CONTROLLABILITY ANALYSIS TO IDENTIFY MANIPULATED VARIABLES FOR A GLYCOSYLATION CONTROL STRATEGY

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N-linked glycans affect important end-use characteristics such as the bioactivity and efficacy of many therapeutic proteins, (including monoclonal antibodies), *in vivo*. However, achieving a precise glycan distribution during manufacturing can be challenging because glycosylation is a non-template driven cellular process, with the potential for significant uncontrolled variability in glycan distributions. As important as the glycan distribution is to the end-use performance of biopharmaceuticals, to date, no strategy exists for controlling glycosylation on-line. In this work, we present a controllability analysis for glycosylation as a first step toward establishing an online glycosylation control strategy. We first assessed the theoretically achievable extent to which the very complex process of glycosylation is controllable. Once theoretic controllability was established, we performed experiments to identify appropriate manipulated variables that can be used to direct the glycan distribution of an IgG1 to a desired state. We found that bioreactor process variables such as glucose and glutamine media concentration, temperature, pH, agitation rate, and dissolved oxygen (DO) had significant but small effects on the relative percentage of various glycans. This indicated that the IgG1 glycan distribution was generally robust to even large perturbations of typical bioreactor variables. Conversely, we found that media supplementation with manganese, galactose, and ammonia had significant and large effects on certain glycans. From this work, we determined that manganese can be used as a manipulated variable to increase the relative abundance of M5 and decrease FA2 glycans simultaneously, and galactose can be used as a manipulated variable to increase the relative abundance of FA2G1 and decrease FA2 and A2 simultaneously. As a final test, we applied machine learning algorithms to validate and enrich these findings from a data-centric point of view. The machine learning algorithms provided an avenue to discover unknown relationships and patterns that refined our findings and provided a framework to explore more variables.