Simplifying Progress

Integration of a PAT controlled perfusion approach with a continuous capture of monoclonal antibodies

Thomas Kruse[1*†], Johannes Lemke[1†], Jens Matuszczyk[2], Markus Kampmann[1] and David Pollard[1]

[1] Sartorius Stedim Biotech GmbH/Corporate Research, Göttingen, Germany
[2] Sartorius Stedim Biotech GmbH/Advanced therapies, Göttingen, Germany
[†] These authors contributed equally to this work
[*] Corresponding author: Thomas.Kruse@Sartorius.com

Introduction

Therapeutic monoclonal antibodies (mAb) are used for the treatment of numerous serious diseases as biopharmaceutical drug. Due to their high efficacy, the market for mAb is constantly increasing [1].
In order to meet the demand, intensified upstream (USP) as well as downstream processes (DSP) have become increasingly important. Continuous operation modes offer several advantages like increased productivity and cost savings [2].

1. Experimental approach

In this study, a continuous high cell density perfusion process of a mAb producing Chinese hamster ovary (CHO) cell line was examined. Hollow fiber filter modules in tangential flow filtration (TFF) and alternating tangential flow (ATF) systems were used as cell retention devices and compared to each other. Sequential-multi-column-chromatography (SMCC) was used for a direct, continuous mAb capture (Figure 1).

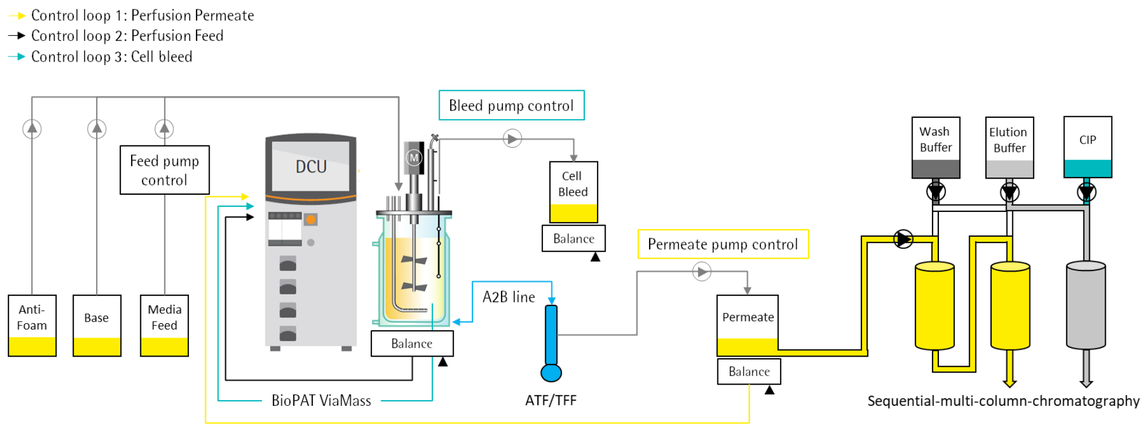

Figure 1: Schematic representation of the integrated USP-DSP approach highlighting the USP perfusion control strategies and the connection to the subsequent DSP.

To ensure a stable operation of the perfusion process, a controlled cell-bleeding strategy was implemented by the usage of an in-line bio-capacitance probe BioPAT®ViaMass (Sartorius, Germany). The steady-state-like operation of the continuous process, with constant cellular properties, enable a linear correlation between the permittivity and viable cell density (VCD). The perfusion rate (PR) was adjusted based on the target VCD and controlled by a gravimetric harvest flow controller, taking the additional cell-bleed flow into account, to maintain a constant cell specific perfusion rate. Simultaneously, cultivation media was added to maintain a constant bioreactor working volume.

Due to the constant loading flowrate of the subsequent SMCC, which was equal to the respective PR, the gravimetric harvest flow controller was set to maintain a constant weight of the surge tank with the perfusion permeate, resulting in a robust USP-DSP integration.

2. Results – Perfusion Process

Perfusion cell retention TFF and ATF systems were used to perform CHO cell cultivations in 2 L Univessel® Glass bioreactors controlled by Biostat® B-DCU (Sartorius, Germany). The systems suitability to support high cell density cultivations were evaluated in a first growth phase up to 80·10^6 cells/mL before the automated cell bleed at 50 or 80·10^6 cells/mL was started (Figure 2). Target VCD was well controlled except deviations caused by operator errors on day 10 (TFF) and 14 (ATF).

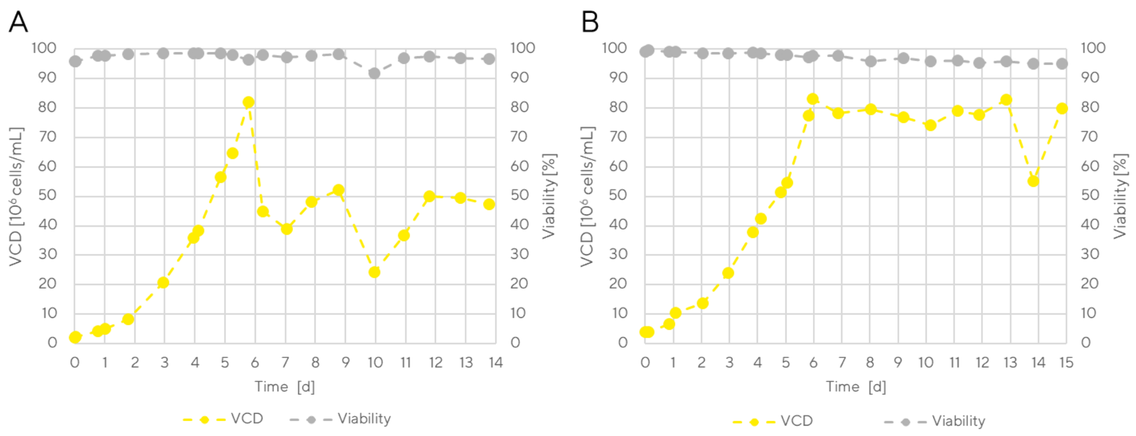

Figure 2: Cell growth and viability of TFF (A) and ATF (B) CHO perfusion cultivations in 2 L stirred tank bioreactors. Automated cell bleed was started on day 6 to maintain a constant viable cell density.

For both cultivations, fast cell growth and constant high cell viability throughout the two-week processes were observed (Figure 2). Both cell retention systems were able to allow for continuous cell-free permeate removal from the bioreactors. Control of the perfusion rate based on target VCD resulted in identical product concentrations of 0.6-0.7 g/L mAb for both, the ATF as well as the TFF process, with similar levels of product retention.

3. Results – SMCC

For the setup of a continuous SMCC process the BioSC™ Lab chromatography system with the corresponding BioSC™ Predict software were used (Novasep, France). The load volume as well as the number and interconnection of the columns were chosen based on previously performed breakthrough curves at different velocities (Figure 3) using an integrated experimental and modeling approach [3].

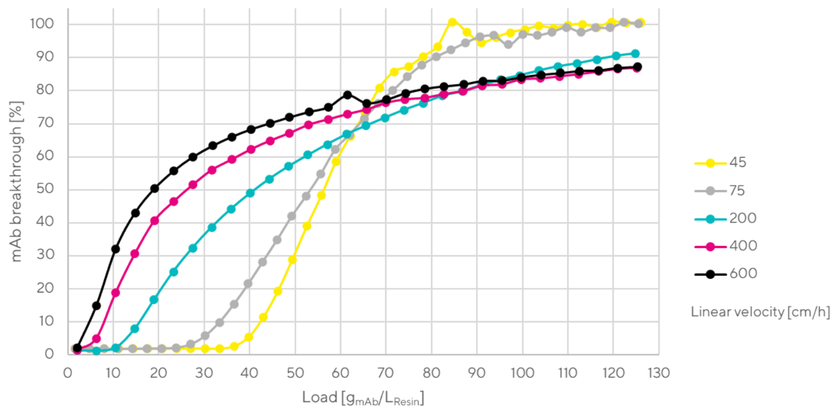

Figure 3: mAb breakthrough curves at different velocities performed with prepacked 5 mL mAbSelect SuRe protein A affinity columns (Cytiva, USA).

For the 50·10^6 cells/mL TFF (3.4 mL/min PR) and the 80·10^6 cells/mL ATF (6.1 mL/min PR) process respectively a 3- (Figure 4A) or 5-column (Figure 4B) SMCC was performed, starting from day 6 of the perfusion process (Figure 2).

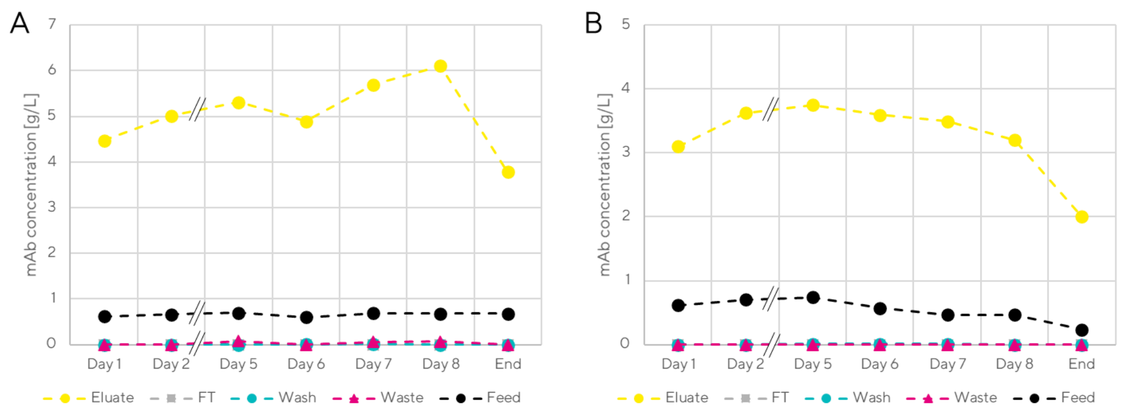

Figure 4: mAb concentration of the continuous SMCC capture process for the 3- and 5-column process. The mAb concentration is given for the eluate, flow-through (FT), wash, waste and feed.

The mAb was successfully captured into the eluate fraction, while no product was observed in the flow-through during the loading phase (Figure 4). It was shown that the SMCC is capable of successfully processing different USP process scenarios with different VCD by making appropriate adjustments to the loading flow rate and volume as well as to the number of columns. Both capture processes were operated for 8 days without any interruption, demonstrating a successful and robust continuous processing of the USP perfusion permeate.

Table 1: Summary of the results for the 3- and 5-column SMCC.

Process setup	3-column	5-column
Purified total mAb mass [g]	33.1	33.9
Obtained productivity [$g_{mAb}/L_{resin}/d$]	272.3	174.6
Yield [%]	97.2	99.7

4. Conclusion

- The applied control strategies allow a robust and automated perfusion process with an easy to adopt integration of a continuous SMCC capture step
- For both, TFF and ATF, the respective target VCD was maintained with a high viability and a continuous cell-free permeate flow
- SMCC processes were adapted to the different VCD of the USP and showed high yields and productivities throughout the continuous capture

References

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