

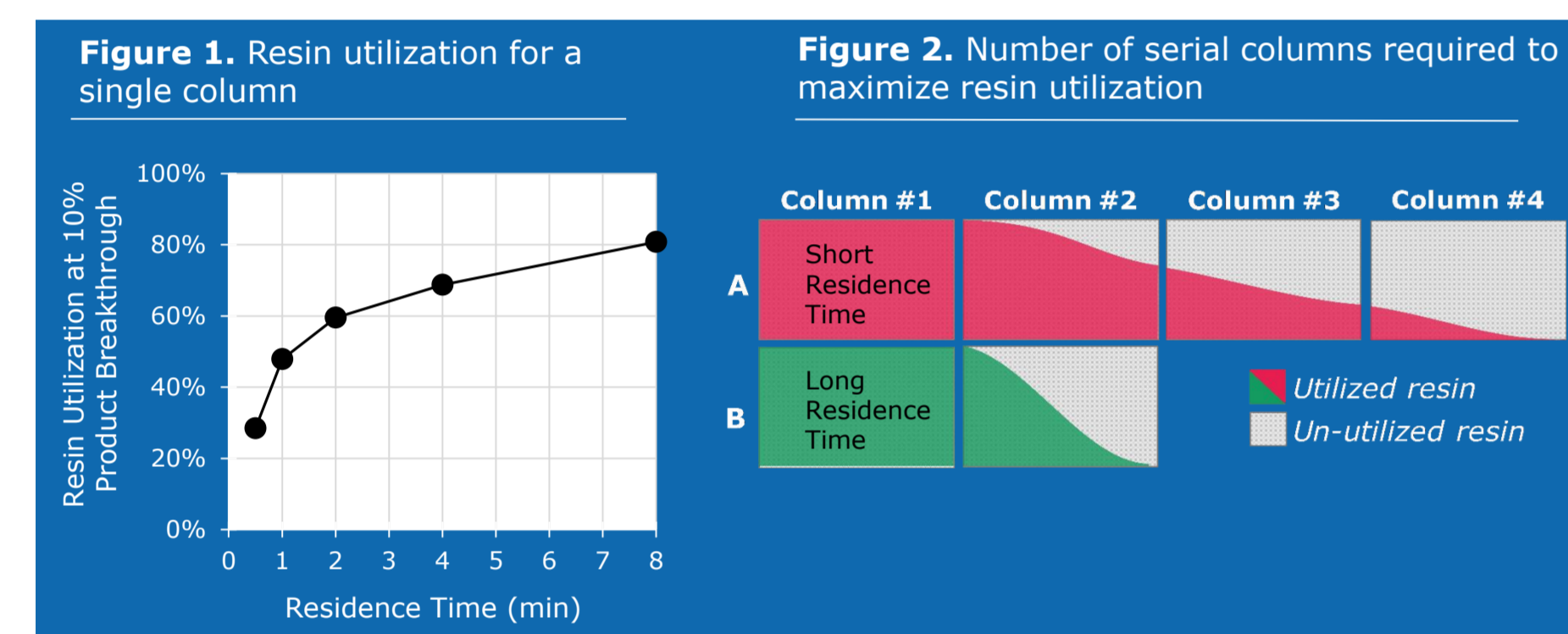
Inline concentration of mAb feed to increase the productivity of a continuous multi-column chromatography capture step

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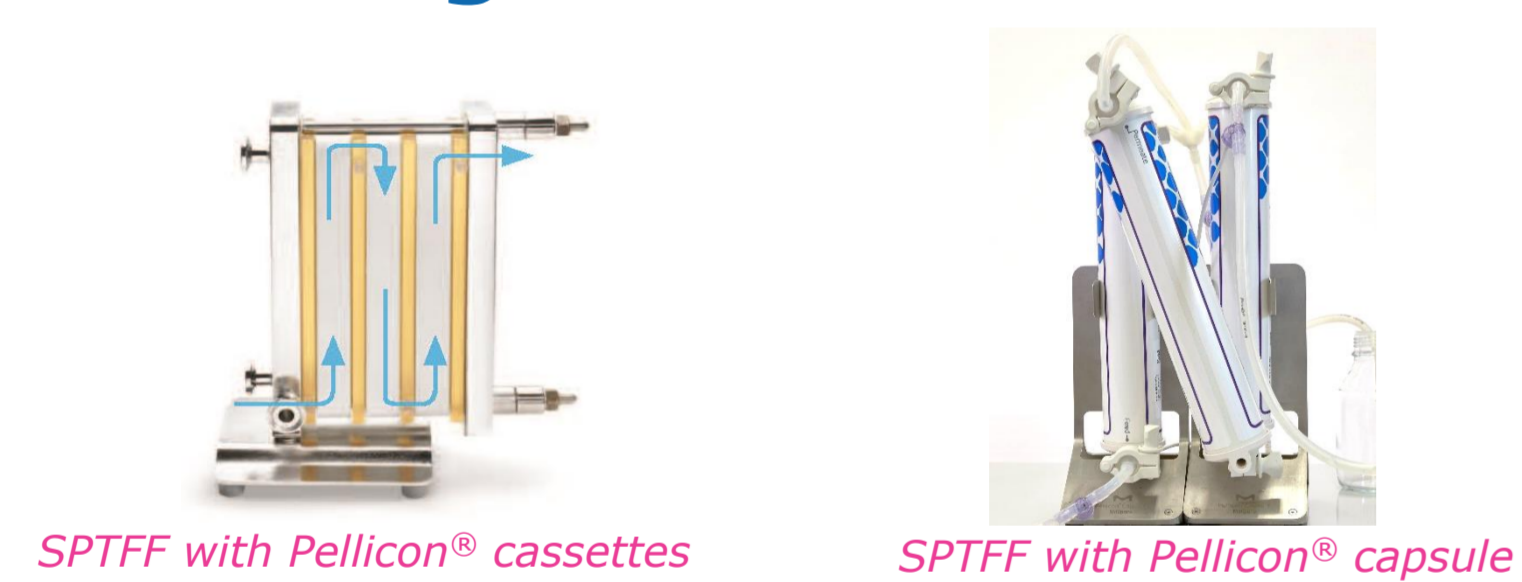
Introduction

In continuous bioprocessing, a perfusion bioreactor may be linked directly to a chromatography capture step. This connection requires the capture step processing rate to conform with the perfusion harvest rate. While a rapid bioreactor harvest rate maximizes production, it also reduces the chromatography step residence time and resin utilization (Figure 1). Multi-column chromatography systems overcome this challenge by loading 2-6 equally sized columns in series to maximize resin utilization at short residence times (Figure 2A)¹. However, a key drawback to this approach is that system complexity increases and resin productivity decreases with each additional column in rotation.

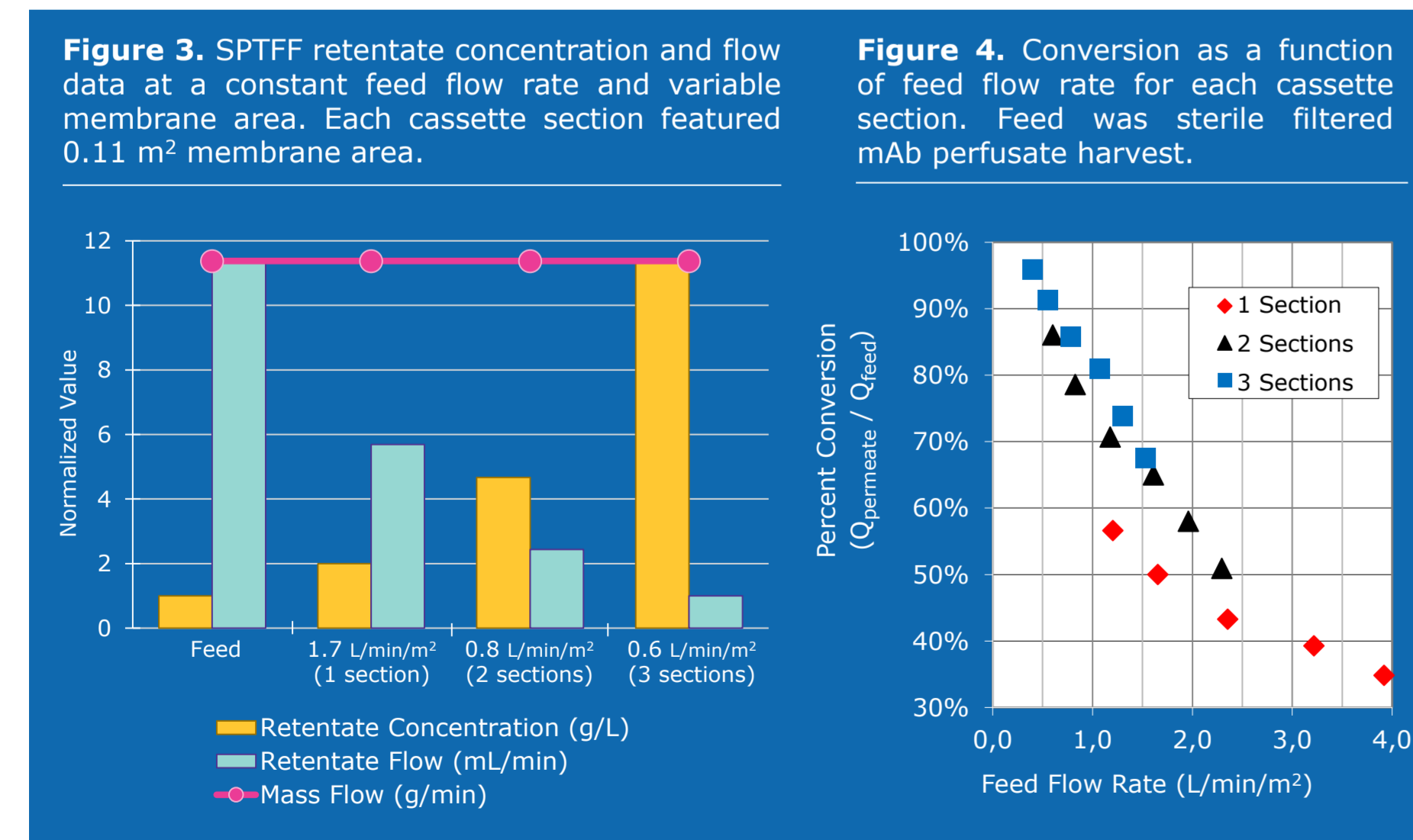
In this work, single-pass tangential flow filtration (SPTFF) is utilized for inline product concentration prior to a multi-column capture step. The volumetric flowrate reduction provided by SPTFF enables long column residence times without affecting product mass flowrate, thus reducing the required number of serial columns for a simpler and more productive process (as illustrated by Figure 2B).



Inline SPTFF concentration enables long column residence time while maintaining mass flow rate



SPTFF is a powerful tool for inline adjustment of product concentrations and volumetric flow rates. Because the retentate's concentration is inversely proportional to its volumetric flow rate, SPTFF does not affect the product mass flow rate (Figure 3). A three-section Pellicon® 3 Ultracel® 30 kD assembly provided >90% conversion for mAb23 perfusion harvest feed (Figure 4).

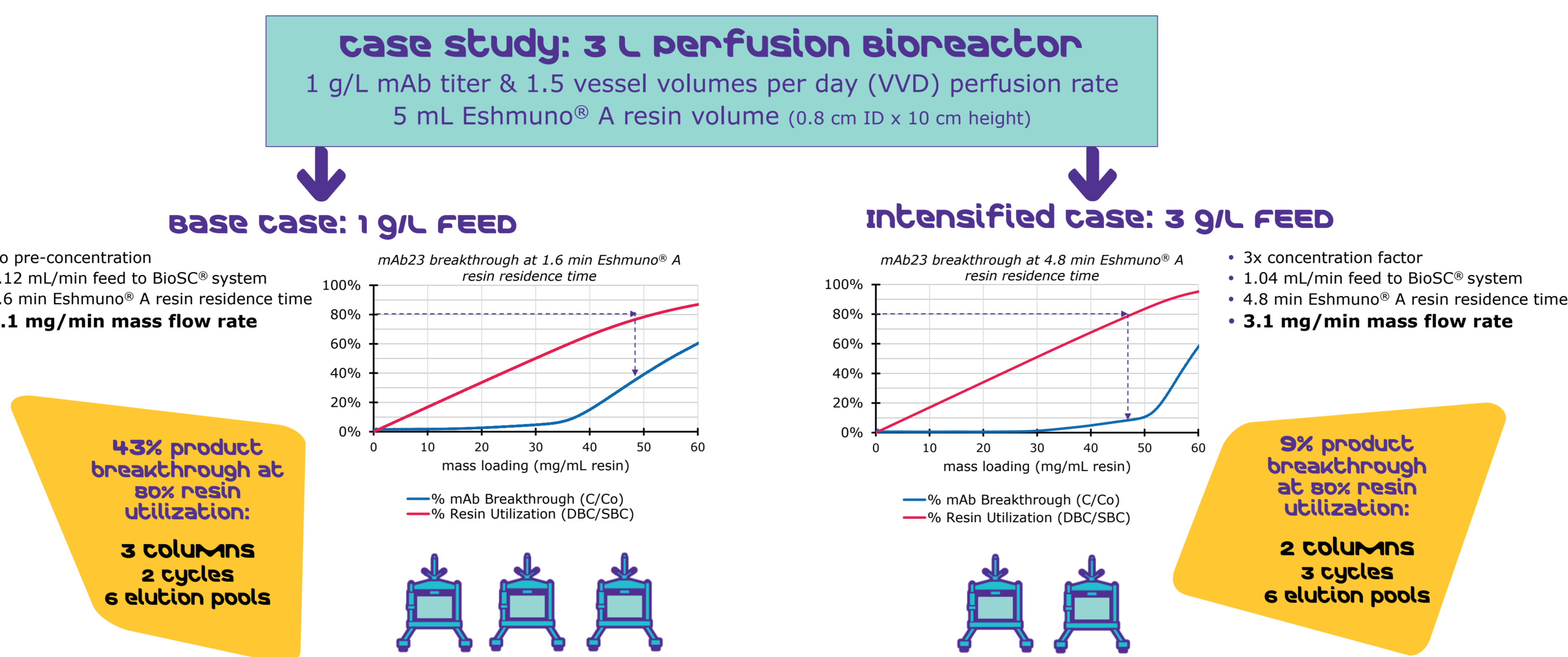


Case Study: Intensified multi-column capture with Eshmuno® A resin

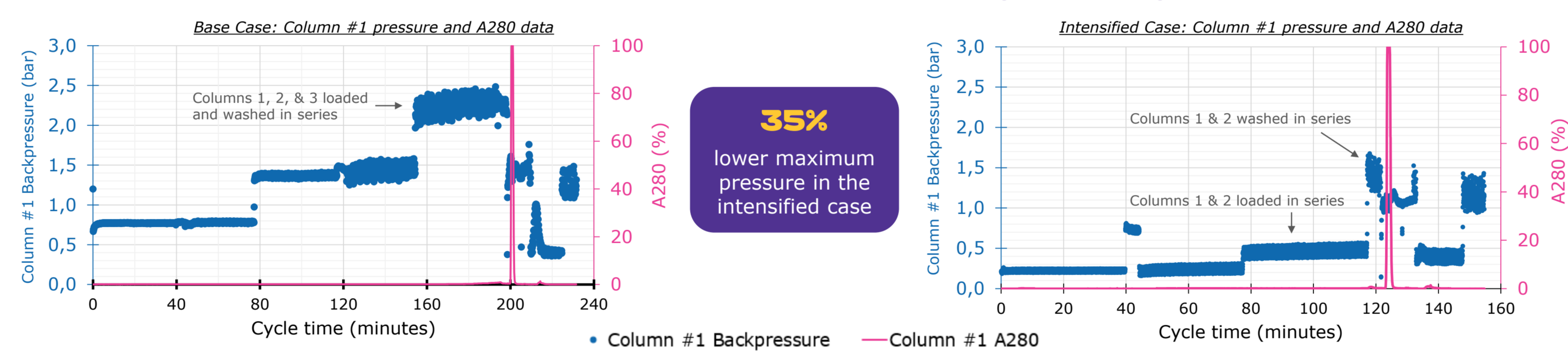
Feed material: mAb23 perfusate was pre-purified by Eshmuno® A chromatography resin to evaluate intensified multi-column capture feasibility. The Eshmuno® A resin eluate was diluted to either 1 g/L or 3 g/L to evaluate the effect of protein concentration on multi-column capture operation at constant mass flow rate.

Equipment: A NovaSep BioSC® system was used to define the column cycling strategy and execute multi-column tests.

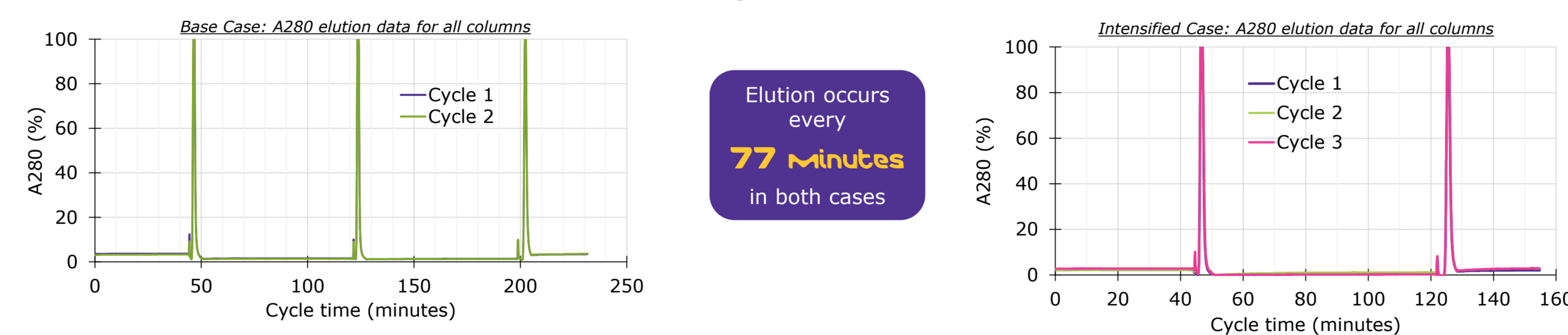
Purification criteria: Target resin utilization was 80%. Non-load steps (wash, elute, strip, CIP) required 38 minutes.



The intensified case uses fewer columns, offering lower system pressures:



Identical steady-state elution behavior:



Improved productivity:

base case		intensified case	
Eshmuno® A resin volume:	15 mL	Eshmuno® A resin volume:	10 mL
Loaded mAb mass:	1.9 g	Loaded mAb mass:	1.9 g
Eluate mAb mass:	1.8 g	Eluate mAb mass:	1.8 g
Steady state process time:	7.7 hr	Steady state process time:	7.7 hr
Total process time: <i>Includes time into and out of steady state</i>	13.4 hr	Total process time: <i>Includes time into and out of steady state</i>	12.2 hr
Productivity:	8.8 g/L/hr	Productivity:	14.3 g/L/hr

62% Resin productivity increase

Process Modeling Data

BioSC® Predict software was used to model the impact of SPTFF feed concentration on multi-column capture step productivity and column requirements (Figure 5). Fixed parameters were perfusion bioreactor volume (10 L) and column diameter (1.5 cm). Variable parameters were bioreactor titer, SPTFF concentration factor, perfusion rate, and column length. The model shows that SPTFF concentration improved productivity for all cases.

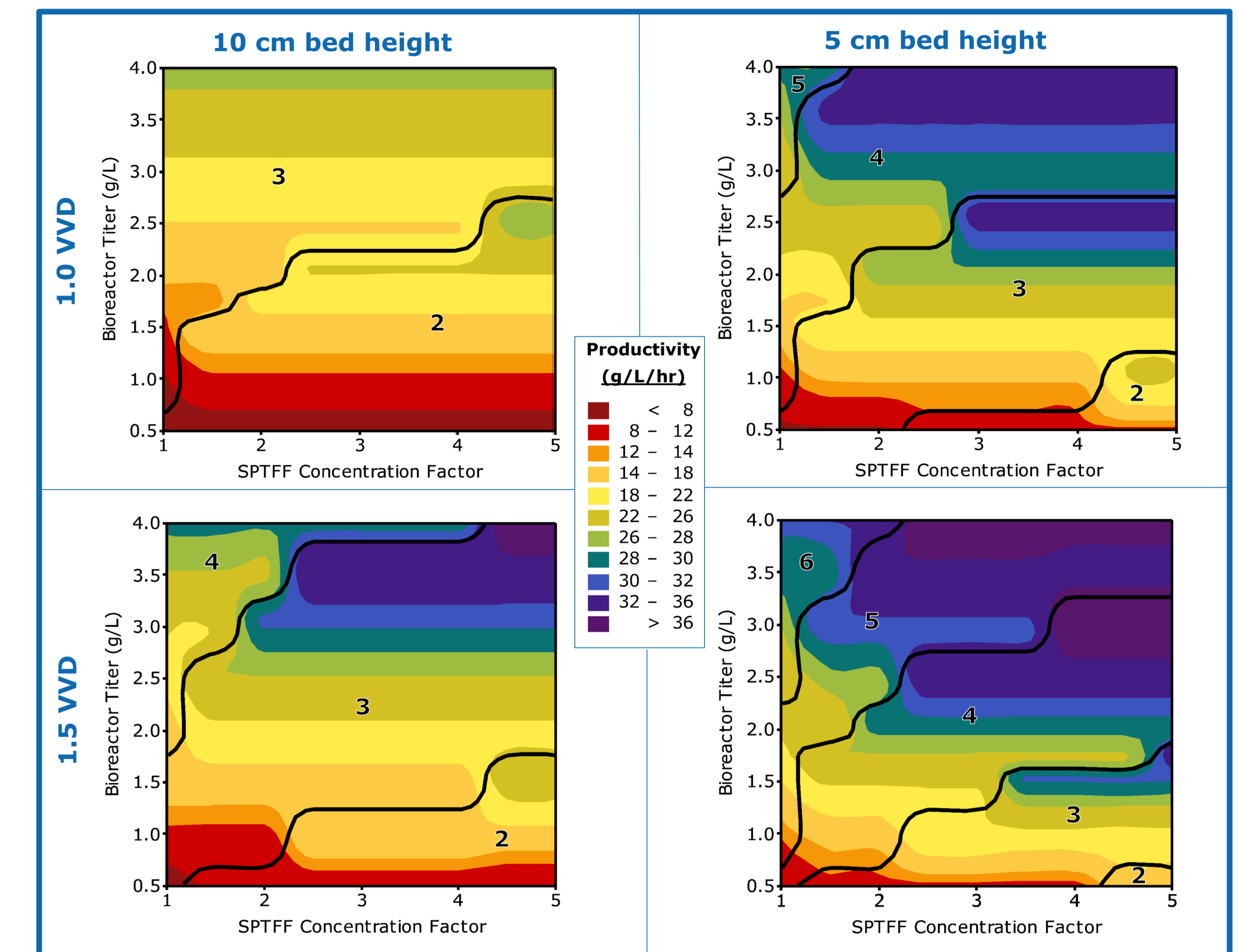


Figure 5. Process modeling data. Numbers within contour plot indicate the amount of columns required for 80% resin utilization.

Summary

Multi-column bind & elute chromatography can be intensified by including inline SPTFF concentration upstream of the capture step. Case study data show that the intensified approach reduced the number of columns required for cycling, resulting in the following benefits:

- **Less resin to process the same mass of mAb in the same time:** 33% reduction in resin capital cost contributes to a 62% increase in resin productivity.
- **Reduce system complexity:** Fewer columns require less valves for a simpler multi-column capture step.
- **Shrink equipment requirements:** 35% reduction in system pressure and 66% reduction in load volumetric flowrate allow more to be done with smaller system pumps.

References:
1. Mihlbachler, K. "Chapter 12: Multicolumn continuous chromatography". *Continuous Biomanufacturing, Innovative Technologies and Methods*. Ed. Ganapathy Subramanian. Weinheim: Wiley-VCH, 2018. 323-368.