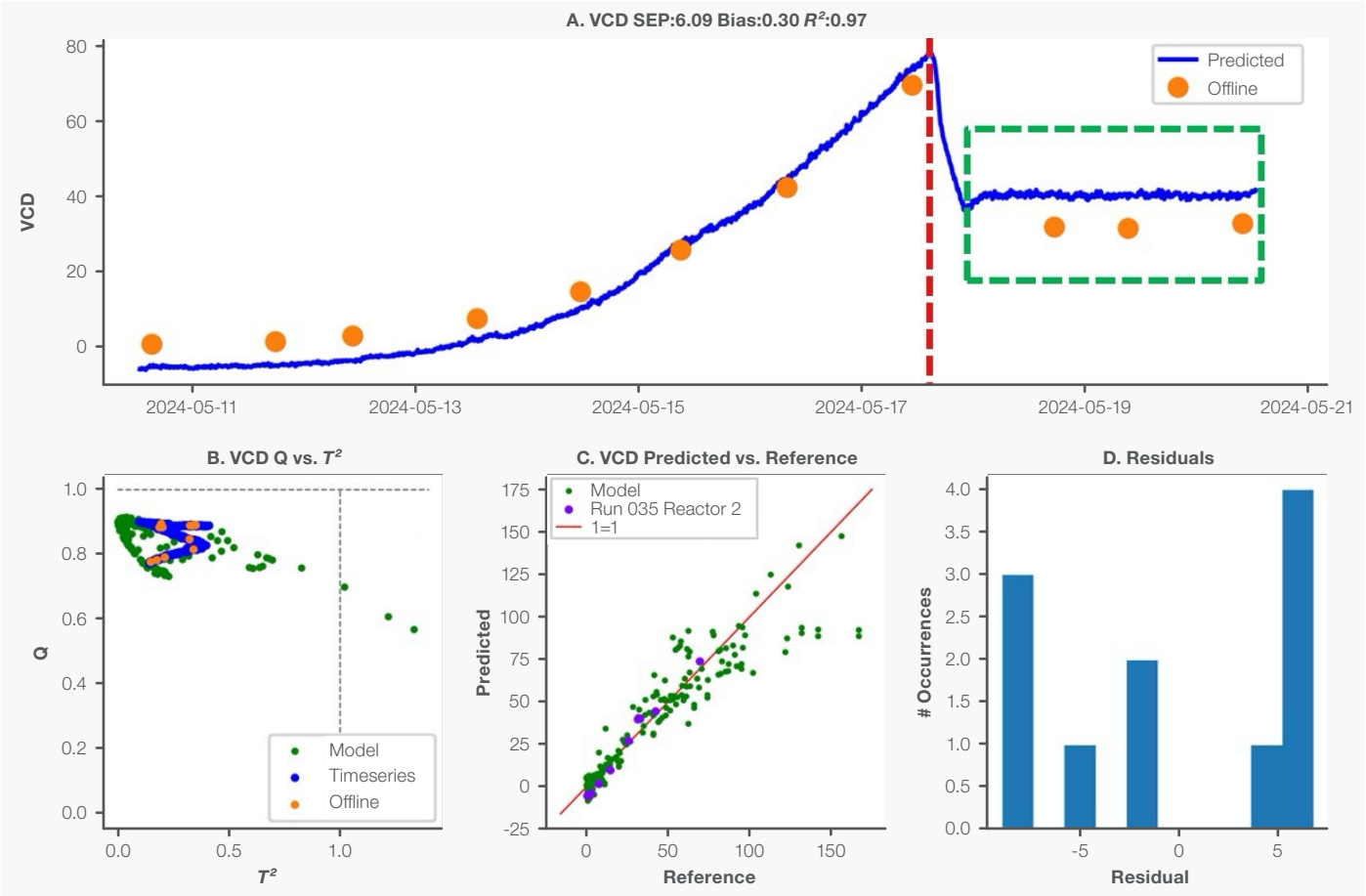

Figure 3. Showing reliable feedback control of cell bleeding in perfusion using the predicted VCD by Raman process analyzer. The *red dotted line* marks the onset of the logic for cell bleeding. The *green dotted rectangle* shows the robust feedback control of cell bleeding based on VCD prediction by process Raman to maintain the VCD of ~40 million cells/mL for four consecutive days.

Conclusions

This study demonstrated a novel approach to controlling VCD through cell bleeding in a perfusion mode bioreactor. The control logic was initiated using the feedback loop of accurately predicted VCD through the Raman process analyzer. This capability equips users with unprecedented control over their processes through multiple feedback loops, as previously demonstrated for automated glucose feeding.[8] By leveraging these multiple feedback loop capabilities, users may effectively reduce process variation, optimize their processes, and ultimately enhance product quality and yield. As a result, using process Raman as a monitoring and control tool has enormous potential to improve bioprocessing processes significantly.

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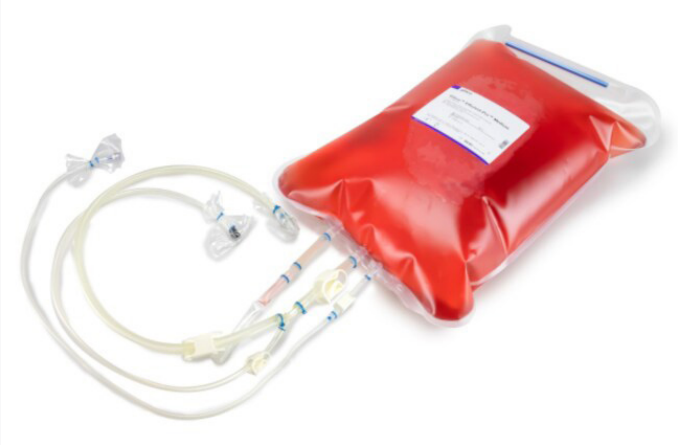
Other Thermo Fisher Scientific products used in this study



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