

## ONLINE CONTROL OF CELL CULTURE REDOX POTENTIAL PREVENTS ANTIBODY REDUCTION

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The phenomenon of monoclonal antibody (mAb) interchain disulfide bond reduction during manufacturing processes has been reported widely across the biotechnology industry. Reduction results in loss of product, requires more complex purification processes, and leads to reduced stability of the final drug product. Here, we show the development of a control system to prevent mAb reduction in the bioreactor based on the cell culture redox potential. Cell culture redox potential indicates the reducing/oxidizing potential of the extracellular environment. This work describes the process development for an IgG<sub>2</sub> mAb where a high level of mAb reduction was observed at harvest. Further analysis revealed reduced mAb in the bioreactor prior to harvest. To understand the impact of reducing/oxidizing environment on mAb reduction, the process was run with several different conditions including increased concentrations of metal ions, 2-mercaptoethanol, glutathione, cystine, and an increased DO set-point. Samples were taken from these bioreactors on days 12 and 14 and the amounts of intact mAb in the cell culture supernatants were quantified. Many of these bioreactors were run with redox probes that allowed monitoring of the cell culture redox potential. Analysis of the data revealed a clear correlation between the cell culture redox potential and mAb reduction. Based on this information, we identified a threshold redox potential above which the mAb remained intact and below which there was significant and highly variable amounts of reduced mAb. Using this knowledge, we developed three control schemes to mitigate mAb reduction. These control methodologies functioned by increasing the concentrations of dissolved oxygen (DO), Cu, or both DO and Cu to maintain the redox potential above the threshold value. The redox control strategies based on the addition of Cu or Cu and DO maintained the cell culture redox potential above the threshold value and prevented mAb reduction, whereas the control strategy based on DO control alone was insufficient to maintain the redox potential above the threshold value and had high levels of reduced mAb (Figure 1). Importantly, the redox control strategies did not significantly impact the cell growth, viability, mAb production, or product aggregates. This method of using on-line cell culture redox potential can be used to predict the likelihood of reduction occurring in the bioreactor and evaluate the effectiveness of new mitigation/control strategies; it can also be extended to prevent mAb reduction from occurring during or after the harvest. Finally, the methods described in this work to control mAb reduction would ensure simpler purification processes, improved product quality, and prolonged drug product stability compared to processes with uncontrolled reduction.

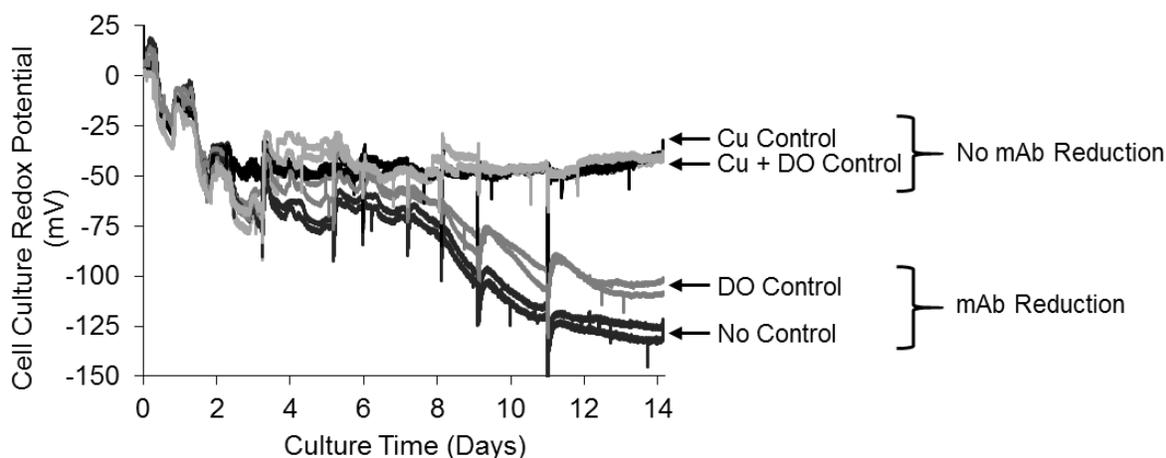


Figure 1 – Online Cell Culture Redox Potential