Simplifying Progress

The RABBIT jumps ahead, Rapid A BioSMB Biolayer Interferometry Technology

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1. Introduction

Monoclonal antibodies (mAbs) are nowadays fundamental in treating a wide range of severe diseases, including cancer, infections, autoimmune disorders, and inflammatory diseases. [1]. Due to the high specificity, activity and fewer side effects compared to conventional drugs, the market for mAbs rises continuously [2]. Ongoing improvements in the upstream process, such as higher mAb titers, have shifted the bottleneck in mAb production to the downstream process (DSP). Consequently, there is an increasing demand for DSP process intensification technologies to overcome manufacturing bottlenecks. Semi- and fully continuous processes are gaining traction in both industry and academia due to their cost-effectiveness and efficiency. However, the complexity of these processes, throughput limitations and the need for adaptive control strategies present significant hurdles.
Continuous multi-column chromatography (MCC) offers several benefits, such as increased productivity, reduced buffer consumption, and lower production costs, all within a smaller equipment footprint. However, MCC processes commonly rely on predefined loading volumes and flow rates. This requires several assumptions like a constant flow rate and known mAb concentration of the feed solution, that all columns have the same dynamic binding capacity (DBC) and that the DBC remains constant throughout the process. These challenges could be overcome by a dynamic loading based on breakthrough detection of the mAb.

2. Experimental approach

This study introduces an enhancement of a recently developed MCC approach (RC-BioSMB, [3]) that leverages convective diffusive membrane adsorbers (MA, Sartobind® Rapid A) by a dynamic loading approach based on continuous at-line biolayer interferometry measurements [4]. This approach addresses the limitations of conventional UV spectroscopy for dynamic loading, such as low specificity and high background noise from media components, by detecting mAb breakthrough with greater specificity. A continuous production setup was established by incorporating a continuous virus inactivation and sterile filtration (Figure 1). Setpoint variations of the permeate flowrate were successfully addressed by a dynamic flow control throughout all unit operations as well as a dynamic loading of the RC-BioSMB process. These advanced control strategies were implemented by interfacing the involved equipment with an overarching custom-made software component, using a low-code platform, which provides a more user-friendly and cost-effective automation solution.

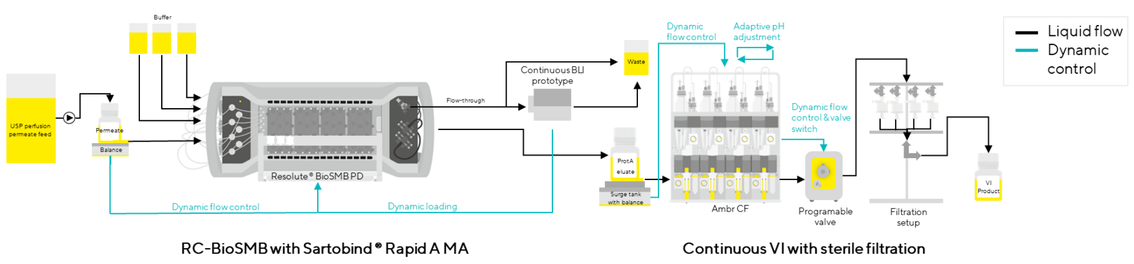

Figure 1: Schematic representation of the integrated continuous Upstream- and Downstream process

3. Continuous at-line BLI measurements for mAb breakthrough detection

BLI is a label-free technology based on the interference pattern obtained from the combination of white light reflected from an internal reference surface and a bio-layer. The high affinity and consequently binding of the analyte to the immobilized molecule at the biocompatible surface result in an increased thickness at the surface of the biosensor, which in turn is measured as concentration dependent signal by the change in the light interference (Figure 2A, [4]). The mAb breakthrough of the SMB capture process was specifically detected in the flow-through by a continuous BLI prototype (Figure 2B), using a biosensor with immobilized protein A.

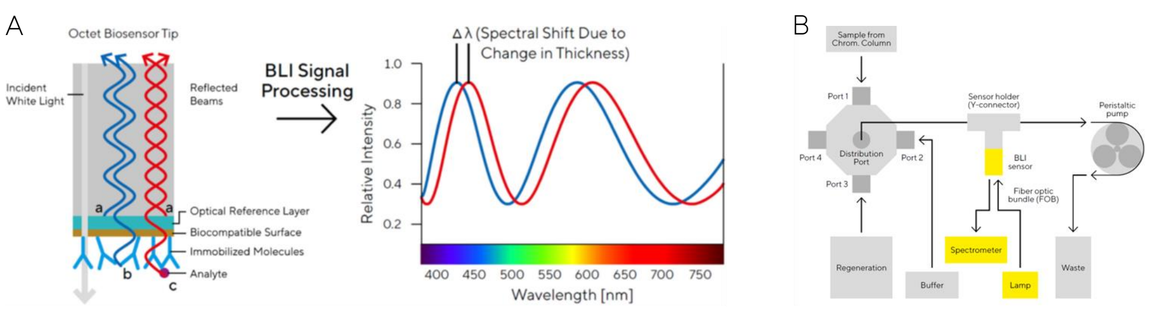

Figure 2: (A) An optical fiber used for BLI and a typical optical signal. **(B)** Schematic representation of the flow paths within the continuous BLI prototype

A complete breakthrough curve of the mAb from the MA was performed and analyzed off-line by size exclusion chromatography as well as at-line by UV spectroscopy and the continuous BLI prototype (Figure 3). It becomes apparent that the offline measurement corresponds very well with the maximum binding rate of the BLI signal and that the mAb breakthrough can be detected easily (the slightly higher signal of the BLI is due to its higher sensitivity). In contrast, a breakthrough can hardly be recognized by the UV signal, as the background is significantly higher here.

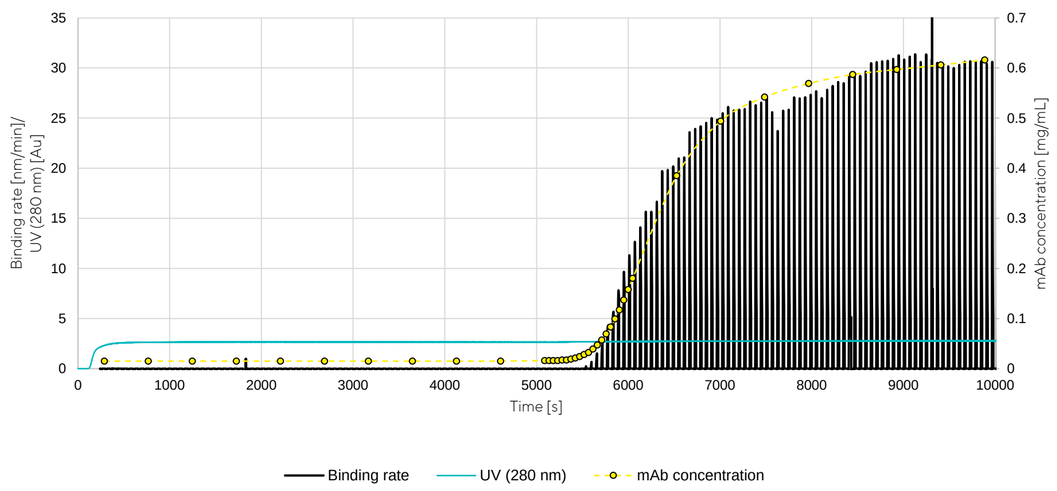

Figure 3: Breakthrough curve of the mAb from the Sartobind® Rapid A MA analyzed off-line by size exclusion chromatography with an HPLC as well as at-line by UV-spectroscopy at 280 nm and the continuous BLI prototype.

4. Results – Dynamic loading and flow control

Setpoint variations of the permeate flowrate were successfully addressed by a dynamic flow control throughout all unit operations as well as a dynamic loading of the RC-BioSMB process (Figure 4). In total 56 loading steps were performed by the MCC chromatography system within approximately 2 days. An enhanced membrane utilization was obtained while simultaneously minimizing product losses. (below 0.02 mg/mL in the flowthrough fraction) despite the steep mAb breakthrough of the membrane adsorbers (Figure 3).

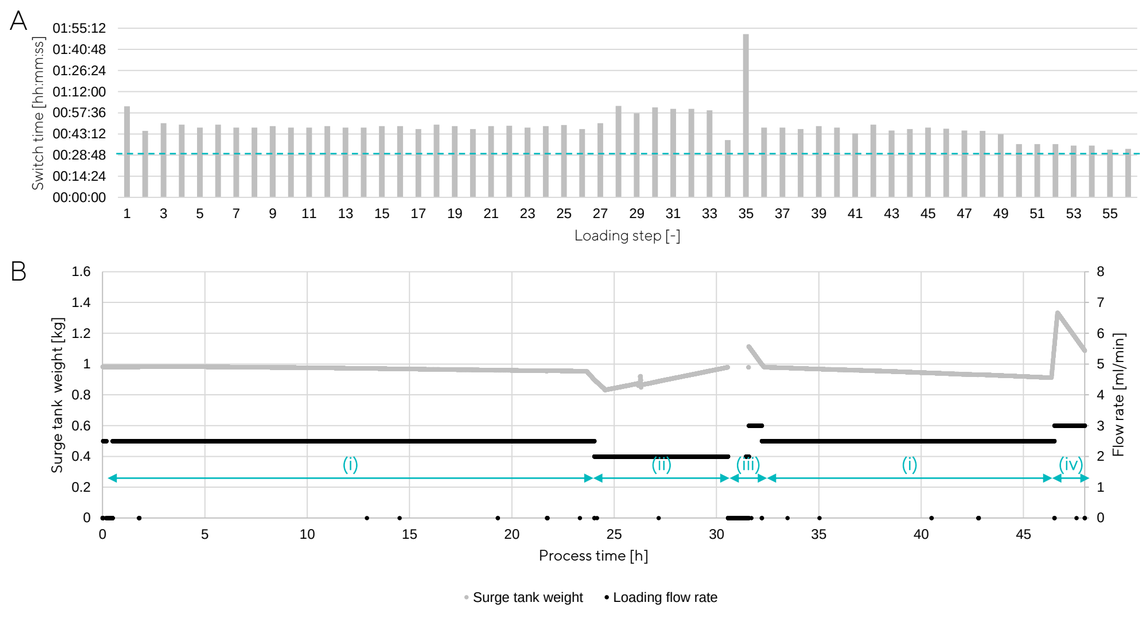

Figure 4: (A) Switch time for the loading step of the MCC chromatography system. The minimum loading time, where no measurement during the loading step was performed, is indicated (29 min, turquoise dashed line). **(B)** Permeate surge tank weight and the MCC loading flow rate. Setpoint variations for the permeate flowrate were: (i) permeate flowrate equal to MCC loading flowrate, (ii) lower permeate flowrate, (iii) connection loss to balance, (iv) higher permeate flowrate.

5. Results – Analytics

Throughout the complete process a high mAb yield of 87 % with simultaneous high removal of process-related impurities like host cell proteins (3.3 log removal to 70 ppm, Figure 5) was obtained . Moreover, The convective diffusive MAs enabled a high productivity of 697 g L-1 d-1 for the RC-BioSMB process.

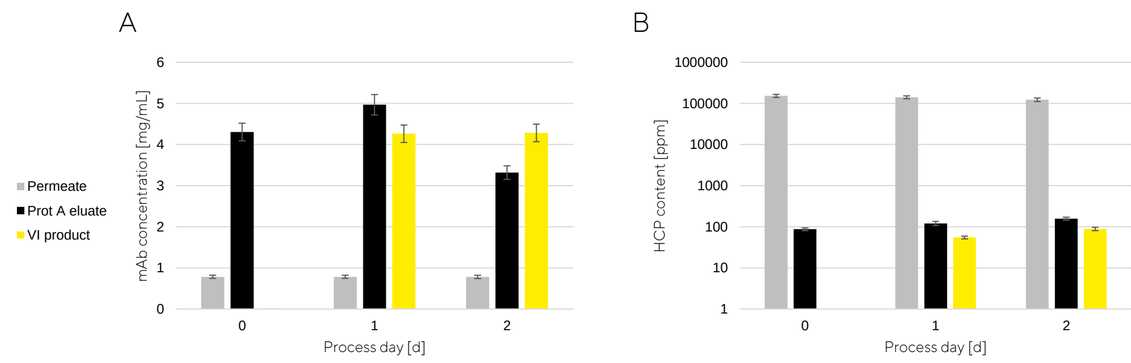

Figure 5: Analytical results of the mAb concentration **(A)** as well as the HCP content **(B)** in the permeate, prot A eluate and VI product.

6. Conclusion

A robust continuous process was operated for several days, enabled by an overarching custom-made software component, with a high yield and purity of the mAb. The results of this study and the novel employed approaches demonstrate substantial potential for the establishment of adaptive continuous manufacturing of biopharmaceuticals.

- Dynamic, automated adjustment to process deviations of the USP (permeate flowrate) or within the DSP by advanced control strategies
- Robust maintenance of surge tank volumes between the different unit operations
- Highly specific and sensitive mAb breakthrough detection by the continuous BLI prototype
- Enhanced membrane utilization without significant product loss by the dynamic loading
- Potential extension of this technology to the chromatographic purification of other biological modalities, such as recombinant proteins or viruses

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