**EVOLUTION OF TFF-BASED PERFUSION: A PATH TOWARDS NON PRODUCT SIEVING AND DIRECT CHROMATOGRAPHY INTEGRATION**

Nuno D.S. Pinto, Biologic & Vaccines, Bioprocess Research & Development, Merck & Co., Inc., Kenilworth, NJ
nuno.pinto@merck.com

Key Words: Monoclonal antibody, CHO cell culture, microfiltration, tangential flow filtration, product sieving.

Manufacturers of therapeutic proteins are becoming increasingly interested in continuous bioprocessing. Via uninterrupted medium exchange, perfusion cell culture yields higher volumetric productivity than traditional batch/fed-batch processes with 2 to 5 fold higher cell density and cultivation duration. In addition, an uninterrupted removal of therapeutic proteins promotes a desired alignment with integrated continuous purification processes to eliminate large hold tanks.

Hollow fiber tangential flow filtration (TFF) is commonly used for cell retention in perfusion cell culture. In these perfusion systems the major limitation can be inefficient or decaying product sieving. This increases product's retention time in the bioreactor, which may detrimentally affect the target protein quality attributes and decrease yield.

Herein, three commercially available TFF membranes are employed in high cell density CHO perfusion for more than 21 culture days. This work highlights the learnings obtained from interrogating various filter characteristics (e.g., pore size, chemistry) as well as operating conditions. The resulting understanding led to a solution with high mass transfer (>90% sieving) and particle free permeate stream (turbidity <10 NTU) to allow direct loading of a chromatography capture step. This work not only showcases a TFF perfusion toolbox, but also demonstrates that continuous processing is possible with TFF-based perfusion cell culture.

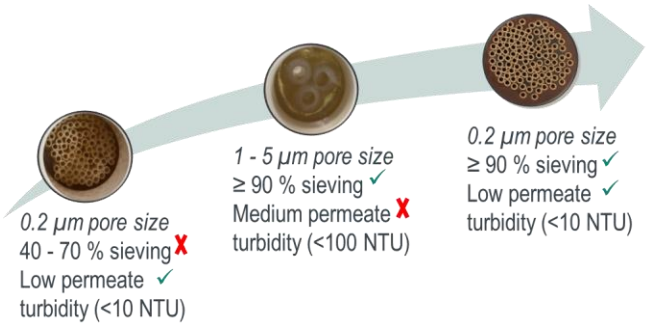


*Figure 1 – TFF perfusion evolution to overcome product sieving.*