DEVELOPMENT OF A HIGHLY CONCENTRATED PERFUSION MEDIUM SUPPLEMENT TO DECREASE MEDIA DEMAND LEVERAGING A NEWLY DESIGNED 250ML SINGLE USE PERFUSION BIOREACTOR

Mona Bausch, Merck
mona.bausch@merckgroup.com
Luis Ayala, Merck
Long Pham, Merck
Channing McLaurin, Merck
Lisa Wolowczyk, Merck
Jana Mahadevan, Merck
Jochen B. Sieck, Merck

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Media demand and logistics are considered a roadblock for the increased adoption of perfusion processes for the manufacturing of biologics. To overcome this challenge, a concentrated nutrient solution (termed VVD Reducer) for CHO based processes has been developed to decrease this medium demand when added to the perfusion process (Fig. 1). Through leveraging statistical experiment design and multivariate data analysis, raw material concentrations were optimized in this supplement with regards to relevant process performance criteria including cell density, productivity, lactate, or ammonia formation.
For application of this supplement, a process was developed leveraging different fully controlled small-scale systems. One of these bioreactor systems presented in this study was a customized 250 mL steady state perfusion bioreactor which serves as an alternative solution for performing dynamic and steady state perfusion in small scale. Due to the reduced head plate space, the configuration of sensors and tubing had to be optimized to allow a proper operation comparable to classical perfusion benchtop bioreactors, and a specific perfusion operation had to be developed. The bioreactors are equipped with a biomass sensor allowing biomass control, a hollow fiber-based cell retention device, level sensors, and a single-use pump. The DASbox® Mini Bioreactor System (Eppendorf) is used to monitor the bioreactors and contains pump modules for feed of media and base, removal of cells (bleed) and cell-free harvest. It could be shown that this novel perfusion bioreactor works comparable to Breez™ perfusion bioreactors, which operate with a working volume of 2 mL.
This system was then used to determine critical cell specific perfusion rates ($CSPR_{crit}$), which means the minimum perfusion rate allowing steady-state perfusion processes using solely basal perfusion medium and a push-to-low approach as described by Konstantinov (2006). In the next step, this process was run with the previously determined critical perfusion rate, and the supplement was used to decrease the perfusion rate further from that. It could be shown with different CHO clones that using the developed supplement, the perfusion rate can be decreased by at least 30 % without losing the steady state of the culture demonstrating the potential to reduce volumetric media demand in upstream manufacturing processes.

Reference: Konstantinov K, Goudar C, Ng M, Meneses R, Thrift J, Chuppa S, Matanguihan C, Michaels J, Naveh D. The "push-to-low" approach for optimization of high-density perfusion cultures of animal cells. Adv Biochem Eng Biotechnol. 2006;101:75-98. doi: 10.1007/10_016. PMID: 16989258.

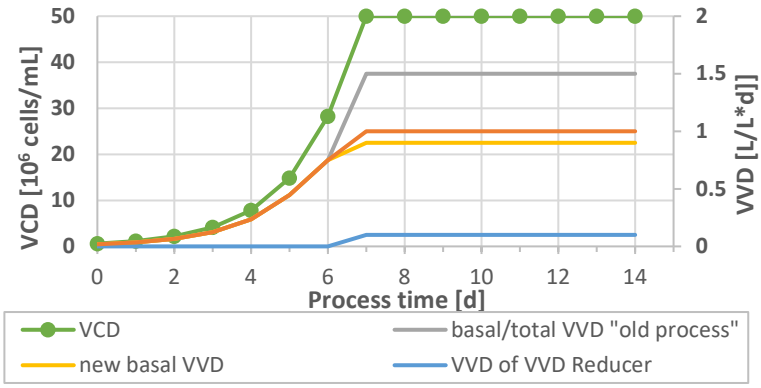

Figure 1 – Concept of VVD Reducer Application
*(VCD=Viable cell density; VVD=Perfusion rate in $L_{Medium}/L_{Bioreactor}$*d)*