BOUNDARY OF OXIDATIVE AND OVERFLOW METABOLISM (BOOM) CONTROLLER FOR CHO CELL FEED CONTROL

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Key Words: Metabolic state, controlled feeding, off-gas sensing

There is limited literature for CHO cell cultures with low batch glucose concentrations (Gowtham et al. 2017; Lu et al. 2005; Wong et al. 2005). Work like Xu et al. (2016) and Berry et al. (2016) have shown positive results for controlled fed-batch cultures at low glucose concentrations following standard high glucose (5-6 g/L) batch cultures. However, the Xu et al. (2016) and Berry et al. (2016) approaches still accumulate lactate. Controlling glucose earlier could potentially avoid lactate accumulation and lead to even greater improvements in culture outcomes. The objective of this project was to develop an advanced feed controller for CHO cell cultures that maximizes cell growth by maintaining the culture in a state of maximal oxidative metabolism while minimizing overflow metabolism. The Boundary of Oxidative and Overflow Metabolism (BOOM) controller periodically manipulates the feed rate while monitoring online signals to gauge the remaining oxidative “space”, in order to decide whether feed can be increased while remaining in oxidative metabolism. The Oxygen Uptake Rate (OUR) is the primary signal of interest, since it plateaus when a culture shifts from oxidative to overflow metabolism, encoding vital information about metabolic state. This project’s approach is different from past work in that the batch glucose concentrations is much lower (on the order of 1 g/L), the glucose and/or glutamine feeding begins very early in the process, and glucose feed is triggered/controlled by the off-gas sensing of the metabolic state instead of a targeted glucose concentration.

During early runs several chemistry effects were observed directly due to the bolus feed additions interfering with the media-dissolved gas equilibrium. For example, a bolus feed that only contained 5 mM bicarbonate, resulted in an observed short sharp decrease in CO2 off-gas as the feed absorbed CO2 from the 5% CO2 sparge gas. Continuous feeding was introduced in subsequent runs as a means to mitigate disrupting the media-dissolved gas-equilibrium and disturbing the off-gas sensing. In order to have effective continuous feeding, the feed pump used a pulse width modulation (PWM) with a 10-minute period to allow extremely low effective feed rates required for the 1-L vessel. Two runs were used to demonstrate that the PWM feed pump could provide these very low pump feed rates for the 1-L vessel containing as little as 500 mL media. Initial glucose concentrations between 0.6 to 2.0 g/L were used (compared to 8 g/L glucose in the standard media formulation). Feedings have started between 6- and 20-hour post-inoculation. Distinct qualitative and quantitative differences have been observed in the corresponding oxygen uptake rate (OTR) responses due to the feeding spikes, suggesting that metabolic state can be detected. The development of the state estimator to control glucose feeding will be presented.