CHO STABLE POOL FED-BATCH PROCESS DEVELOPMENT OF SARS-COV-2 SPIKE PROTEIN PRODUCTION

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Key Words: Stirred-tank Bioreactor Process Development, Oxygen Uptake Rate, Feeding Strategy, Bio-capacitance, Process Analytical Technologies (PAT)

Technology transfer is a fundamental and critical part of process development. Important bioreactor hydrodynamic characteristics such as volume, overhead gas flow rates, volumetric power input (P/V), impeller type, aeration strategies (VVM) and sparger type must be selected based on key performance attributes to ensure a smooth scale-up. Additionally, adequate control must also be obtained for the chosen dissolved oxygen (DO) set point so as to reduce heterogeneity in the culture run. In this investigation, process development of an inducible CHO stable pool expressing SARS-CoV-2 spike protein in 1.8 L benchtop stirred-tank bioreactors (DASGIP parallel bioreactor system) is detailed. Open pipe sparger was employed as to generate smooth DO control for oxygen uptake rate (OUR) estimation through overhead gas analysis. Various DO levels and aeration air caps were studied to determine their impact on IVCC (integral viable cell concentration), culture longevity and endpoint product titers. Once hydrodynamic conditions were tuned to an optimal zone, various feeding strategies were explored to increase culture performance. Feeding based on current culture volume, feeding based on constant feed per cell, feeding based on OUR signal, feeding based on bio-capacitance signals were tested and compared to standard bolus addition every 2 days. It was shown that increased IVCC (1.36-fold increase), longevity (extended culture time by 5 days) and protein yield (2.8-fold increase) were observed in dynamic feeding strategies when compared to static bolus addition. Interestingly, lactate consumption was observed in dynamic feeding strategies while this was not observed in standard bolus addition. Alternatively, some dynamic feedings strategies (based on current culture volume) increased ammonia concentrations twofold when compared to bolus addition suggesting an overabundance of amino acids addition beyond metabolic need. However, these increased concentrations did not negatively impact culture performance. The study emphasizes the importance of designing feeding strategies around metabolically relevant signals such as OUR or bio-capacitance signals. This entails that feed flow keeps up not only with total cells within the reactor but with total biovolume and metabolic activity. Additionally, online monitoring signals can then be leveraged to construct automatic feeding protocols that do not require constant operator input.