Metabolomic Characterization of a Perfusion Bioprocess System using Pyxis™ – A Machine Learning Tool for Absolute Quantitation

Erin Weisenhorn[1], Luke S. Ferro[2], Jack Howland[2], Matthew Stebbins[1], Jonathan Mene[1], Rosemichelle Marzan[1], Timothy Kassis[2], Ana S.H. Costa[2], Jefferson G. Pruyne[2], Devesh Shah[2], Joshua D. Lauterbach[2], Tian Cai[2], Jennifer M. Campbell[2], Geoffrey Horner[1]

[1] *Just Evotec Biologics. 401 Terry Ave N. Seattle, WA 98109*
[2] *Matterworks Inc. 444 Somerville Ave Somerville, MA 02143*

Introduction

Biologics manufacturing typically uses engineered Chinese hamster ovary (CHO) cells to produce folded and glycosylated antibodies which requires considerable time and effort to grow and maintain cells at optimum conditions. As metabolites provide the most direct readout of physiology, a quantitative understanding of cell metabolism can enable optimization of growth conditions for improved titers or increased production duration. Mass spectrometry is the premier tool for metabolite measurement; however, transforming raw data into accurate quantitative measurement requires both expertise and extensive sample preparation. Here we demonstrate the ability of simple sample preparation using universal calibrators and a novel machine learning algorithm to rapidly provide biological insight into bioprocessing samples.

Methods

Eight bioreactors expressing a monoclonal antibody were grown for twenty-five days using a perfusion process. At ten time points throughout the run, 10 million cells were collected and washed in cold phosphate buffered saline before flash freezing. Two media compositions, and two target cell densities were compared across the runs (Table 1). With technical replicates, this process yielded a total of 160 samples. Samples were extracted using a solution of 50:30:20 methanol, acetonitrile, water, and StandardCandles™ at final dilution of 5E6 cells/mL. The data was analyzed on a Thermo Scientific Orbitrap Exploris® 120 with polarity switching at Matterworks using a 6.5-minute HILIC LC method. The raw files were uploaded to the Pyxis™ application and absolute concentrations were returned in minutes.

Table 1. Experimental design collected from day 4 to day 25.

Reactor	Media	VCD Target
1	A	High
2	A	Low
3	A	Low
4	A	High
5	B	High
6	B	Low
7	B	Low
8	B	High

Results - Bioreactors

The bioreactors showed differences in VCD corresponding to the predicted target. Viability levels decreased in all reactors past day 15, with a sharper decrease in reactors with a high VCD target. The shaded areas of each graph denote the standard deviation between reactors.

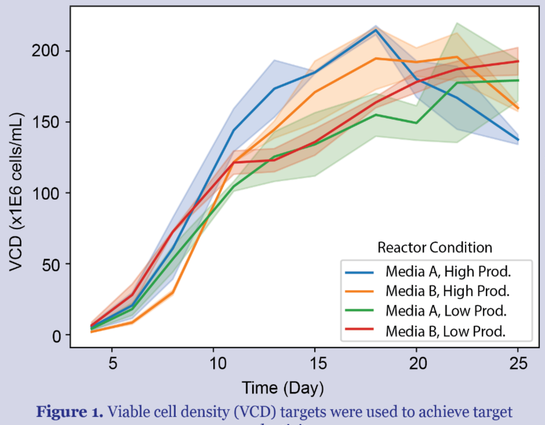

Figure 1. Viable cell density (VCD) targets were used to achieve target productivity

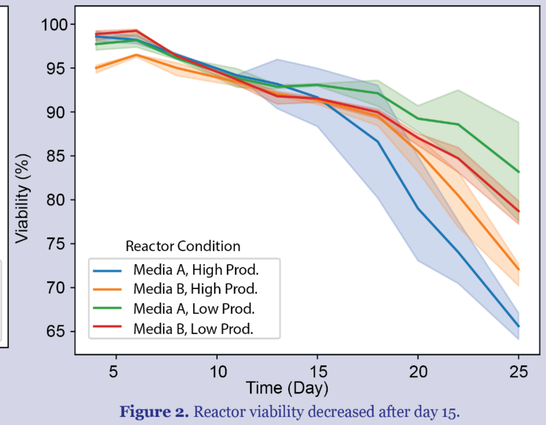

Figure 2. Reactor viability decreased after day 15.

Results - Metabolomics

The results calculated with Pyxis were compared to an orthogonal method using mass spectrometry and a traditional standard curve. We saw most metabolites fall along a y=x line showing good agreement between Pyxis and an orthogonal method although some metabolites are shifted (Figure 3). Metabolites that fall off the line include histidine and fumaric acid which will require further optimization. Pyxis™ reported absolute concentrations for 82 analytes from a range of annotated KEGG pathways including the TCA cycle, vitamins, glycosylation precursors, and nucleotide synthesis (Figure 4). This analyte coverage allows for rapid biological insight into cell health. Many metabolites detected are ingredients in cell media, providing a lever for future optimization. Using the Pyxis data and dimensionality reduction techniques such as Unsupervised Uniform Manifold Approximation and Projection (UMAP) differences in the reactors were resolved. Pyxis' data showed a change in the metabolome over the run (Figure 5).

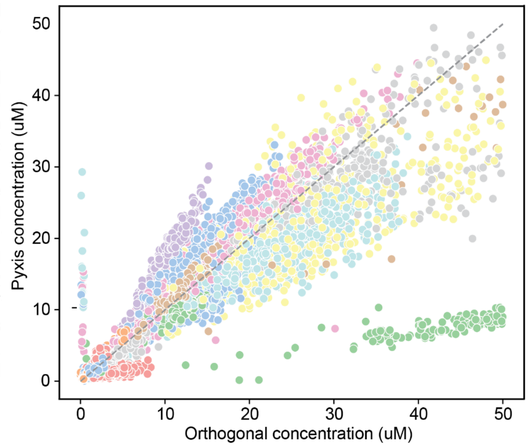

Figure 3. Accuracy data comparing concentrations calculated in Pyxis with an orthogonal mass spectrometry method. Each color represents a different metabolite

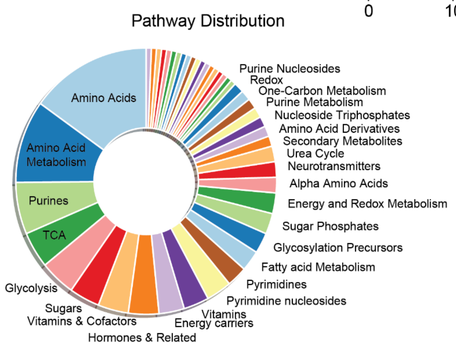

Figure 4. Pathway distribution of metabolites identified with Pyxis™

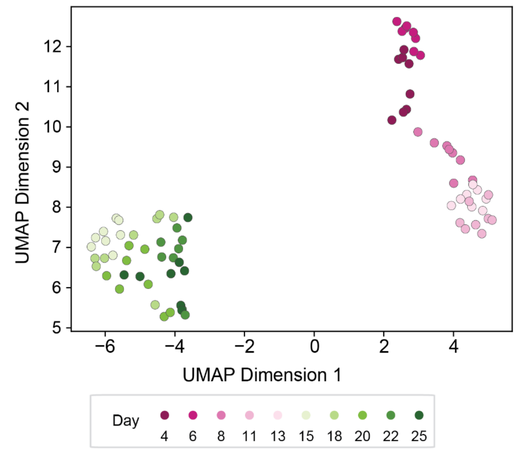

Figure 5. UMAP analysis of all samples show reactors separating by day. When cells reach the target VCD there is a clear metabolic shift as cells are no longer in the exponential growth phase.

Conclusion

The largest shifts were observed at day eight and day fifteen which correspond to the time at which cells are dividing exponentially before reaching peak VCD (Figure 1).

Amino acids are a fundamental media ingredient and some of the most frequently tracked. Bioreactors grown with media 'A' have higher levels of amino acids in the media including asparagine (Figure 6) which is detected in our results. We also were interested in comparing the results from Pyxis™ with a standard targeted assay. Spent media from the same bioreactors was analyzed using a Waters Q-Tof. We observed the same overall trend in asparagine concentration with a sharp initial decrease followed by a plateau. Reactors grown in media 'A' also had higher levels of asparagine, although the absolute concentration differed as the samples came from spent media. These results provide further confidence in the Pyxis™ results which can be reproduced with another method.

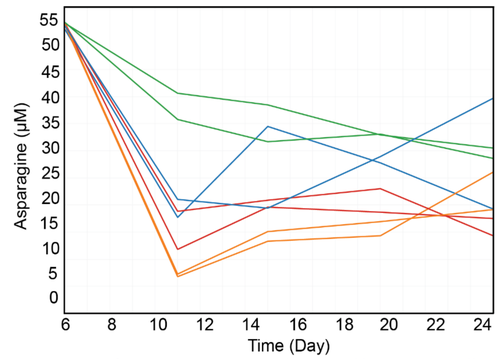

Figure 6. Asparagine levels reported by Pyxis™ in cell pellets

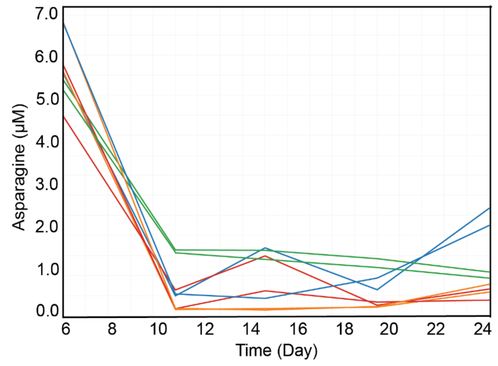

Figure 7. Asparagine levels reported by a targeted mass spectrometry method in spent media. Results not calculated with Pyxis™

Adjusting the glycan profile of biotherapeutics is often of high importance to modulate effector function. Bioreactors with a lower productivity target, and grown with Media A have higher beta-galactose levels on the Fc glycan after day 20 (data not shown). This same set also had higher UDP-Galactose after day 20 (Figure 8). UDP-Galactose is a substrate for glycosyltransferases which transfer the galactose moeity to the glycan on a nascent polypeptide. There is a slightly higher uridine level in Media A compared to Media B. This may result in the higher UDP-Galactose levels observed. Uridine was also measured and no difference was observed between reactor conditions (data not shown). In this experiment metabolite levels in spent media were not measured so we can not tell if uridine was differentially depleted in media. Monitoring intermediates in glycan formation such as UDP-Galactose, UDP-GlcNAc, GDP-mannose, and fucose could provide insights on shifts in glycan profiles that are often observed during a run. As these metabolites are not directly added to the media, identifying reactor conditions or media components which modulate glycan precursors and the resulting glycans will be a focus for future work.

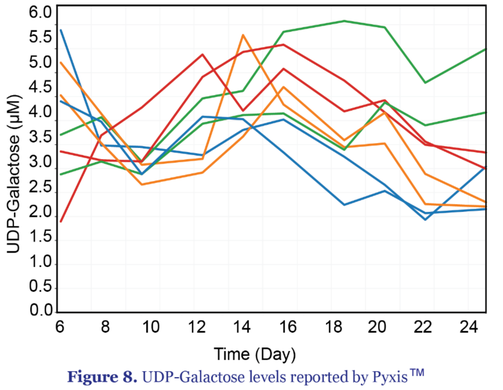

Figure 8. UDP-Galactose levels reported by Pyxis™