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## **SMALL-SCALE COMPARISON OF PSEUDOPERFUSION FEEDING STRATEGIES USING BASAL AND CONCENTRATED FEED MEDIA**

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**Key Words:** pseudoperfusion process, CHO cells, monoclonal antibody, feeding strategy, basal and concentrated media

Perfusion has long been the industrial choice for the production of unstable proteins, and nowadays is being intensively studied also for stable proteins due to its ability to keep high viable cell densities and high volumetric productivities over long operation times. However, conventional perfusion is fed with basal medium at high dilution (or perfusion) rates, causing a dilution of the protein of interest (POI) and generating large volumes of harvest to be processed in the purification steps.

In this work, small-scale pseudoperfusion experiments were performed to compare the conventional perfusion strategy using basal culture medium (TC-LECC, Xell AG) with a feeding strategy employing a concentrated feed medium (TCX2D, Xell AG) that was originally developed for fed batch processes. The aim was to evaluate if the concentrated feeding strategy could equally enable high cell concentrations and high viabilities, but at higher dilution rates and potentially yielding higher product titers.

The cell line CHO DP-12 (ATCC CRL-12445), producing a recombinant monoclonal antibody, was cultivated in spin tubes, and a daily medium exchange to mimic perfusion was initiated in the mid-exponential of growth phase. TC-LECC basal medium and TCX2D concentrated medium were fed at perfusion rates chosen according to their initial glucose concentration, in the range of 0.15 – 1 vvd and 0.05 – 0.35 vvd, respectively. Both processes kept the cell viability higher than 60% for approximately 15 days. Maximum viable cell density of  $20 \cdot 10^6 \text{ mL}^{-1}$  was reached on day 6 for both processes. During the exponential phase, both feeding strategies provided a sustainable cell growth, though the post-feeding glucose measurements were not equivalent. MAb volumetric productivities were the same for both processes, and these results will guide the implementation of a controlled-fed process in a perfusion bioreactor.