Culture

Whitepaper

Ensuring Data Quality Through Mass Balance Calculations in Culture Biosciences' Cloud Bioreactors

Helen Luo[1], Kris Tyner[1], Skye Vandeleest[1], Leron Perez[1], Masaki Yamanda[1], Collin Edington[1] Cayley Hoyer[1], Katie Madler[1], Max Gilbert[1], Matt Ball[1]

[1]Culture Biosciences, 269 E Grand Avenue, South San Francisco, CA 94080, USA

The Importance of Mass Balance Calculations

In order to make improvements to a cell line and the cell culture process, it is important to have a thorough understanding of carbon fluxes within a bioreactor. While volumetric measurements can be informational, such as titer in g/L, they do not provide information around the total amount of product made or total loss of carbon to byproducts such as CO2. Without calculation of mass balances and use of mass-based measurements, it is difficult to gain more than a cursory estimate of clone performance and product fluxes.

Measurements taken throughout a batch to accurately track total mass additions and losses enable the calculation of mass-based metrics from the batch. Culture Biosciences' automated cloud bioreactors are equipped with hardware and software features that allow for accurate tracking of mass (both liquid and gas), which provides a more comprehensive assessment of cell line performance and opportunities for both clone and process improvements.
Introduction

In order to make improvements to a cell line and the cell culture process, it is important to have a thorough understanding of carbon fluxes within a bioreactor. While volumetric measurements can be informational, such as titer in g/L, they do not provide information around the total amount of product made or total loss of carbon to byproducts such as CO2. Without calculation of mass balances and use of mass-based measurements, it is difficult to gain more than a cursory estimate of clone performance and product fluxes.

Measurements taken throughout a batch to accurately track total mass additions and losses enable the calculation of mass-based metrics from the batch (or run?). Culture Biosciences' automated cloud bioreactors are equipped with hardware and software features that allow for accurate tracking of mass (both liquid and gas), which provides a more comprehensive assessment of cell line performance and opportunities for both clone and process improvements.
Introduction

Introduction

To truly understand both cell line performance and the impact of bioprocess adjustments it is critical to know the amount, kinetics, and efficiency of molecule production; namely, titer, rate, and yield. A comprehensive understanding of these metrics allows for an assessment of the commercial feasibility of a process and informs where improvements to a mammalian cell line and cell culture process should be directed.

Throughout a mammalian cell culture process, there are numerous inputs and outputs that together contribute to performance. A cell culture run may begin with a batch medium, into which cells are inoculated and start to grow. Once the initial carbon source is exhausted, cells may be maintained in a nutrient-limited state with the addition of various feeds. The pH is maintained with the addition of base or CO_2 control, and mass may be removed as samples to take measurements or as part of a continuous process. In addition, other factors such as liquid evaporation and uptake or evolution of gasses may impact mass entering or leaving the reactor. All of these factors impact the total mass in the reactor, which in turn determines the total amount of product in the system. nputs and outputs to a batch run are described in **Figure 1**.

Measurements Required for Calculation of a Mass Balance

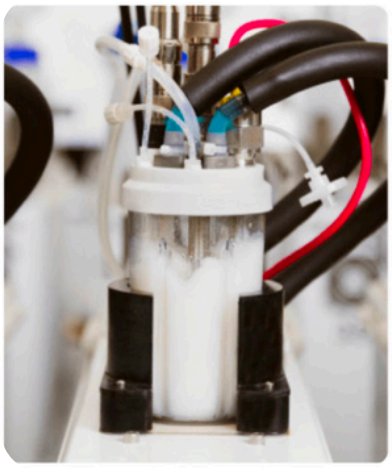
Inputs

O_2 delivered (g)

Initial process mass (g)

Manual additions (g)

Mass pumped (g)(corrected for evaporation)



Outputs

Vessel evaporation (g)

CO_2 evolved (g)

Manual removals (g)

O_2 in offgas (g)

Mass pumped (g)

Final vessel mass (g)

For all cell culture runs at Culture Biosciences, the following inputs and outputs are always measured:

O_2 delivered: Each reactor is fitted with two Mass Flow Controllers (MFCs), which measure the amount of each gas (Air, O_2) delivered.

Initial Process Mass: The initial mass of each batch is measured prior to the initiation of each process.

Manual Additions: The mass of all manual additions, such as inoculum of addbacks are measured.

Mass Pumped: Scales on up to 5 feed bottles allow for the mass added (accounting for evaporation from bottles and tubing) to each reactor throughout the batch to be measured. Any mass pumped out of the reactor is also accounted for.

Vessel Evaporation: The amount of evaporation over the course of the batch is continuously estimated in real time by custom software.

Manual Removals: The mass of all removals, such as samples, are measured

Final Reactor Mass: The final process mass of each batch is measured.

Accounting for all of these inputs and outputs is necessary to accurately measure the total amount of product in the reactor.

Without an accurate measurement of total product by mass (in contrast to a volumetric measurement of titer, which does not account for reactor volume), calculated performance metrics will be inaccurate. This, in turn, could lead to an inaccurate estimate of clone performance, which at its worst could lead to the development of a batch process that is not commercially viable.

Measurement of all these inputs and outputs requires the installation of additional equipment that is not typically included in traditional, off-the-shelf bioreactor systems. Culture's cloud-based bioreactor platform has been developed from the outset with the importance of mass- based measurements in mind. Culture's flagship 250mL bioreactor matches the leading industry standard reactors in working volume and small scale system features, while simultaneously boasting additional fit-for-purpose cloud connectivity and data science features that drive data driven analysis. An example of a distinguishing feature of Culture's system is that each reactor is equipped with hardware to accurately measure and seamlessly report into a cloud database every input and output for every cell culture process. Custom control software enables all inputs to the reactors to be delivered accurately and all manual additions or removals to be tracked for error and risk mitigation. Together, these features allow mass balances to be calculated across a range of organisms and cell culture processes, giving customers high-quality mass-based data measurements. These comprehensive datasets can then be used to calculate mass-based metrics that reflect the true performance of a cell line within a production process.

Materials And Methods:

A Summary 0f Culture's Tracktracking Infrastructure for Mass Balance

Gas Delivery and Evolution

Each of Culture's bioreactors are fitted with four mass flow controllers (MFCs) capable of delivering between 0 - 45 mmol/min of gas. These, as with many of Culture's measurements, are reported online as a volumetric mea- surement (in this case SCCM), but are converted to mass for mass balance calculations. Each flow controller can be calibrated for a variety of different gasses (O_2, CO_2, N_2, etc.) so that all input gasses into the bioreactor are accurately measured. By tracking gas in, these measure- ments allow oxygen uptake (g) and carbon dioxide (g) evolved from cells to be calculated for each production run. This can be observed in **Figure 2,** where the cumula- tive amount of O2 taken up and CO_2 evolved are recorded over the course of a run and the final values are used in the mass balance calculations. As seen in this graph, the values vary significantly between conditions or clones, and are required for accurate mass balance calculations.

Evaporation Studies

Cell culture processes rely on aeration and agitation in order to efficiently mix the culture medium and transfer oxygen into solution. These, combined with the temperatures required for optimal growth and production by different organisms, can result in the saturation of air exiting the reactor with evaporated liquid. Despite the use of condensers to condense liquid vapor so that it does not exit the reactor, significant vessel mass can still be lost to evaporation. Failure to account for this loss results in an inaccurate assessment of the mass, and therefore total product, in the production reactor.

Accounting for the loss of liquid to evaporation can improve the estimation of reactor whole cell culture fluid mass and total product throughout the production. However, a number of reactor-specific conditions such as condenser temperature and reactor back pressure can impact evaporation. To account for these, evaporation under a wide variety of conditions in Culture's bioreactors was empirically determined

Materials And Methods: Continued

Reactors were filled with 200mL of PBS and were incubated over a range of temperatures from 4 - 37°C, airflows of 2.25 - 13.5mmol/min, and agitation ranging from 750 - 3500rpm. The mass evaporated was measured over six days of incubation and the experimentally derived values were used to build a response surface methodology model of evaporation.

Measurement of Gas Uptake and Evolution

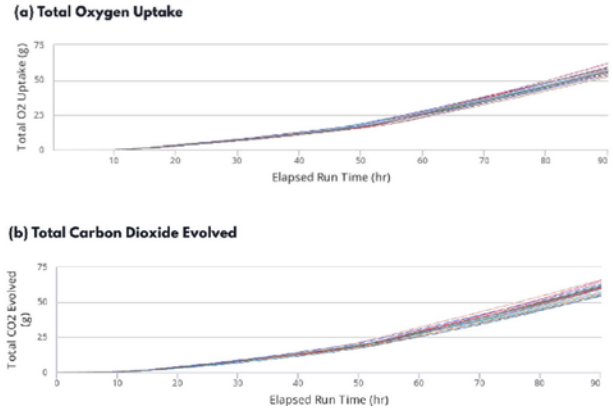


Figure 2: Measurements of flow rates and off-gas concentration enable calculation of the mass of (a) oxygen taken up and (b) carbon diozide evolved by organisms in the reactor over the duration of the cultivation.

Model of Evaporation From Bioreactors

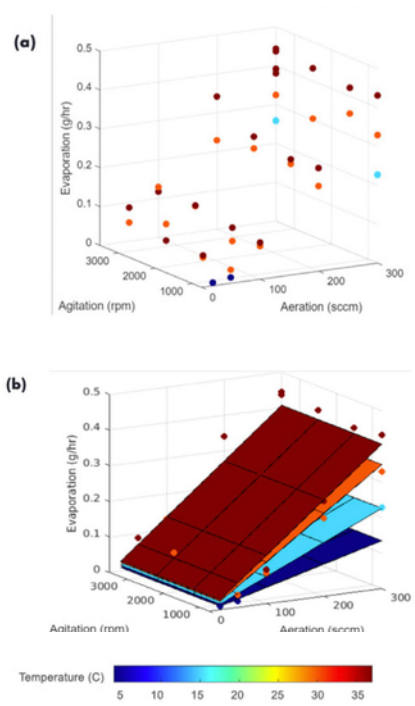


Figure 3: (a) Evaporation rates were measured in Culture's reactors under a number of different conditions (b) and results were used to calculate a RSM of evaporation to be incorporated into experiments.

The model generated can be described by the following formula:
Evaporation=A*(total gas flow)*(temperature)+B*(agitation)+C*(total gas flow)

Where
A, B, and C are coefficients derived from the experiment data, and result in a predictive model with an R^2 of 0.968. Each of the coefficients quantifies the relative impact of each factor in the model. In Culture's reactors, as can be seen from the RSM model in **Figure 3b**, the primary factor impacting evaporation from the reactor is the total gas flow through the reactor. Temperature has a large impact over a wide range of temperatures, but a much more modest impact under biologically relevant conditions for most organisms, and interacts with the gas flow rates to impact evaporation.

Materials And Methods: Continued

Agitation had the lowest impact of factors tested on evaporation. This model is then applied to online data in real time **(Figure 4)**, providing a more accurate estimate of the whole cell culture fluid mass at any given time point. While this model does not account for changes in evaporation due to changes in volume, type of liquid media, or the presence of cultured cells in the media compared with the 200mL of PBS in the reactor used in each condition, it does provide a better estimate of reactor mass at a given time point than without an evaporation correction factor.

Changes in setpoints **(Figures 4 a, b, c)** impact evaporation throughout the batch **(Figure 4d)**, which are reflected in changes in slope of the total vessel evaporation line **(Figure 4e)**. This mass of evaporation is then applied to correct the cell culture medium mass to enable accurate calculation of the media mass at a given time point. In this example, evaporation was even estimated during a cooled hold before inoculation over the first portion of the experiment.

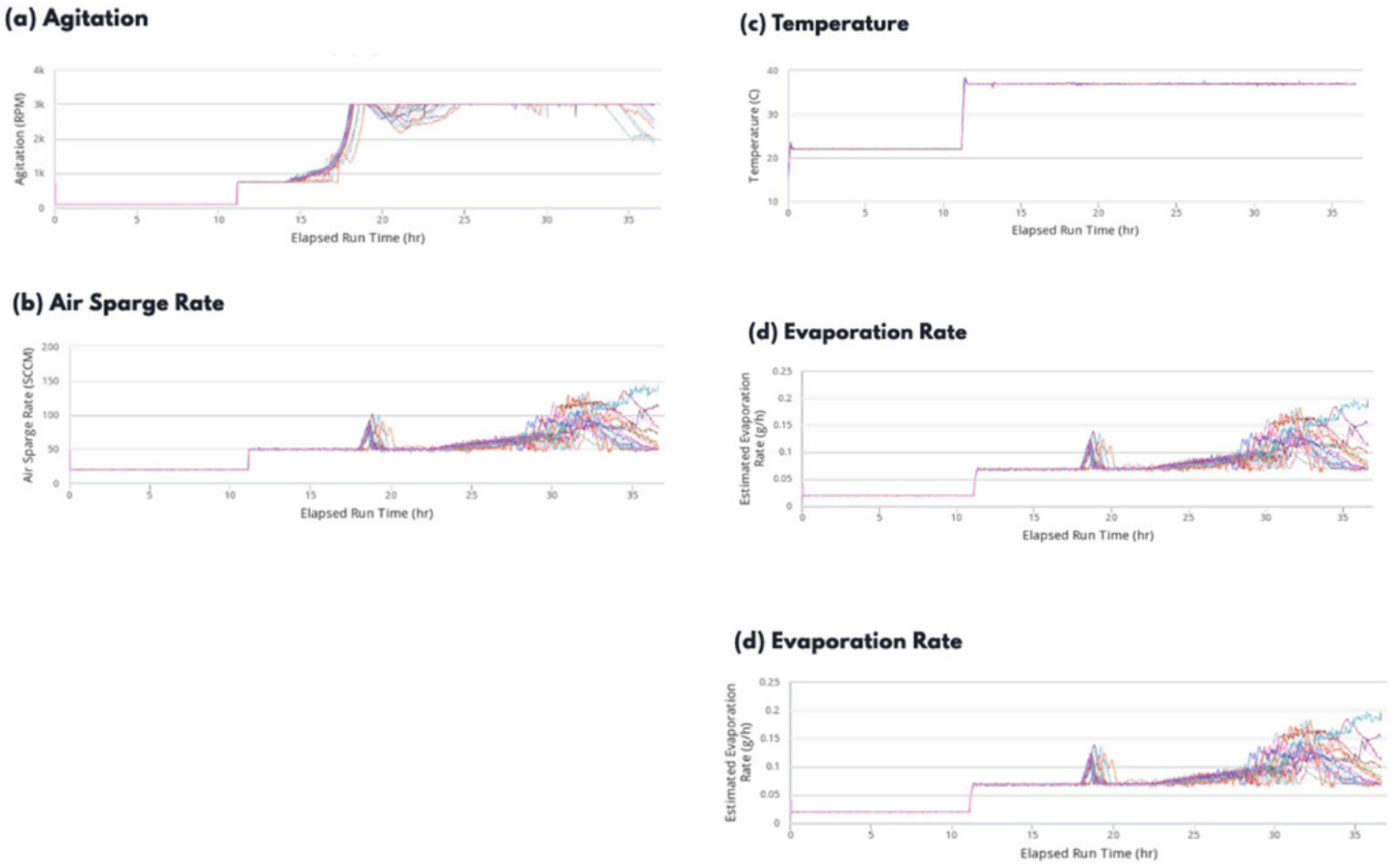


Figure 4: Evaporation corrections are applied to a cell culture run based on agitation (a), airflow (b) and process temperature (c). When changes are made to the setpoints that impact evaporation, the corresponding changes in evaporation rates are applied within the run (d) and incorporat- ed into the total evaporation over the course of the cell culture run (e).

In an analysis of over 500 batch runs (**Figure 5a)**, ranging from 22 hours to 120 hours in length and using various feeding strategies, the median calculated evaporation from the vessels was 4.95% of the total final media mass, representing a significant amount of the total whole cell culture fluid volume. However, depending on the conditions of the process, evaporation can account for a much larger proportion of the total whole cell culture fluid. Failure to account for this loss in analytical measurements leads to an overestimate of the mass in the reactor, impacting mass balance closure (**Figure 5b)** and increasing the potential to yield misleading results.

Fluid Delivery Into Production Reactors

A common method of fluid delivery to reactors is through the use of peristaltic pumps. These pumps provide a simple and economical method for delivery of feeds, acid, and base over a wide range of flow rates. They can also be programmed to change pumping rates over the course of a run.

However, delivery of feeds via a peristaltic pump is prone to error, with actual feed rates often deviating significantly from the setpoint. Feed rates can be influenced by factors as nuanced as how a feed line is threaded through the pump by an individual operator or the length or age of the pump tubing. In addition, unless pumps are regularly calibrated with the relevant solutions they are pumping, deviation from the setpoint can increase over time between calibrations. It is not uncommon for feed quantities delivered via a peristaltic pump to accumulate errors of 5 - 10% from setpoint over the course of a run. These deviations can significantly change results by impacting derived calculations calculated on a volumetric basis such as yield, or by altering cell line physiology itself.

A more precise manner of delivering feeds to a bioreactor is by feed weight. Traditionally, scales are expensive and require a large lab footprint, limiting the feasibility of this approach for many labs. In contrast, each of Culture's reactors is equipped with individual load cells for each feed, and can accommodate up to five individual feeds.
This capability, described below, typically results in feed delivery within +/- 3% of the intended setpoint over the course of the batch, with real-time feed correction based on weight measurements.

Each feed rate is programmed within a production protocol using the peristaltic pumps fitted to the reactor. The load
cell measures the mass of each feed bottle and compares that to the predicted mass based on the commanded
pump flow rate. Polypropylene feed bottles were selected to deliver feeds, as bottles with the smallest mass serves to minimize errors in mass measurements.

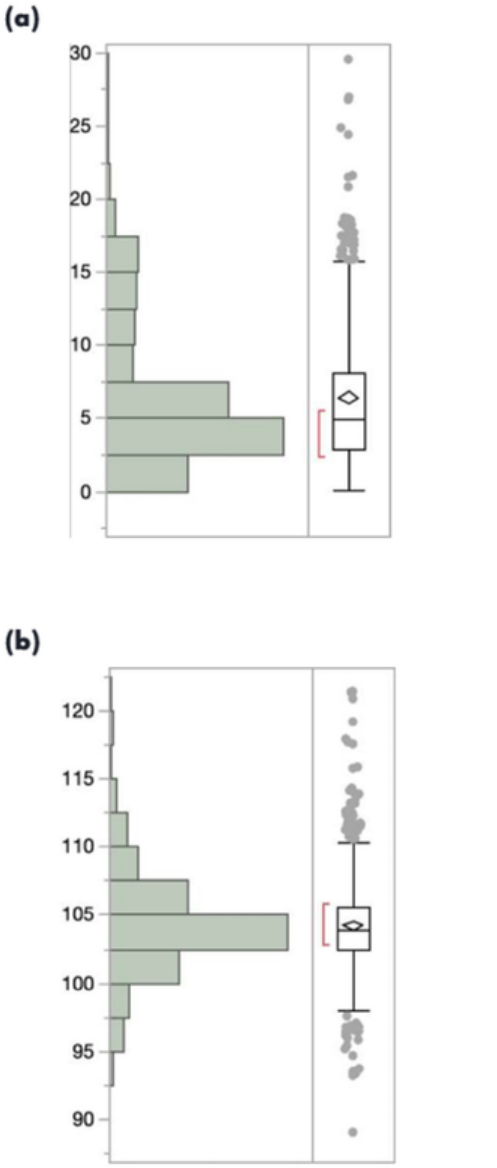


Figure 5: (a) Evaporation from production reactors as a percentage of total final whole cell culture fluid mass was calculated from over 500 individual batch runs and a variety of production processes. (b) Evaporation accounts for an average of 4.95% of the reactor mass and failure to account for this loss results in an inaccurate mass balance.

A custom developed control algorithm determines whether the actual feed delivered deviates from the intended flow rate based on the bottle weight and inputted liquid density,and provides feedback to adjust the pump speed to match the intended flow rate accordingly.

This can be visualized in **Figure 6**, where a constant flow rate setpoint was programmed into the reactors (Figure 6a), but the actual feed rates are constantly being adjusted based on feedback from the load cells in order to achieve the intended setpoint **(Figure 6b)**.

To further improve liquid delivery, additional features have been incorporated into the control system in order to ensure world class precision in liquid delivery to the reactors. The feed control algorithm contains custom logic to reject false signals from environmental scale noise as well as accidental bumps. The algorithm also calculates fluid delivery deviations based on an array of continuously calculated scale deltas.

This minimizes any errors that could occur from manual adjustments that were made after the scales were tared, and also allows the algorithm to adapt to any continuous changes in the hardware such as the peristaltic tube slowly loosening or wearing down.

Like the production reactor, liquid additions to the vessel may evaporate. This is especially relevant for commonly used volatile liquids such as ammonium hydroxide and methanol. To this end, similar studies to the one described above were performed to empirically determine the evaporation rate of commonly used liquids from liquid delivery bottles. As the liquid type and density are inputted into each production recipe, these evaporation rates are also incorporated into the mass-based feed algorithms so evaporated liquid is accounted for in load cell signal outputs, further increasing precision.

Weight based additions (with mechanisms to protect against and account for load cell disturbances during setup and the run), as well as accounting for liquid class and evaporation in the production recipe together allow for accurate liquid delivery, typically within +/- 3 % of intended delivery rates. This not only results in accurate estimation of liquid delivery, but minimizes physiological variability attributed to imprecise feed delivery.

Weight Based Adjustments to Feed Rates

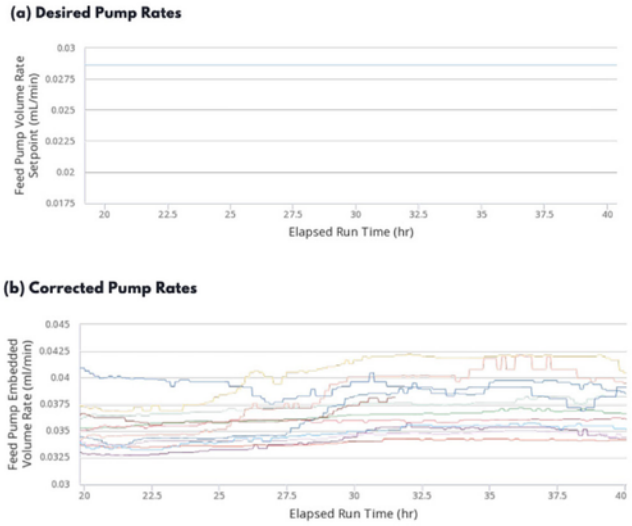


Figure 6: Weight based adjustments to feed rates. Feed pump setpoints are programmed into each reactor (a), and pump rates are continually adjusted based on feedback from load cells under each feed bottle (b).

Liquid Evaporation From Feed Bottles

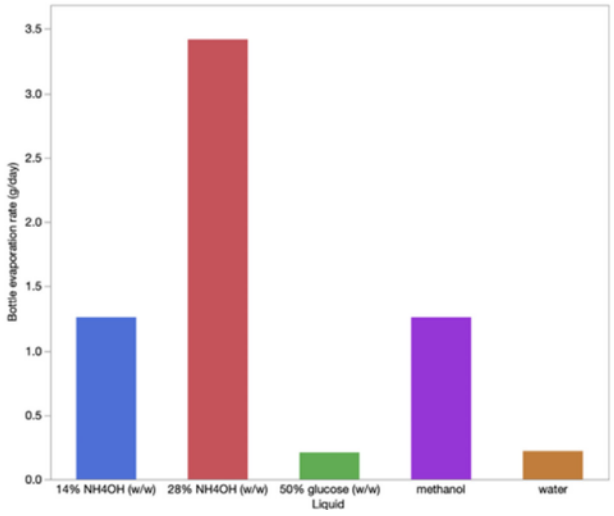


Figure 7: Evaporation from feed bottles can account for a significant portion of the feed and varies widely based on the liquid type. The evaporation rate of commonly utilized liquids was quantified by measuring the loss of mass over a period of time.

Manual Removals and Additions

Throughout a production run, samples are routinely removed from the reactor to take intermediate measurements that provide a more comprehensive understanding of cell line performance. Over the course of a cultivation, this volume can represent a significant proportion of the total cell culture fluid. These masses, along with any manual additions, must be accounted for in order to account for mass entering and exiting the batch.

Culture tracks all sample removal and manual additions to the reactor and these amounts are accounted for in mass balance calculations. Additionally, these manipulations are tracked to maintain an accurate volume estimate throughout the batch. Consequently this enables other variables and calculations, such as feed rates, to be consistently adjusted based on an accurate reactor volume at any given time.

Results and Discussion

Together, the features described above allow all of the inputs and outputs from the reactor to be measured. There are a number of different mass balance calculation methods that can be used in order to track inputs and outputs from the reactor, or to estimate the final cell culture fluid mass, which is then compared with the actual.

Culture's method tracks all mass added to and leaving the reactor. With these values, a mass balance for each production run can be calculated using the following formula:

$$\%\ Mass\ balance = (\Sigma mass\ out \div \Sigma mass\ in)\ x\ 100$$

Where

$$\Sigma\ mass\ in = Initial\ process\ broth\ mass\ (g) + net\ manual\ additions\ (g) + total\ mass\ pumped\ (g) + oxygen\ uptake\ (g)$$

$$\Sigma\ mass\ out = final\ process\ broth\ mass\ (g) + net\ manual\ removals\ (g) + Carbon\ dioxide\ evolved\ (g) + evaporation\ (g)$$

In an analysis of over 500 runs, the mass balance closure across multiple organisms and cell culture processes ranging from 22 - 120 hours were analyzed. The distribution of mass closure across these runs is shown in **Figure 8a**, below. From these data, the mass balance closure was between 97% and 103% in over 75% of the runs **(Figure 8b)**.

Together, these results demonstrate the ability of Culture Biosciences' cloud bioreactors to deliver the highest quality cultivation data, beyond the capabilities of the traditional industry leading systems on the market. Throughout a run, customers can visualize a live mass estimate of each reactor **(Figure 9)**, which incorporates all the calculations described above.

Fermentation Mass Balance Closure

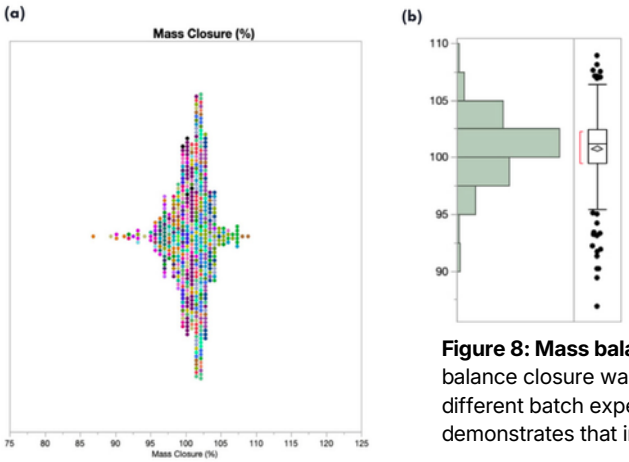


Figure 8: Mass balances were calculated from over 500cell culture runs. (a) Percentage mass balance closure was calculated using the % Mass Balance equation described above. Colors denote different batch experiments and processes. (b) A distribution of % Mass Closure from these runs demonstrates that in excess of 75% of runs fall between 97% and 103% mass closure.

As it is unfeasible to place 250ml reactors on load cells in order to have an accurate real-time process mass, it is challenging to approximate the mass of the reactor at any given time during the batch. Standard workflows often only measure whole cell culture fluid mass at the conclusion of the run, leaving midpoint measurements without an associated cell culture fluid mass. In contrast, the mass estimate provides insight into the mass of the reactor at any point during the run. In turn, this allows midrun measurements to be combined with an estimated real-time cell culture fluid mass in order to have a more accurate estimation of total product in the reactor. This enables customers to do more with their production data; instead of relying on only endpoint measurements based on an accurate mass, they can have greater insight into the entire duration of their runs.

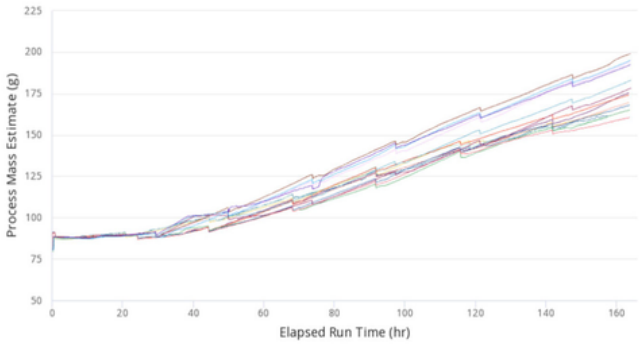
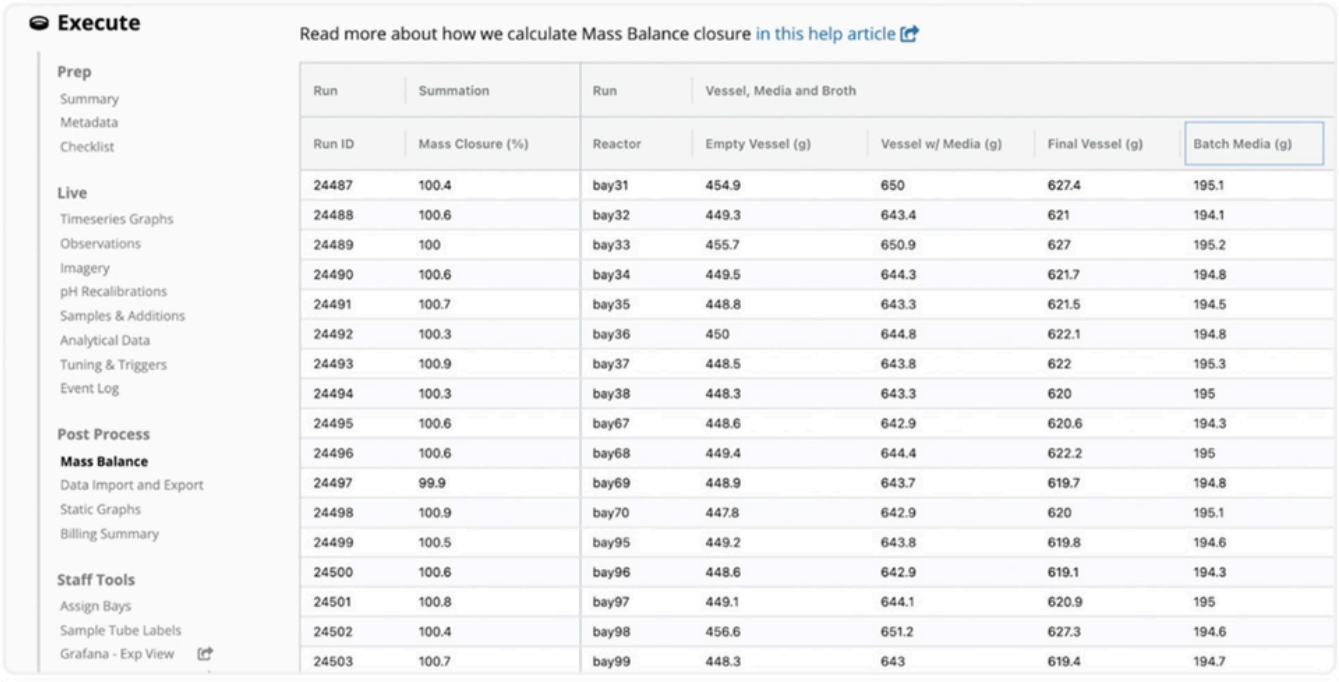


Figure 9: In these representative plots, an estimate of the mass of each reactor can be visualized over the entire production run. Manual additions and removals, such as inoculation and samples can be easily visualized, as well as changes in the reactor mass due to evaporation or addition of feeds.

Mass Balance Closure Data



Run	Summation	Run	Vessel, Media and Broth			
Run ID	Mass Closure (%)	Reactor	Empty Vessel (g)	Vessel w/ Media (g)	Final Vessel (g)	Batch Media (g)
24487	100.4	bay31	454.9	650	627.4	195.1
24488	100.6	bay32	449.3	643.4	621	194.1
24489	100	bay33	455.7	650.9	627	195.2
24490	100.6	bay34	449.5	644.3	621.7	194.8
24491	100.7	bay35	448.8	643.3	621.5	194.5
24492	100.3	bay36	450	644.8	622.1	194.8
24493	100.9	bay37	448.5	643.8	622	195.3
24494	100.3	bay38	448.3	643.3	620	195
24495	100.6	bay67	448.6	642.9	620.6	194.3
24496	100.6	bay68	449.4	644.4	622.2	195
24497	99.9	bay69	448.9	643.7	619.7	194.8
24498	100.9	bay70	447.8	642.9	620	195.1
24499	100.5	bay95	449.2	643.8	619.8	194.6
24500	100.6	bay96	448.6	642.9	619.1	194.3
24501	100.8	bay97	449.1	644.1	620.9	195
24502	100.4	bay98	456.6	651.2	627.3	194.6
24503	100.7	bay99	448.3	643	619.4	194.7

Figure 10: At the conclusion of each run, mass balance data for each reactor is published in Culture's custom data analysis tool, Console.

At the conclusion of each run, mass balance data for each run is easily accessible through Culture's data analysis tool, Console (**Figure 10)**. This transparency enables customers to have confidence in the quality of data provided for each run.

Conversely, mass balances can be used as a simple diagnostic tool; runs that fall outside expected mass balance metrics can be highlighted for quality investigations. This evel of visibility for quality control purposes is a feature that allows Culture to provide high quality data and reduce release of results that may be inaccurate and therefore confounding to customers.

Combining this data with additional analytical measurements based on the mass of the whole cell culture fluid, performed either by Culture or by customers, allows for calculation of carbon balances and fluxes, which can provide additional insight into opportunities for cell lines and process optimization.

Contact us
contact@culturebiosciences.com
culturebiosciences.com