

## **A SYSTEMATIC APPROACH FOR PROCESS DEVELOPMENT AND QUALITY CONTROL IN CONTINUOUS PERFUSION CULTURES**

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Biopharmaceutical industry is facing numerous challenges. Increased cost pressure, changing markets, and the need for more manufacturing flexibility are main drivers for a gently mindset change: the switch from batch to continuous production of biopharmaceuticals. Mammalian cell perfusion cultures represent not only a valuable tool for the production of therapeutics, but also for process intensification of state-of-the art fed-batch processes. Considering N-stage perfusion processes, the knowledge on time and cost effective development and scale-up procedures to achieve a reliable reactor operation and desired product characteristics is still limited. However, a robust and optimized reactor operation is a prerequisite for a successful end-to-end integration production facility.

In this study a comprehensive optimization framework for mammalian cell perfusion cultures was defined. In a first step, rapid evaluation of suitable operating conditions in small scale perfusion cultures allowed to identify key process parameters to facilitate steady state operation and process control with a given media formulation. A further refining was performed in a stirred tank perfusion bioreactor setup by sequential screening of different steady state set points while targeting improved product yields and desired product quality characteristics. Minimizing cell specific perfusion rate, as well as varying perfusion rate and viable cell density set point at a suitable CSPR were evaluated targeting optimal and robust operation and product quality control. The tuning of key cell culture parameters led to an improved performance and control of the perfusion culture. Especially, the decrease of the cell specific perfusion rate prevented excessive cellular growth and reduced significantly the loss of product in the bleed stream. Constant patterns of product quality attributes such as N-linked glycosylation and charge isoforms were observed within each steady state. Overall, product quality distributions remained quite stable and did not vary between different operating set points. In a last step, the use of additional media supplements might provide an additional tool to effectively control and tune product quality towards desired distributions.

Overall, this study presents a systematic approach for the development of intensified continuous cultures and underlines the potential of perfusion cultures to simultaneously achieve high productivities while tuning towards desired characteristics of consistently expressed therapeutic proteins.