Culture

Whitepaper

Accurate Cell Culture Characterization Enabled by Culture Biosciences' Cloud Bioreactors

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Abstract

Determining accurate performance metrics from a cell-culture experiment (such as doubling time, production rate of therapeutic modality, and substrate consumption rate) is essential for cell growth characterization and process performance quantification. Culture Biosciences currently offers clients access to cloud-connected bioreactors with 250mL and 5L working volumes. This cloud nativity allows all process variables (e.g. pump flow rates) to be available as high fidelity *continuous* time-series data. Using data analysis and processing, the amount and timing of *discrete* volume change events (such as sampling) is estimated. Combining these data streams in an automated analytics engine enables rapid and accurate cell culture characterization. Grouping across different axes (such as process or clone) enables researchers to extract insights from their runs with Culture Biosciences.

Overall the Culture Biosciences platform provides a more accurate, automated and accessible view into fed batch bioprocessing experiments, particularly into key performance metrics related to cell growth, viability and productivity. The ability to combine historical data, live bioprocessing sensor data and offline analytical data makes it possible for Culture Biosciences clients to quickly and accurately derive unique insights into their research.

Introduction

The use of fed-batch cultures is foundational to both upstream development and manufacturing of bio-therapeutic modalities. Typical development cycles involve tens of experiments, each lasting for days, where design parameters – media compositions, clones, feeding strategies, control strategies – are varied with the goal of identifying their impact on the quantity and quality of the therapeutic modality and/or the robustness of a cell clone to process conditions. Time-series data generated by individual experiments is then analyzed (individually or grouped) to quantify performance metrics such as doubling time, and nutrient consumption rates.

Traditionally, these analyses are conducted through software tools such as spreadsheets or statistical analysis packages, where a lack of automated data ingestion

and visualization tooling limits the number of times the analysis is performed and the types of views that can be generated (such as via grouping across different metadata identifiers). Furthermore, the mathematical foundation behind these metrics typically relies on assuming a constant volume (or minimal volume change throughout the experiment) – assumptions that break down for fed batch cultures lasting several days with multiple sampling and bolusing events.

Here, using a simulated dataset for Chinese Hamster Ovary (CHO) growth, we show how working with Culture Biosciences' platform enables seamless development of data analysis workflows. Additionally, we show how a fine-grained timestream of pumped volumes during the course of an experiment can be integrated into the analysis together with discrete events such as sampling.

We show how by grouping individual run level insights across different axes, insights on cell-line and/or media can be drawn to guide future experiments and knowledge transfer to downstream manufacturing.

Methods

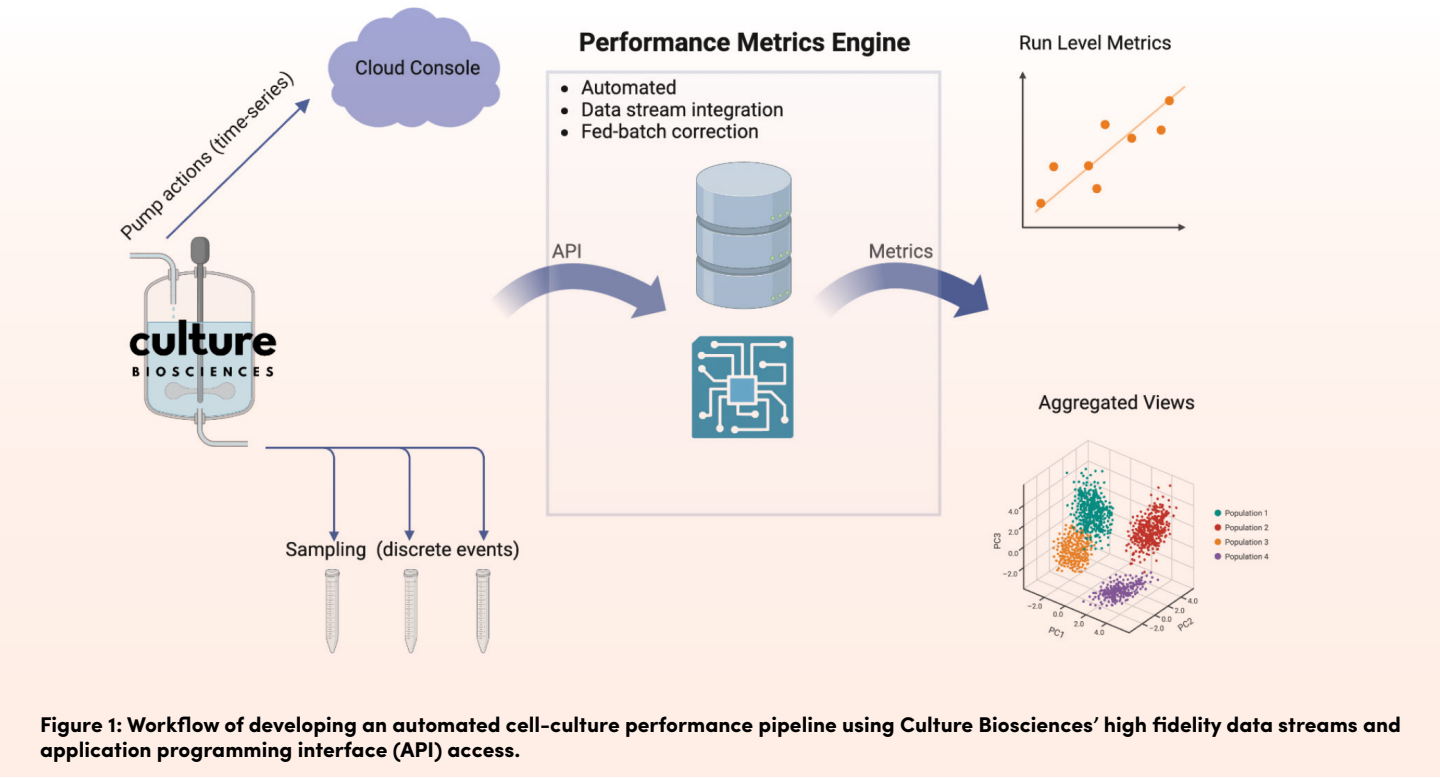


Figure 1: Workflow of developing an automated cell-culture performance pipeline using Culture Biosciences' high fidelity data streams and application programming interface (API) access.

Culture's platform provides an all-inclusive resource with proprietary hardware, operational team, and a SOC-2 compliant online data management system. Using proprietary algorithms that integrate data of different fidelities (pump sensors - continuous, samples - discrete, evaporation rate - modifiable estimate at the start of a run), these unified data streams are made available to clients.

Here we use a simulated dataset of a CHO cell culture to generate these datastreams. Previously, we released an open source package InsiliCHO[1] that implements a literature model of CHO cell growth along with operation variables, such as the concentration of glucose in feed, frequency and days of feeding etc. Using similar logic as to what is contained in this open source package, we generate a datastream of volume changes, glucose measurements and cell densities to create an example dataset for this whitepaper.

[1]Modeling Cell Line Dynamics for Process Development; February 24, 2023; https://blog.culturebiosciences.com/insilicho-modeling-cell-line-dynamics-for-process-development

The volume changes in the dataset arise from three sources – glucose boluses (timing determined by protocol), nutrient bolus (single, fixed time) and sampling events (daily frequency). The simulated data streams are shown in Figure 2. Since a high fidelity estimated volume estimate is available, the timing and amount of addition and sampling events can be reconstructed to generate an event series.

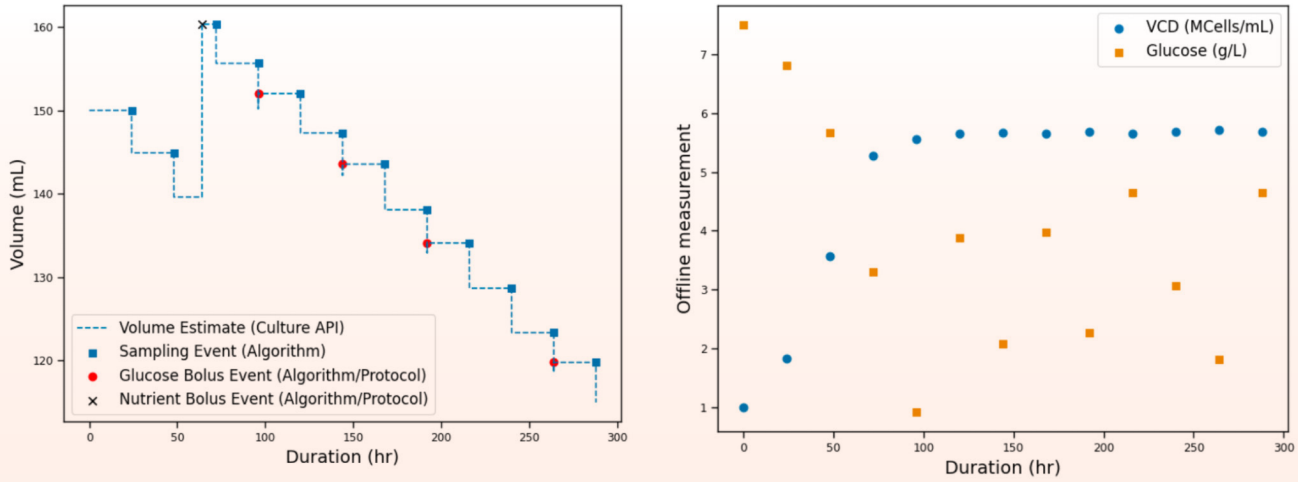


Figure 2: Simulated dataset used in this whitepaper. (left) Volume estimate available as a continuous time-series, while sampling and bolus events are deduced from data and protocol knowledge. (right) Offline measurements, such as Viable cell density (VCD, million cells/mL) and glucose concentrations (g/L) are measured at discrete time points after sampling reactor contents. Both discrete offline measurements and continuous volume estimates are accessible through Culture Biosciences' API.

Applications

Using Culture Biosciences' application programming interface (API), data from offline measurements (e.g., viable cell density) can be pulled into an analytics pipeline. These information sources are integrated in the performance metrics engine (see schematic) to compute a piecewise growth rate/doubling time (Figure 1). The timeseries of doubling times can be automatically parsed to identify the end of the exponential growth phase of a culture. For instance, a rapid drift/increase in doubling time at ~*75* hrs in Figure 3 marks the end of this growth phase. As an aside, Culture Biosciences' API also provides high fidelity pH and temperature setpoint data (not shown) which can optionally be used to identify phase shifts to assist with this analysis.

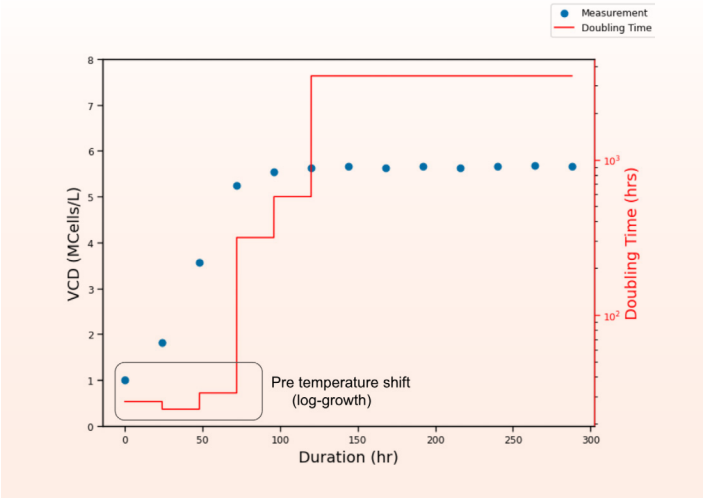


Figure 3: Automated computation of doubling times and identification of log growth phase.

Application: Grouping for Insights

Performance metrics generated from individual experiments can be visualized in many different ways to enable insight generation and knowledge extraction. For instance, for clone screening, data can be grouped over cell-lines to highlight the performance of each clone. Similarly, for media screening or process robustness needs, data can be viewed through a media composition or process metadata lens to understand the performance and statistical significance. Working with a cloud native platform that is guaranteed to provide data in a consistent schema allows for comparison of runs across multiple experiments enabling a "build once, analyze anytime" analytics engine. For example, the doubling time for a large number of individual experiments is grouped and visualized in Figure 4 enabling comparison of performance of batches of individual experiments.

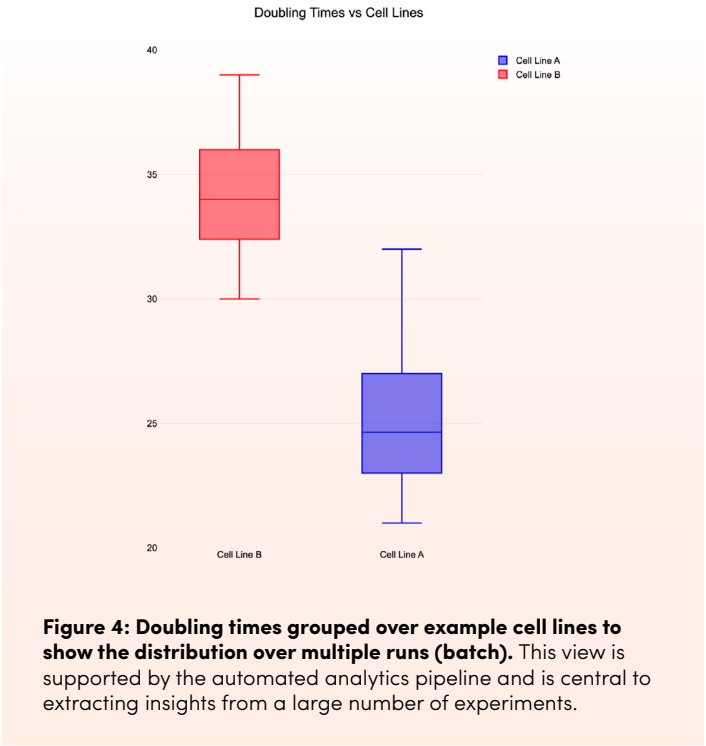


Figure 4: Doubling times grouped over example cell lines to show the distribution over multiple runs (batch). This view is supported by the automated analytics pipeline and is central to extracting insights from a large number of experiments.

Application: Data Enrichment for Sparse Measurements

Another application of high quality data access is the ability to enrich sparse data streams. Using a volume correction method to account for sampling and addition changes, the sparsely measure glucose data can be put into the context of all the actions taking place during the course of the experiment[2]. For instance, the repeated glucose bolusing events lead to a sharp rise in glucose and feeding of glucose free media leads to a sudden decrease. Both kinds of events are precisely accounted for with the knowledge of media and feed compositions, and the event stream constructed using the volume estimate available from Culture's API.

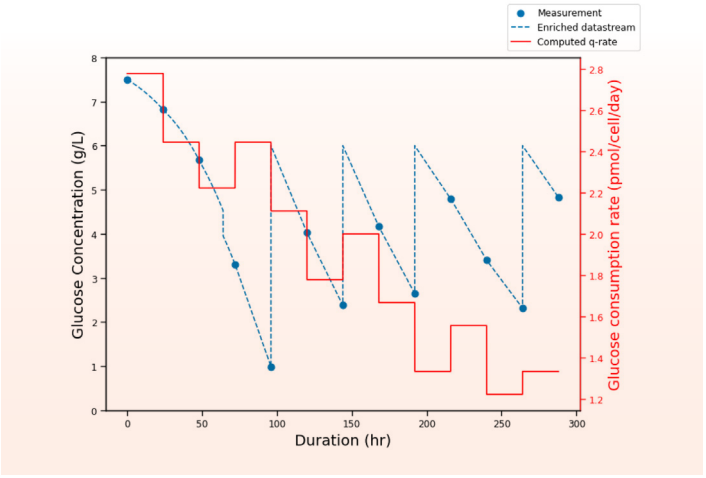


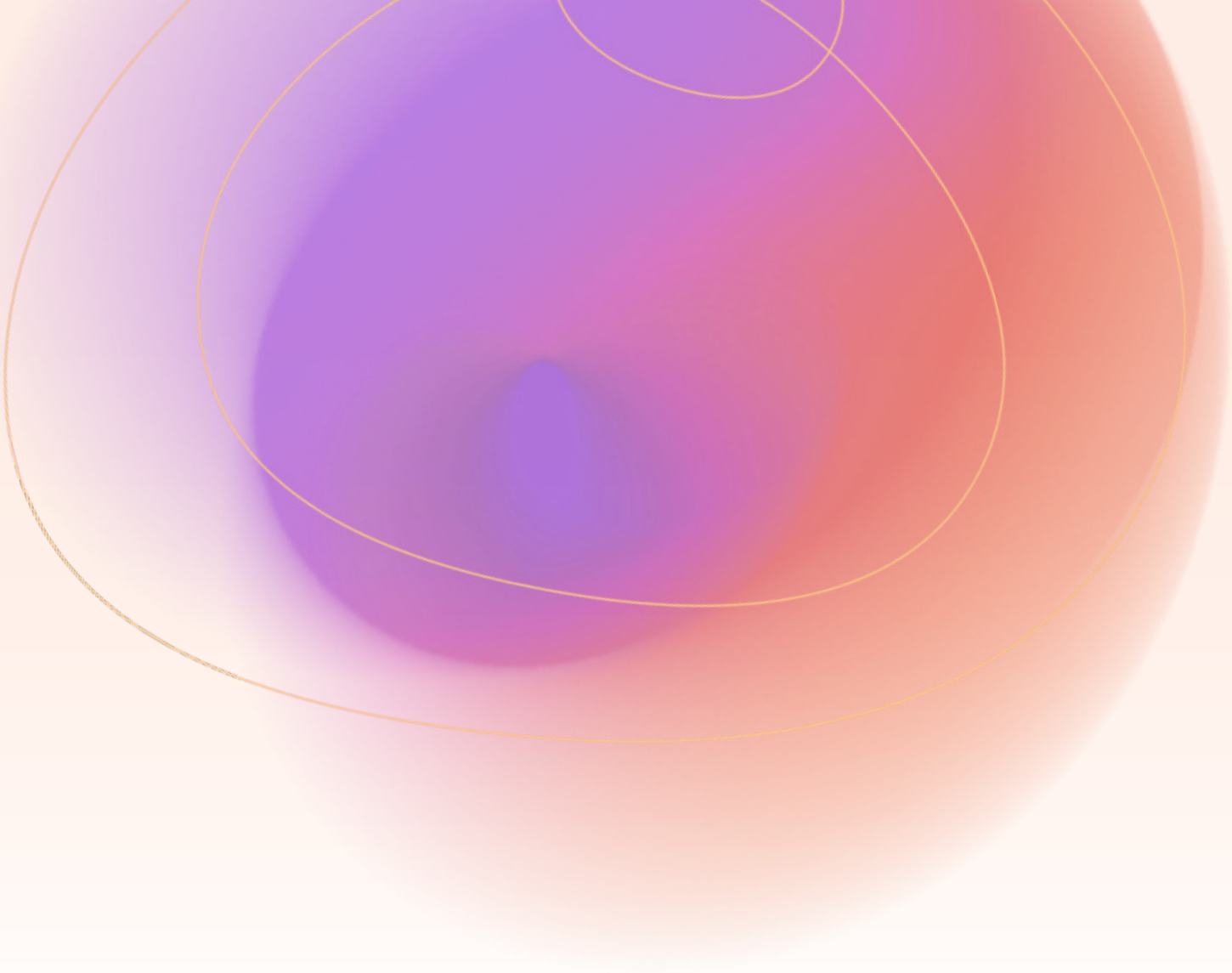
Figure 5: Imputed glucose concentration (blue line, left axis), measured glucose concentration (blue points) and computed glucose consumption rate by cells (red, right axis). The imputation of glucose data is made possible with knowledge of feeding and sampling events as shown in Figure 2, the q-rate calculation accounts for these events.

[2]The Mysterious Case of the Missing Glucose; August 23, 2023; https://blog.culturebiosciences.com/the-mysterious-case-of-the-missing-glucose

Conclusion

Computing performance metrics on bioprocessing experiments is a key step in knowledge extraction. In contrast to batch systems, fed-batch cultures undergo many volume changing events that must be integrated into a single data stream to infer these metrics accurately. Using simulated example data from CHO cell culture experiments, we demonstrated how working with Culture Biosciences can help streamline and automate these evaluations, leading to faster and more accurate knowledge extraction from cell culture experiments.

Clients can calculate growth rates, consumption rates and compare variability in cell lines when working with Culture Biosciences. The connection of online process data with analytical data, as well as Culture's API, makes it possible to rapidly analyze bioprocessing data and derive insights from experiments at Culture.



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