

Nutrient Feed Control of Fed Batch Mammalian Cell Culture Runs with In-Line Cell Density Monitors

Introduction and Motivation In mammalian cell culture, the availability of adequate nutrient feed (such as glucose) determines cellular multiplication and growth. Therefore, controlling the nutrient feed in a bioreactor is critical to optimizing the environment in which mammalian cells are grown. Improper nutrient feed can result in lower yield, or even a failed production run. For example, if excess glucose is fed to a bioreactor, the cellular metabolic activity increases and results in excess lactic acid, which in turn limits cell production, so that a suboptimal cell density is achieved from the run. In contrast, if the nutrients can be fed at a rate that holds the stoichiometric ratio of glucose consumed and lactic acid produced at the optimal set point, then the cell growth process becomes highly efficient, all

feed becomes converted to biomass, and product yield is maximized. Therefore, knowing the growth rate of the cell mass, as well as the cell mass concentration, is critical to developing an optimal nutrient feed strategy.

Traditionally, the nutrient feed supplied to a bioreactor has been based on models whose input parameters include estimated oxygen uptake rates, off-line cell density measurements, as well as data from previous growth runs. In this whitepaper, we will show that using only these three input parameters for controlling nutrient feed and production yield can lead to sub-optimal results. Real-time, in-line measurements of cell density and cell mass growth rates should also be provided to the growth models.

Using Time Stamps and Cell Density Thresholds to Trigger Feed Events

Some cell culture facilities have developed sufficient understanding of their processes and control of their bioreactor environments to conclude that their optimal feed strategy is to simply trigger a nutrient feed event using a specific time, at which a target cell density is assumed to have been achieved. For example, the final cell density and expression achieved by a process might be optimized if the nutrient feed is done at a 100 hour time stamp, which is thought to correspond to a cell density target of 1.5 MM cells/mL. However, the feed time required for optimal yield is by no means a “fixed” value. It depends on the media used in the bioreactor, the particular strain of the cell line, and the purity of the overall feedstock. These parameters can affect the growth rate so that the target cell density can be achieved slightly earlier or later (based on elapsed time) than the predictive models. The feed event time stamp then becomes inaccurate. Ideally, the actual cell density would be measured and compared to a target value.

More sophisticated controls and models do trigger the nutrient feed event based on the target cell density itself, by incorporating data from off-line cell density measurements. Unfortunately, off-line cell density measurements are known to be relatively inaccurate, especially at the low cell concentrations found in the early stages of cell culture, when proper timing of nutrient feed is most important. Off-line measurements typically suffer from dilution and/or sampling errors, and the inherent inaccuracy of off-line cell counting techniques at low concentrations. In-line optical density measurements, on the other hand, provide real-time information on cell density (and hence cell growth rates). Moreover, laser-based sensors such as the Finesse TruCell, can achieve excellent measurement precision even at low cell concentration. Figure 1 illustrates that in-line cell density measurements are more accurate than their off-line counterparts, especially at low cell concentrations. Therefore, real-time, in-line optical density measurements can provide unique data to improve process control decisions for nutrient feed.

Figure 1
Lab Grab Sample versus TruCell Measurements

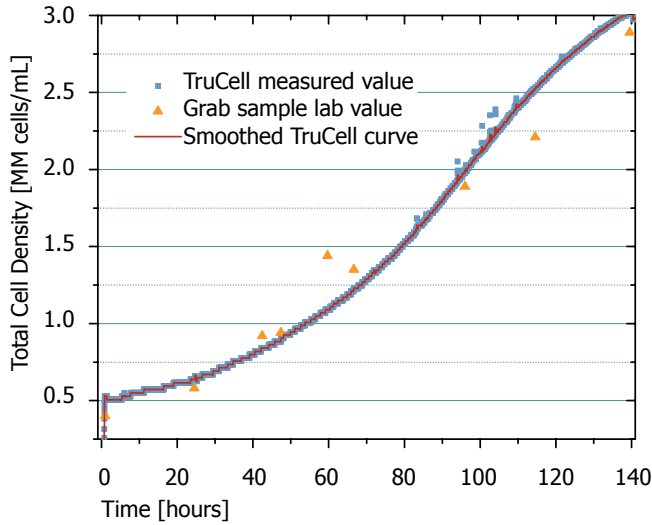


Figure 2
Typical Mammalian Cultures with Different Feed Times

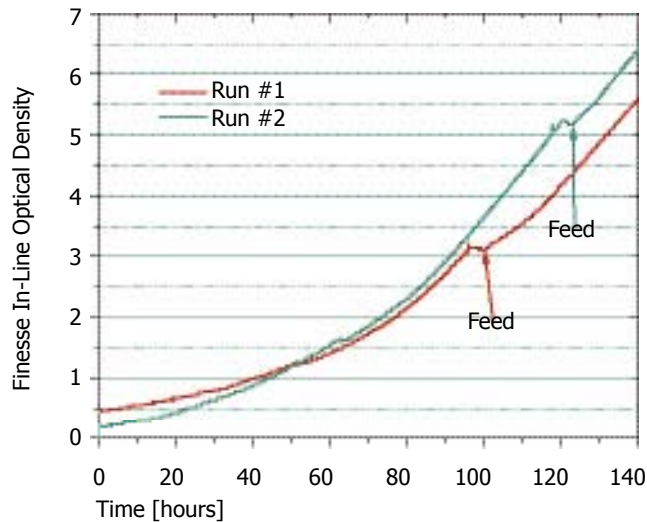
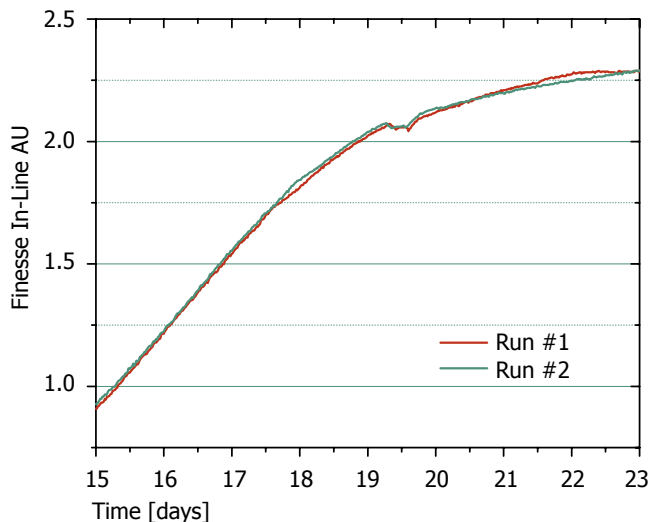


Figure 3
Consecutive Mammalian Cell Culture Runs using TruCell for Nutrient Feed Timing.



Note the excellent reproducibility.

Some typical Chinese Hamster Ovary (CHO) cell growth curves from fed-batch reactors are shown in Figure 2. Off-line cell density measurements taken once per operator shift were used to trigger the nutrient feed event. From Figure 2, we observe that both the feed time and cell density change from run to run. Specifically, the nutrient feed time in the second run was delayed by ten hours, and occurred at a cell density more than fifty percent higher than that in the previous, “identical” process.

The delay in obtaining off-line results, as well as the general inaccuracy of the method results in the observed lack of process repeatability. This problem is unfortunately prevalent in many cell culture facilities, but could be easily remedied by implementing an in-line, real-time optical density measurement.

Improving Nutrient Feed Control using Optical Density Measurements

Using advanced in-line optical (cell) density monitoring, researchers and engineers can more deeply understand the effects of nutrient feed on early stage cell growth, and develop more sophisticated predictive models. They can also develop and implement more advanced control strategies for nutrient feed, to thereby improve the yield and repeatability of fermentation and cell culture processes.

Figure 3 illustrates the improvement in process control that can be achieved by adding cell (optical) density measurements to a nutrient feed control system. The run-to-run reproducibility is excellent, and demonstrates that a good model for both nutrient feed timing and growth rate was achieved. The overall production yield became more reproducible.

We recommend adding a real-time, in-line cell (optical) density measurement to the process control as a fourth control parameter. Such a sensor can optimize the nutrient feed strategy, and minimize overall process variability. Specifically, we recommend our TruCell probe for improving nutrient feed control strategies.