MODELING THE EFFECT OF BIOREACTOR pH ON CHINESE HAMSTER OVARY (CHO) CELL METABOLISM AND SITE-SPECIFIC N-LINKED GLYCOSYLATION OF VRC01

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Optimization of Chinese Hamster Ovary (CHO) cell based monoclonal antibody production processes require optimization of media development, cell line development, bioreactor operation, process monitoring, process control and careful monitoring of product's critical quality attributes (CQA) such as N-linked glycosylation. Media optimization typically involves specifying the concentrations of more than 50 components. Optimal fed-batch bioreactor operation requires optimizing the pH, temperature, dissolved oxygen and feeding schedule. Optimizing the process performance using experiments is an expensive proposition. It has been demonstrated in the literature that models can be used to optimize the process while minimizing experimental effort. However, it is difficult to use models to optimize the overall process as the majority of models in the literature are built with the goal of specifically optimizing one of the above key aspects. There is a need to develop models incorporating all those process parameters to capture the important interactions between them.

The work here aims to overcome these drawbacks of literature models by developing a detailed model that can be used to optimize bioreactor operation while keeping track of N-linked glycosylation and change in media requirements under different pH conditions. More specifically this work targets the integration of the effect of pH on a model of CHO cell metabolism and site-specific N-linked glycosylation. VRC01 producing CHO cells were grown at pH of 6.75, 7 and 7.25 in fed-batch mode in a 1 L Eppendorf bioflo 120 bioreactor system. Cell density, viability, glucose, lactate, 18 amino acids, ammonia, titer, nucleotide sugar and N-linked glycan structures were measured at each condition to develop a database for model regression. The model for metabolism was developed by integrating a combined kinetic and stoichiometric model for metabolism. The integrated model was developed by using semi-empirical kinetic expressions to determine uptake rates of a few metabolites and these uptake rates were used as constraints to generate the solution of the detailed stoichiometric model by using metabolic flux analysis. The effect of pH was empirically incorporated into the kinetic expressions of the model for metabolism. Through the experimental data it is evident that changes in pH led to depletion or accumulation of different metabolites. This leads to suboptimal media performance. The VRC01 mAb contains N-linked glycans at Fab region and the Fc region of the mAb. The glycan fractions on both sites were measured and found to be very different. The stirred tank reactor-based model for N-linked glycosylation has been modified to model the glycans at multiples sites of the mAb. This model is used to understand why there are differences in glycan fractions across sites and different bioreactor pH values. The proposed model is then used to determine the effect of pH on nutrient requirements as well as product quality, providing a platform to optimize pH and media formulation while tracking CQAs.

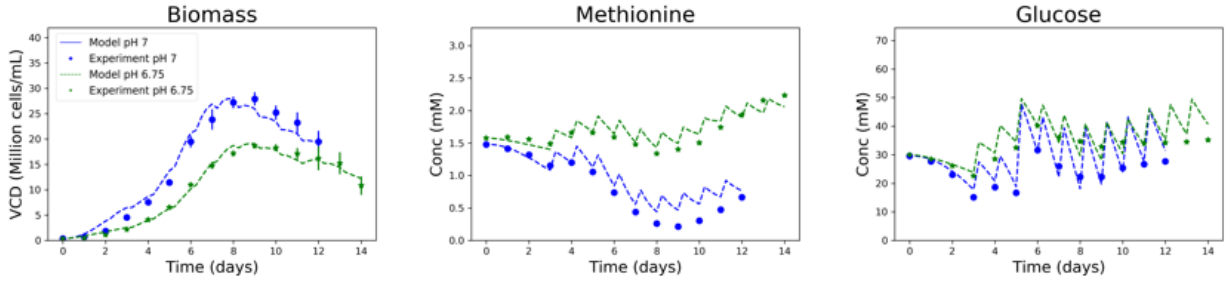

Figure 1 – Modeling the effect of bioreactor pH on CHO cell growth, methionine concentration and glucose concentration in a fed-batch process.