CELL BEHAVIOR IN HIGH DENSITY PERFUSION PROCESSES

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Key Words: perfusion, high density, CHO, protein quality, metabolic profile.

Intensified upstream perfusion processes with cell densities well above $50 \times 10^6$ cells/mL are being applied or considered by the majority of pharmaceutical industries. A market survey performed internationally showed that by 2026 intensified upstream processes will be incorporated in commercial manufacturing of more than 30% of mAb related drugs.

In order to better understand high density perfusion processes, we compared the cellular state and metabolic behavior of two different CHO cell lines producing either an IgG or a fusion protein in fed-batch and high density perfusion. In this part of the work we compared not only specific growth and productivity, but also protein quality profiles. We then modified specific protein quality attributes in perfusion and evaluated the relationship between the modulators and the perfusion rates.

Cellular state and metabolic behavior are not only affected by the nutritional environment, but are also greatly affected by shear stress. Consequently, one of the challenges when working with high density cultures is to supply adequate oxygen to the culture. To increase the kLa of our benchtop bioreactors, while minimizing shear stress, we used a combination of very high power input ($>100\text{W/m}^3$) and a dual sparger, cascading the use of macro and micro bubbles. By using this strategy, we were able to reduce shear stress, while maintaining cultures at high viability. However, even sub-lethal levels of shear stress have been reported to generate a cellular response that can reflect in cellular and metabolic changes, potentially reducing growth, productivity and product quality (1, 2).

To better understand the effect of sub-lethal shear stress in the culture, we evaluated the cellular profile of a CHO cell line producing a fusion protein in steady state perfusion cultures at $50 \times 10^6$ cells/mL (with high and low shear stress) and at $80 \times 10^6$ cells/mL at a CSPR of 35pL/cell/d. Cell cycle, apoptosis, central carbon flux analysis and redox were monitored during these cultures. The results of this study provide an improved understanding of cellular behavior in intensified perfusion processes that will help develop methods for economic production of biologically active molecules.

References:
https://etd.ohiolink.edu/rws_etd/document/get/osu1206393724/inline