**204c A Simple Strategy to Custom-Optimize Feed Streams for Fed-Batch Processes**

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Since it often takes a long time to develop a high-producing process for the manufacture of therapeutic monoclonal antibodies, pharmaceutical companies are often faced with balancing process productivity against time-to-clinic. To get to the clinic faster, sub-optimal processes are often utilized to manufacture material for early stages of clinical trials. Consequently, major changes to the process in later stages of clinical trials are often required to meet the increase in demand. Implementing major process changes increases the risk of changes in product quality and therefore risk failing product comparability. Clearly, it is desirable to be able to quickly develop high-producing, scaleable processes to support early-phase clinical trials without jeopardizing time-to-clinic.

A simple strategy was developed for custom-optimization of any generic fed-batch process. This strategy involves two aspects 1) custom-balancing the amino acids (AAs) in the feed medium and 2) adjusting the feed rate daily to meet the consumption needs. This is an iterative process in which the existing feed is modified to reflect the nutrient requirements of the cells using consumption data from previous experiments. If successfully implemented, this process maintains a constant concentration of nutrients throughout the cell culture process, thus avoiding cell death due to nutrient depletion or accumulation of potentially toxic feed components. This, in turn, results in higher cell concentrations, longer viability, and higher overall productivity. While modest productivity gains can be achieved by applying either aspect of the strategy, both aspects of this strategy need to be implemented for significant productivity improvements.

The first feature of the strategy is the design of the well-balanced feed medium, *i.e.* custom-balancing the AA concentrations in the feed medium. The following algorithm summarizes this process:

1. Take daily samples during a cell culture bioreactor run. Analyze the spent media for amino acid composition.

2. Using material balance, calculate the consumption rate of each amino acid while the cells are in the log growth phase, and normalize it to the consumption rate of an easily measurable nutrient, such as glucose.

3. Determine the concentration of each amino acid in the feed medium relative to that of glucose. Adjust the AA concentration in the feed as needed so that the ratio of AA/glucose concentration is same as the ratio of AA/glucose consumption (calculated in step 2).

4. Apply the new feed and take daily samples for spent media analysis. Repeat steps 2 and 3, as relative consumption rates may shift with improved feed and resulting higher cell concentrations and metabolic by-product accumulation. Several iterations may be needed to fine-tune the medium composition so that a constant AA concentration throughout the culture is achieved.

The second feature of the strategy involves the design of a customized feed strategy, *i.e.* adjusting the feed rate daily to meet consumption needs. Daily feed rates are determined by applying glucose measurements to the simple algorithm below, which is based on basic material balance principles to target a pre-determined glucose concentration:

$$\text{Feed rate}_{(i+1)} = (\text{Glucose fed}]_{(i)} + \Delta[\text{Glucose}]_{(i)} + \text{Glucose to recover}_{(i)})/\Delta t$$

where

$\text{Glucose fed}_{(i)} = \text{Feed rate}_{(i)} * \text{Glucose concentration in feed}$

$\Delta[\text{Glucose}]_{(i)} = [\text{Glucose}]_{(i)} - [\text{Glucose}]_{(i-1)}$

$\text{Glucose to recover}_{(i)} = \text{Target glucose level} - [\text{Glucose}]_{(i)}$

By implementing this strategy, we have been able to realize over a 100% increase in productivity with two different NS0 cell lines within three iterations. Depletion of critical nutrients or overfeeding of potentially toxic feed components was avoided by better balancing the feed medium and adjusting the feed rate to meet the consumption demand. This led to higher Xv and longer viability, thus resulting in greater overall IVC relative to a non-customized process. Although the work presented here focused on customizing the concentration of amino acids in the feed medium, it is likely that other classes of nutrients also play critical roles. Future work will involve the customization of vitamin and trace elements in the feed medium.