APPLICATION NOTE

Testing of GEA kytero® single-use pharma separator at ProBioGen AG in perfusion mode

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Short Abstract:

The GEA kytero® 500 single-use disk stack separator was successfully implemented in a 15-day, 100 L perfusion process using a CHO cell line producing a monoclonal IgG1 antibody. The system enabled reliable and continuous cell retention and harvest of clarified, product-containing media, while maintaining high cell viability and stable antibody productivity throughout the run. The GEA kytero® 500 proved to be a robust and efficient solution for long-term perfusion, contributing to a stable and productive biomanufacturing process without risk of cell retention filter clogging. Furthermore, the newly released GEA kytero 10®, the world's smallest single-use disk-stack centrifuge, was tested for its perfusion performance.

About GEA

GEA has been instrumental in advancing mechanical separation technologies optimizing downstream production in areas such as human blood proteins, vaccination, mammalian cell culture, enzymes and hormones. Centrifugal separation plays a key role in the recovery of active pharmaceutical ingredients (APIs).

GEA enhances the efficiency and sustainability of production processes in line with the company's purpose: "Engineering for a better world".

About ProBioGen

ProBioGen is a globally recognized and experienced Contract Development & Manufacturing Organization (CDMO) and technology provider offering **INTELLIGENT SOLUTIONS** for **BIOPHARMACEUTICALS**.

Our value proposition is based on our five values: Innovation, Passion, Partnership, Solutions and Fearless. That means for you, a reliable and honest cooperation at eye level and that through: Respect. Transparency. Trust.

1 Content

2 Introduction

Perfusion cell culture has gained increasing attention in biopharmaceutical manufacturing as a powerful strategy for the production of complex biologics, including monoclonal antibodies (mAbs). Unlike traditional fed-batch processes, where nutrients are supplied intermittently and the product is harvested only at the end of the cultivation, perfusion enables the continuous addition of fresh media and simultaneous removal of spent media and product.

This approach enables a stable culture environment, supports higher cell densities and prolonged culture durations, which can lead to significantly higher overall product yields and improved process consistency [1]. Perfusion is particularly attractive for processes involving unstable or difficult-to-produce molecules, as the continuous removal of product from the bioreactor can help reduce product degradation and improve product quality [2]. Additionally, the constant culture environment created by steady-state conditions often results in greater cell viability.

Advancements in cell retention technologies have further expanded the applicability of perfusion by offering robust, scalable, and user-friendly solutions. The systems reduce shear stress on cells, minimize cleaning requirements, and support flexible manufacturing models. These characteristics are important key advantages in an industry increasingly focused on efficiency, speed, and adaptability. Among the most prominent solutions for perfusion are Alternating Tangential Flow (ATF) systems, retention filters (used in rocking bioreactors), and centrifugal-based cell separators. [1]

ATF systems rely on membrane-based separation, where the culture fluid flows tangentially across a filter surface. A diaphragm pump alternates flow direction across the hollow fiber membrane to reduce membrane fouling. The pore size of the membranes used in ATF systems is usually between 0.2 µm and 0.5 µm, depending on the application. For mammalian cell cultures, a pore size of 0.2 µm is commonly used to retain cells while allowing proteins, nutrients, and metabolic by-products to pass through freely. ATF is well-known for its scalability, ability to support high cell densities, and gentle handling of cells. However, it can be sensitive to fouling over time and often requires more complex system integration. [3]

In rocking bioreactor systems, cell retention filters are usually integrated into the disposable bag and positioned near the bottom or side. These filters enable continuous or semi-continuous perfusion by allowing cell-free supernatant to be harvested while retaining viable cells within the bioreactor. The nominal pore size of such integrated filters in rocking bioreactors is 7 µm. Retention filters offer a simple, compact, and user-friendly solution, especially for small-scale and single-use applications. They are easy to operate and gentle on cells. Therefore, they are mainly used for the N-1 seed train step. On the downside, they face limitations in scalability and long-term performance, particularly as cell density increases and the risk of clogging rises.

Centrifugal-based cell separators operate continuously by using density differences to separate cells from the medium. These systems are particularly attractive for large-scale manufacturing because they avoid membrane fouling and can be run continuously. Nonetheless, they may subject cells to higher shear stress and often involve more complex operation and maintenance. However, the market launch of single-use separators such as the GEA kytero® has eliminated these disadvantages, as they achieve excellent separation using disk-stack technology with significantly lower centrifugation rates and are maintenance-free thanks to modern drive technologies.

Each of these technologies has distinct advantages and limitations. The choice of cell retention method depends on various factors including process scale, cell line characteristics, desired throughput, and regulatory considerations.

A collaborative project between GEA Westfalia Separator Group GmbH and the Upstream Processing Department of the ProBioGen AG was agreed upon to evaluate the GEA kytero® 500 perfusion performance. In this context, a 15-day, 100 L perfusion run was performed using a CHO cell line producing a monoclonal IgG1 antibody, with the GEA kytero® 500 single-use separator serving as the cell retention device. The following sections describe the implementation, performance, and outcomes of this process, demonstrating the potential of modern perfusion systems in meeting the demands of next-generation biomanufacturing.

To evaluate scalability and system performance under comparable conditions, parallel small-scale perfusion experiments were conducted using the same CHO cell line in two different 1 L bioreactor setups: one equipped with an Alternating Tangential Flow (ATF) system, and the other using a perfusion-ready rocking bioreactor with an integrated cell retention filter. An additional perfusion process was initiated using a 10 L bioreactor with the GEA kytero® 10 separator, which is the smallest single-use disk-stack centrifuge launched in 2025.

3 Materials and methods

3.1 Experimental setup

Four perfusion processes were performed using three different perfusion techniques mentioned above. The objective was to evaluate and compare cell retention strategies under standardized conditions (Figure 3-1). The evaluated cell retention devices included the GEA kytero® single-use separator for 100L (kytero® 500) and 10L (kytero® 10) scale, the ATF 2 (Alternating Tangential Flow) for 1L scale culture and a 1L rocking bioreactor system with integrated cell retention filter.

Each perfusion process followed the same general workflow. Perfusion medium was continuously supplied to the bioreactor to maintain optimal nutrient levels and support high cell densities. Simultaneously, cell-free harvest stream was withdrawn through a cell retention device, which allowed clarified, product-containing medium to be collected while cells were retained and recirculated back into the reactor. Two complementary strategies were employed: dynamic perfusion driven by a target cell-specific perfusion rate (CSPR) to support biomass expansion during the ramp-up phase, and then steady state perfusion, where biomass was maintained either naturally through the imposed dilution rate or through targeted cell bleed when required.

With regards to cell bleed a small portion of the culture is regularly removed to avoid excessive density that may impair viability or productivity. The rate of bleed is determined based on viable cell density (VCD) measurements. Although bleed can be applied with any perfusion system, it is typically more critical for membrane-based retention devices such as ATF, which maintain very high cell-retention efficiencies and therefore provide fewer inherent mechanisms for passive biomass removal. The kytero® centrifugation-based system on the other hand offers distinct advantages when bleed is needed: by tuning sedimentation behaviour, biomass can be controlled with reduced reliance on dedicated bleed streams, thereby minimizing product loss.

In the present study, cultures were primarily operated using a dilution-rate-driven strategy, avoiding the use of bleeding and allowing the culture to stabilize naturally at the cell density at which the growth rate matched the imposed dilution rate. This strategy proves particularly effective when implemented with the kytero® system. In this setup, cell debris is efficiently removed from the culture whereas in other systems with small pore sizes, such debris often accumulates and remains within the culture vessel. The enhanced removal of cellular waste in kytero®-based processes contributes to a more stable culture environment and supports robust cell growth and productivity.

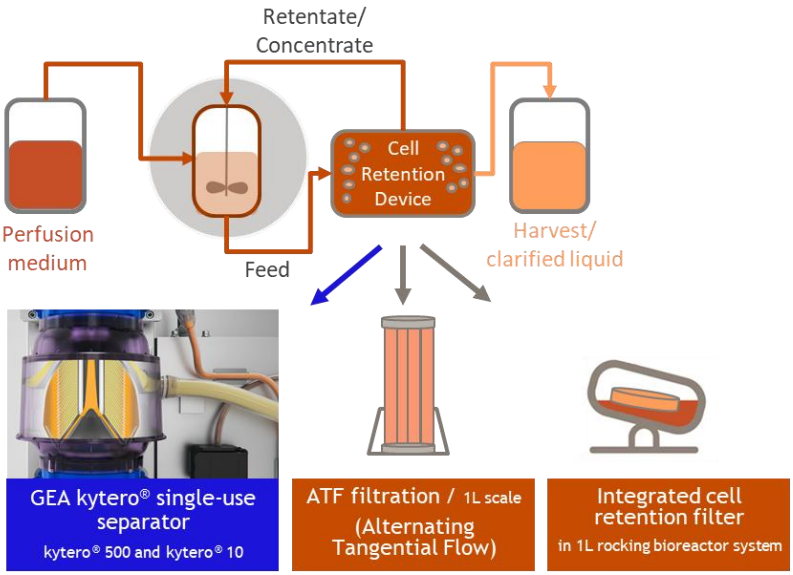

Figure 3-1: Schematic perfusion experimental setup

3.2 Process parameters

To ensure the highest possible comparability between processes, the following target values were defined for the perfusion process. These general parameters are summarized in Table 3-1.

Table 3-1: Target Process Parameter

General parameter	Target value
Process mode	Perfusion
Product type	IgG1
Process duration [days]	≥ 14
Seed viable cell density (VCD) day 0 [cells/mL]	≥ 2.0 E6
Temperature Shift to 34°C at VCD [cells/mL]	≥ 60 E6
Target VCD [cells/mL]	≈ 100 E6
Target viability [%]	≥ 85
Expected perfusion rate [vvd]	1

All processes were performed using a proprietary perfusion medium designed and manufactured by ProBioGen.

3.3 Perfusion setup

3.3.1 GEA kytero®

An overview of the GEA kytero® 500 pharma cell separator is given in Figure 3-2 and the main technical parameters are summarized in Table 3-2.

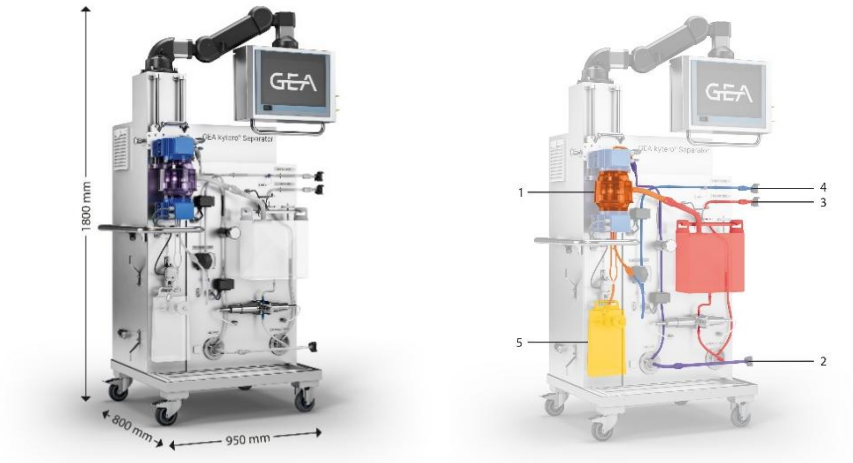

Figure 3-2: GEA kytero® 500 picture and schematic layout: (1) Separating unit (bowl); (2) Cell suspension feed line; (3) effluent line of clear phase to harvest bulk; (4) effluent line of solid phase / cell mass; (5) post production drain bag.

The GEA kytero® cell separator relies on GEA's disk stack technology with a proven track record for safe, reliable and efficient biopharma manufacturing processes during the last decade. The compact and movable skid comes with fully integrated turbidity and flow meters, pumps, tubes, connections, control module and is batch ready in 5 minutes. The patented breezeDrive® is the only drive system without bearing or mechanical seals – the failsafe system to prevent impurities in the product.

Table 3-2: GEA kytero® 10 and GEA kytero® 500 main technical parameters

Instrument parameter	GEA kytero® 10	GEA kytero® 500
Dimensions (LxWxH) [mm]	450 x 200 x 575	950 × 800 × 1.800
Expected flow rate [L/h]	2 - 6	50 - 150
Weight (net) [kg]	15	280
Power demand	100 V/50 Hz or 240 V/60 Hz	110 V/50 Hz or 230 V/60 Hz
Material of kytero® single-use cartridge (housing and bowl)	Somos Bioclear, BASF Ultracur 3D RG35	Polycarbonate
Material of kytero® single-use path	C-Flex	Silicon, TPE, PE, PP, PSU, PPSU, PC, C-Flex
Integrated sensors	No integrated sensors	Pressure, feed flow rate, turbidity, weight
Centrifugation rate technical range [rpm]	5200 - 13000	2000 - 7500
Used centrifugation rate for CHO perfusion setup [rpm]	6500	3000

GEA kytero® is available in three sizes for the processing of 10L to 2000L. The smallest model, kytero® 10, is optimized for laboratory-scale applications, supporting working volumes between 1 and 10 liters. It is ideal for research and development settings, including small-scale harvest and perfusion studies. The intermediate model, kytero® 500, is designed for pilot and clinical-scale production, capable of handling up to 500 liters. It enables seamless scale-up and is well-suited for early GMP manufacturing and process optimization efforts. At the largest scale, the kytero 2000 is intended for larger clinical-scale or commercial GMP biomanufacturing, with the capacity to process up to 2000 liters of high-density cell culture. The kytero® 500 and 2000 are designed to operate as part of a fully integrated perfusion platform in continuous bioprocessing environments.

Figure 3-3: GEA kytero® 10 to 2000

3.4 Equipment & methods

Table 3-3 summarizes the main equipment including the application and methods.

Table 3-3: Equipment list

Equipment	Type/ Manufacturer	Use	Method
Cell counter	ViCell XR™/ Beckman Coulter	Counting of cells, measurement of viability	automated trypan blue staining procedure Reported parameters: Viability, Total and Viable Cell Density
Biochemical analyzer	BioProfile® Flex2/ Nova Biomedical	Measurement of nutrients, metabolites, gases	MicroSensor™ Card technology with optical measurement and freezing point osmometry Reported parameters: Lactate, pH
Protein A-measurement device	Octet K2 / Sartorius Stedim Biotech	Measurement of antibody concentration	Based on Bio-Layer Interferometry (BLI), real-time, label-free analysis for the determination of kinetics, affinity and antibody/protein quantitation Reported parameter: Titer
Centrifuge	5810/ Eppendorf	Centrifugation of cells for Wet Cell weight determination	Setpoint of 2000g/min for 10min
Single-use stirred-tank bioreactor	XDR-200/ Cytiva with control unit from ISE	Production bioreactor	15 days perfusion process using the kytero® 500
	XDR-10/ Cytiva with control unit from ISE		8 days perfusion process using the kytero® 10
Rocking bioreactor	WAVE25/Cytiva		17 days perfusion process using the integrated cell retention filter
1L bioreactor	DASGIP®/Eppendorf		18 days perfusion process using the AFT 2
ATF Cell retention system	XCell® ATF 2/ Repligen	Perfusion system enables cell retention	

4 Results and discussion

4.1 GEA kytero® perfusion performance evaluation

The GEA Kytero® 500 perfusion process was carried out successfully over the entire cultivation period, operating stably and without any technical complications. The study confirmed the feasibility of implementing centrifugation-based perfusion at this scale. The process was terminated intentionally, due to scale-related constraints rather than technical limitations. During the 15-day operation, approximately 1.500 L of culture medium were processed.

Additionally, the GEA Kytero® 10 system was tested in a perfusion process using a 10 L bioreactor. Here, unfortunately, due to a technical issue the process had to be stopped on day 9, which is why only data up to day 8 is shown.

The performance of the 100L (red) and 10L (purple) perfusion processes is shown Figure 4-1. Graph A presents the Viable Cell Density (VCD), expressed in millions of cells per milliliter, while Graph B shows the corresponding cell viability in percentage. The data provides insights into the dynamics of cell proliferation during the exponential growth phase, potential stress events or nutrient limitations indicated by a drop in VCD, and the gradual decline in viability over time, thereby giving a comprehensive overview of the culture's performance and stability throughout the process.

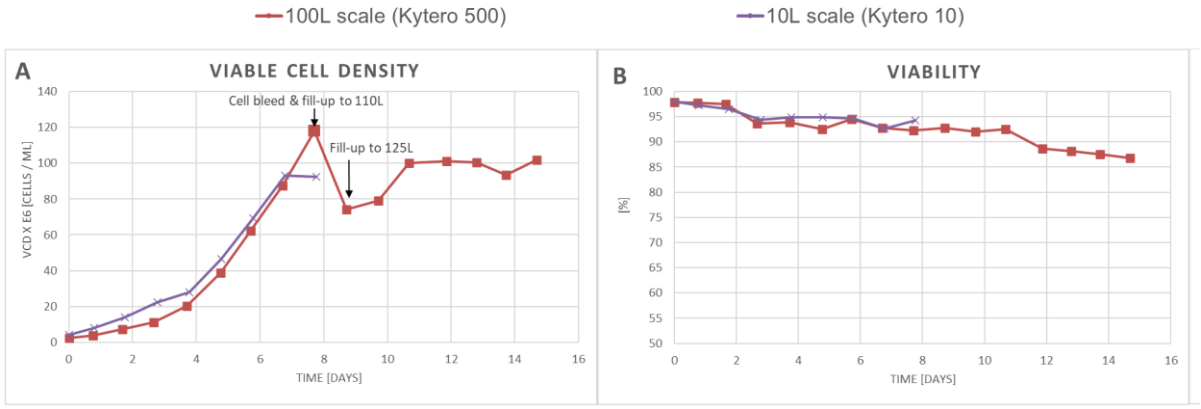

Figure 4-1: Viable Cell Density (VCD) and Viability of 100L perfusion process using the GEA kytero® 500 and 10L process using GEA kytero® 10.

The GEA kytero® 500 process demonstrates an initial exponential growth phase up to day 7, reaching a peak VCD of approximately 120 E6 cells/mL on day 8. This growth phase was followed by a sudden drop in cell density on day 9, to around 75 E6 cells/mL due to a performed cell bleed and gradual increase of bioreactor volume to 110L on day 8 and to 125L on day 9. These measures were taken to limit cell mass build-up and improve overall execution. From day 9 onwards, perfusion was maintained at a fixed vvd rate, with no additional bleeds performed, thereby allowing the cells to recover and stabilize between 90 and 100 E6 cells/mL for the remaining cultivation time. The kytero® 10 process also reached the target VCD of approximately 100 E6 cells/mL by day 7, however, with the first technical issues occurring on day 8, VCD then stagnates before the process was then discontinued.

Cell viability of both processes remained high (>90%) during the initial phase but showed a slight decline over time. A more noticeable drop occurred after day 10 during the 100L perfusion process, with viability falling below 90% and reaching approximately 85% by day 15. However, this progression can be explained by the dilution-rate–driven perfusion strategy, as well as a reduction in the perfusion rate from 1.0 vvd to 0.8 vvd between day 8 and 10 (see Figure 4-2). The effective CSPR was around 0.008 nL/(cell*day) which represents the lower limit of cell supply resulting in a gradual decline in viability.

WCW measurements from the 10L bioreactor show a steady increase from approximately 3% on day 2, to nearly 20% at day 8. This upward trend is coherent with progressive cell growth in the smaller-scale system during the early phase of the process. WCW levels for the 100L bioreactor also started around 3% on day 2, steadily rising and surpassing 25% by day 15. The steady increase of WCW in both systems reflects successful cell retention during perfusion, which is crucial for maintaining high cell densities and optimizing productivity in bioprocessing. The extended data range for kytero® 500 suggests stable operation over a longer duration, highlighting its suitability for larger-scale or longer-term perfusion processes. According to the technical specification, the kytero® 500 and 2000 single-use perfusion set is even suitable perfusion processes of up to 60 days.

In summary, the data emphasizes the scalability of the kytero® centrifuge platform, where both small and mid-sized units effectively concentrate cells over time.

The following settings were selected for the GEA kytero® 10 and GEA kytero® 500 in order to perform the perfusion process at the respective scale.

Table 4-1: Perfusion process settings GEA kytero 500 and GEA kytero 10

Process control	GEA kytero® 10	GEA kytero® 500
Centrifugation rate [rpm]	6500	3000
G-force [g]	940	600
Average circulation flow rate [L/h]	2.0	43.0
Average perfusion medium flow rate [L/h]	0.3	4.7

To assess the efficiency and stability of the GEA kytero® 500 perfusion system, key process parameters such as the Cell-Specific Perfusion Rate (CSPR) and vessel volumes per day (vvd) were monitored over time, as shown in Figure 4-2 below.

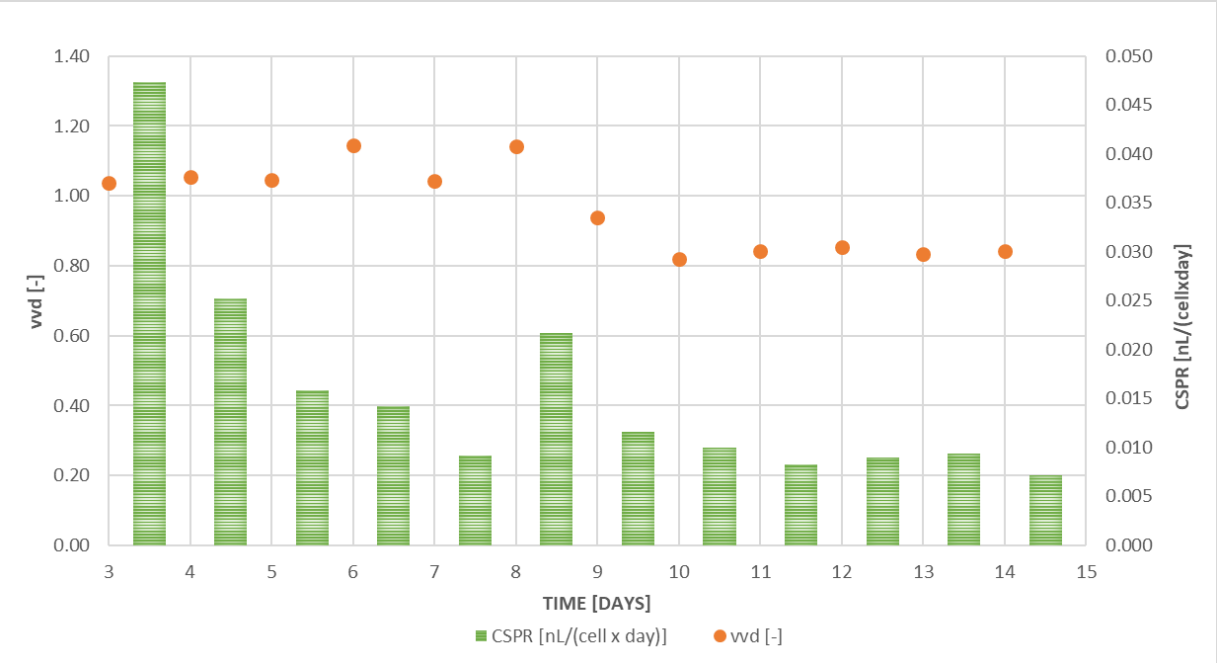

Figure 4-2: Cell-specific perfusion rate (CSPR) and vvd during 15-day perfusion process using the kytero® 500.

Perfusion was initiated on day 2, marking the beginning of the initialization phase, during which process settings were optimized to identify appropriate operating conditions. This is reflected in the elevated CSPR on day 3, reaching approximately 0.45 nL/(cell·day), followed by a decline as the system adjusted to stable operational parameters and VCD increased. From day 3 onwards, CSPR decreased

progressively and stabilized at around 0.01 nL/(cell·day), indicating improved operational robustness. This is in accordance with increasing VCD levels at a fixed perfusion rate (vvd).

The effective vvd remained relatively stable and close to 1.0 until day 8. On day 8, a cell bleed was performed in combination with a gradual increase in reactor volume from 100 L to 125 L until day 9. This change in working volume explains the drop in vvd to approximately 0.80. From day 9 onwards the vvd remained stable around 0.85 which corresponds to an effective CSPR of 0.008 nL/(cell*day), which represents the lower limit of a sufficient cell supply for maintaining a steady state. Overall, the data demonstrates a rapid stabilization of perfusion control following the initialization phase, effective adaptation of the bioprocess and consistent vvd and CSPR throughout the main production phase.

During the centrifugation with the GEA kytero® 500 the online values for turbidity, feed and retentate flow rate were monitored and are shown in Figure 4-3 .

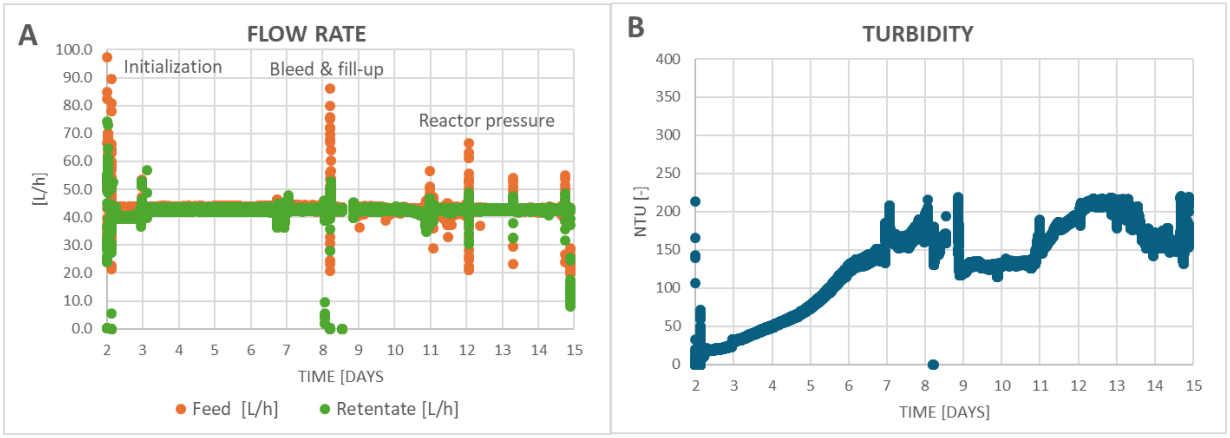

Figure 4-3: (A) Flow rates and (B) turbidity of harvest line post kytero 500®.

Graph A illustrates the volumetric flow rates of feed (orange) and retentate (green). During the initialization phase (days 2-3), the flow rates exhibit variability, initially reaching values close to 90 L/h. Following initialization phase, the flow rates remain stable at around 30 to 40 L/h. Between days 8 and 9, the system undergoes a bleed and fill-up operation, reflected by noticeable fluctuations in both feed and retentate flow rates. After day 8, during the steady-state phase, feed and retentate flow rates stabilize around 40–50 L/h, indicating balanced process operation. Several transient spikes are observed between days 11 and 14, related to reactor pressure adjustment issues. The average circulation flow rate was 43.0 L/h and the average perfusion medium flow, which also shows the Δ between retentate and harvest line, was 4.7 L/h.

Graph B shows the turbidity, measured in NTU. Starting from near-zero values on day 2, turbidity rises continuously during the initial days, reaching approximately 150 NTU by day 6, indicative of increasing biomass in the system. During the bleed and fill-up phase around day 8, a transient decrease in turbidity is observed, consistent with dilution effects from medium replacement and cell removal. Following this, turbidity stabilizes between 150 and 250 NTU, with some fluctuations, reflecting steady cell density maintained in the bioreactor.

The correlation between stable turbidity and flow rates during steady state suggests effective cell retention and consistent biomass concentration, which are essential parameters for successful perfusion culture.

4.2 Comparison of cell retention systems

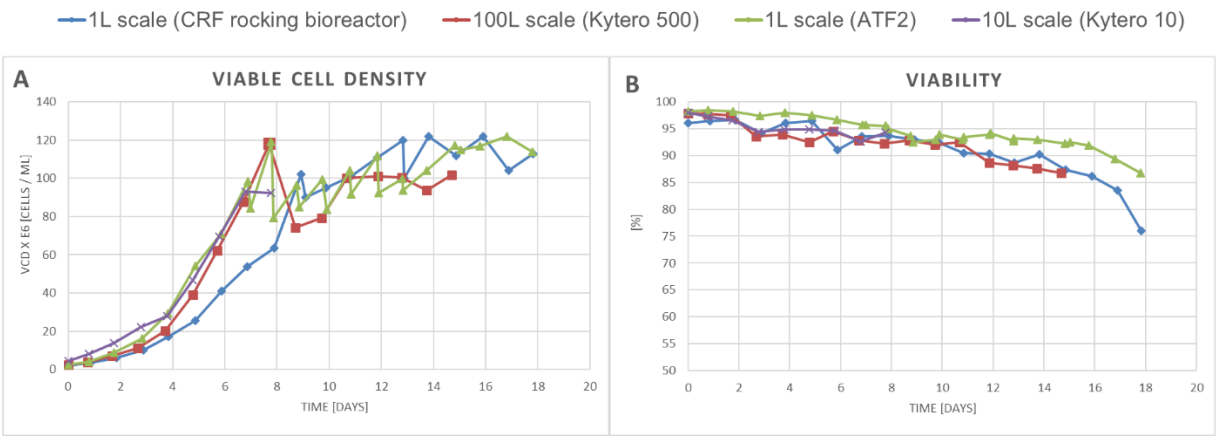

Figure 4-4: Viable Cell Density (VCD) and Viability of 100L perfusion process using the GEA kytero® 500, 10L process using GEA kytero® 10, 1L process using rocking bioreactor with integrated cell retention filter and 1L process using ATF 2 system.

The graphs compare cell culture performance across different bioreactor scales and perfusion systems in terms of viable cell density and viability over time for four setups. All processes demonstrate an initial exponential growth phase up to around day 7, reaching peak or near-peak values thereafter. The 1L ATF 2, kytero® 10, and kytero® 500 processes reached the target VCD (>90 × 10^6 cells/mL) between day 7 and day 9 and maintained this level throughout the cultivation. In addition, for the 1L ATF 2 culture, daily bleeds were performed between day 7 until day 12 to control biomass and ensure better nutrient supply. The 1L CRF rocking bioreactor showed a delayed VCD increase likely due to another medium formulation, but eventually also reached the 90 × 10^6 cells/mL target around day 11–12. The 100L kytero® 500, 1L ATF and 1L rocking bioreactor processes show stable and sustained VCDs around or above 100 × 10^6 cells/mL.

Cell viability over time shows a general downward trend across all scales without significant differences in performance. This is a known behavior of self-stabilizing steady state perfusion setups. 1L ATF process maintains viability consistently above 90% throughout the process, well above the 85% target, indicating better nutrition supply of cells due to higher perfusion rates (see Figure 4-5) and biomass control (bleed). Kytero® 500 (100L) process, 1L CRF rocking bioreactor and kytero® 10 (10L) stay close to or slightly below 90% but remain above 85%. The 1L CRF rocking bioreactor, however, experiences a sharp drop in viability after day 14, falling below 85% by the end of the process due to a clogged cell retention filter.

The following graph illustrates the perfusion rates over a 15-day period for the three different bioreactor setups. The perfusion rate is expressed in vessel volumes per day (vvd).

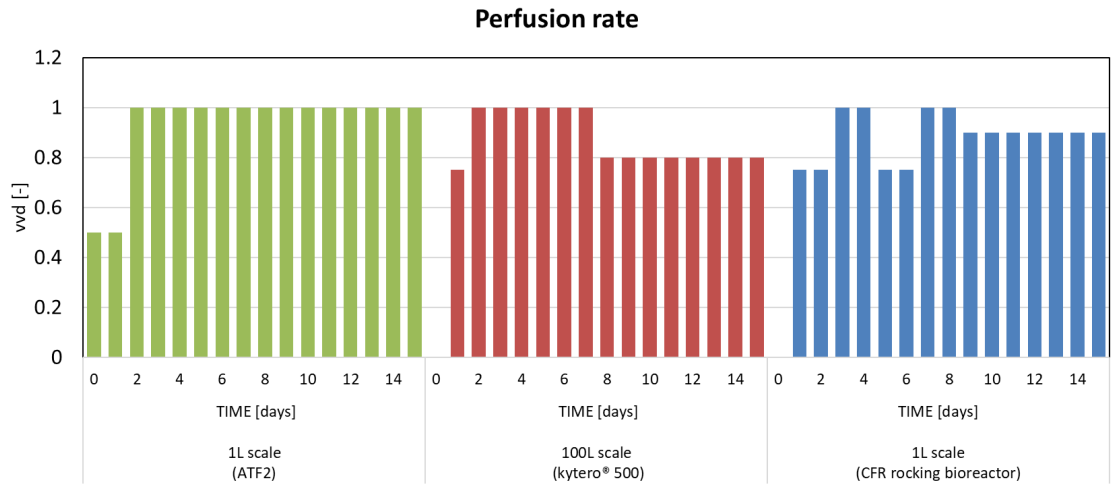

Figure 4-5: Perfusion rate of 100L perfusion process using the GEA kytero® 500, 1L process using rocking bioreactor with integrated cell retention filter and 1L process using ATF 2 system.

In the 1L scale ATF 2 system, the perfusion rate starts at 0.5 vvd on day 0 and was rapidly increased to a stable rate of 1.0 vvd from day 2 onward, maintaining this constant rate throughout the process duration. For the 100L scale kytero® 500 system, perfusion started on day 1 with 0.75 vvd and increased to 1.0 vvd on day 2. After cell bleed and fill-up steps the perfusion rate was decreased stabilizing around 0.8 vvd until the end of the process. For the 1L scale CRF bioreactor perfusion rates started at 0.75 vvd on day 1 and increased to 1.0 vvd on day 3. However, due to a difference in medium formulation, the perfusion rate was decreased again to 0.75vvd for 2 days, to avoid overfeeding the cells. Towards the end of the process, the perfusion rate was adjusted to 0.80 vvd to reflect the perfusion rates operated in the GEA kytero® 500 process.

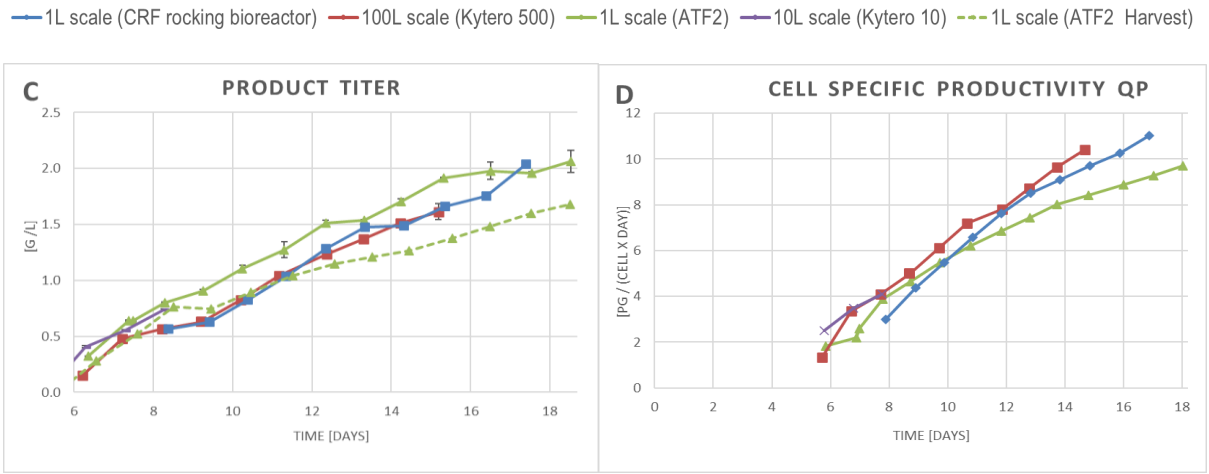

Figure 4-6: Product titer in bioreactor and cell specific productivity of 100L perfusion process using the GEA kytero® 500, 10L process using GEA kytero® 10, 1L process using rocking bioreactor with integrated cell retention filter and 1L process using ATF 2 system. For 1L process ATF 2, harvest titer is also shown (dotted green)

Overall, processes demonstrate a steady increase in bioreactor product titer over time, reaching concentrations between approximately 1.7 and 1.9 g/L by day 15. The 1L process using the ATF (green) shows the highest product titers throughout the duration, reaching approximately 2.0 g/L by day 18. The 100L scale with kytero® 500 (red) and the 1L rocking bioreactor with CRF (blue) demonstrate overall, consistent productivity, with overlapping titers during the entire cultivation. The 1L rocking bioreactor with CRF extended to 18 days, achieving the highest final titer due to filter clogging on day 17. In addition to the process titers within the bioreactor, the titer in the harvest of the 1L scale ATF system is shown (dotted green line). From day 8 onwards, the harvest titer trend runs lower than the

bioreactor trend, indicating that the product is retained by the ATF membrane and accumulates within the bioreactor. This is a phenomenon known to occur with filter-based approaches and where centrifugation shows potential. Nonetheless, despite differences in scale and retention technology, the trends are comparable across all systems, indicating good scalability.

Table 4-2 summarizes the key process performance parameters for 100L scale kytero® 500, 1L scale ATF and 1L scale rocking bioreactor with integrated cell retention filter.

Table 4-2: Key Process Performance Parameter

Key Process Performance Parameter	100L scale Kytero® 500	10L scale Kytero® 10	1L scale ATF	1L scale CRF rocking bioreactor
Bioreactor process volume [L]	100 - 125	8.0	0.8	1.0
Process duration [d]	15	8	18	17
Max. VCD [cells/mL]	118 E6	93 E6	122 E6	120 E6
Min. Viability [%]	87	93	87	84
Mean WCW of stationary phase [%]	20			
Average CSPR in growth phase without bleed [nL/(cell*day)]	0.030	0.029	0.018	0.023
vvd mean value in growth phase without bleed [-]	1.07	1.01	1.03	0.85
Total accumulated product of harvest day 7-15 [g/L]	9.3	n/a	10.1	9.4
Average CSPR in stationary phase without bleed [nL/(cell*day)]	0.008		0.008	0.008
vvd mean value in stationary phase without bleed [-]	0.84		0.96	0.89
Mean turbidity of harvest bulk post cell retention device [NTU]	150		20	40

All tested scales and systems demonstrated comparable process performance. VCD and final cell viability were very similar across all setups. Likewise, the harvested product quantity between days 7 and 15 showed only minor variations and was highly comparable. A stable cell-specific perfusion rate was established in all systems, regardless of scale or the cell retention technology applied. The 100L GEA kytero® 500 process demonstrated the highest level of stability and consistent performance throughout the entire production run. In contrast, both reference technologies displayed a noticeable decline in process stability during the final phase of the process. A distinct characteristic of the ATF process was the progressive accumulation of product within the bioreactor, as indicated by the titer profiles, suggesting an increasing degree of product retention over time. In comparison, the 1 L scale rocking bioreactor equipped with an integrated cell retention filter encountered filter fouling at the later stages of cultivation, indicating limitations in sustained filtration capacity and long-term process robustness. A notable difference was also observed in the turbidity of the harvest bulk, which was significantly higher compared to processes using ATF or the CRF of the rocking bioreactor. All systems and scales showed a very high level of comparability, confirming the robustness and scalability of the perfusion process across different setups.

4.3 Evaluation of complete perfusion setup

The performance of the perfusion processes across the different scales and cell retention technologies was highly comparable. Each system offers specific advantages and limitations, which are summarized in Table 4-3.

Table 4-3: Comparison of Cell Retention Technologies in Bioprocessing Applications.

	GEA kytero® single-use separator	**ATF filtration**	**Integrated cell retention filter** in rocking bioreactor
Flexibility (scale/flow rates)	High	High	Low
Filter clogging/ fouling risk	Low	Medium	High
Clarity post cell retention device	High	Low	Medium
Product loss by bleeding and final bioreactor harvest	Low	High	High
Dual-use perfusion/ cell separation	Yes	limited	No
Costs for single-use consumables	Medium	High	Low

Upon reviewing the comparison, the GEA Kytero® demonstrates the most favorable overall profile. Designed to address the limitations of membrane-based technologies, the kytero® offers a robust, flexible, and efficient solution for both perfusion and cell separation processes. In perfusion bioprocessing, product loss due to cell bleeding is a critical consideration. Bleeding removes a portion of the cells to help maintain certain VCD levels and high viability, but it also results in removal of cell-associated product and a portion of the reactor volume, thereby reducing overall product yield. When using kytero, targeted adjustment of the flow rate ratio of the feed, retentate, and harvest lines can reduce harvest product loss while simultaneously discharging cells. Minimizing product loss while effectively controlling biomass is therefore essential for process efficiency.

Although ATF enables effective cell retention and is widely used in perfusion processes, its performance can be limited at high cell densities. Membrane fouling and increases in transmembrane pressure (TMP) can restrict sustainable perfusion rates and lead to debris accumulation within the bioreactor. Moreover, harvesting cells directly from ATF bioreactors is not possible due to the accumulation of cell impurities at high cell densities, which limits the product yield to the harvest bulk. In contrast, the GEA Kytero® system allows direct cell harvest from the bioreactor vessel, providing a significant operational advantage. The kytero® centrifuge supports dual-use functionality, enabling both perfusion and continuous cell separation within the same platform. This integrated approach has been successfully demonstrated in prior collaborations with GEA Westfalia Separator Group GmbH and the Upstream Processing Department of the ProBioGen AG, showcasing its potential to streamline upstream processing. (Link to harvest Application Note: *https://www.gea.com/en/products/centrifuges-separation/centrifugal-separator/clarifier/kytero/*) Unlike systems that rely on membranes, the kytero® avoids the risk of fouling or blockage, contributing to higher process reliability and less downtime.

However, a notable drawback of the kytero® system is the relatively high turbidity of the harvest bulk. In perfusion systems using ATF modules or integrated cell retention filters in rocking bioreactors, the membranes typically feature very small pore sizes, often in the range of 0.2 to 7 µm. These fine pores effectively retain not only viable cells but also cellular debris and subcellular fragments within the bioreactor. As a result, the harvest stream remains cell-free and exhibits low turbidity, which is an advantage for certain downstream processing steps, particularly in clarification and filtration. However, the mode of retention also leads to the accumulation of cell debris, host cell proteins (HCP), and DNA inside the bioreactor over time. Such buildup can negatively impact cell viability, product

quality, and overall process performance, especially in extended perfusion runs. In contrast, the GEA kytero® utilizes a mechanical separation principle that does not fully retain small cell fragments or debris. Consequently, a portion of these components is removed from the reactor along with the harvest. While this may lead to increased turbidity in the harvested material, it offers a notable benefit. The bioreactor environment remains cleaner, as DNA, HCP, and cell debris do not accumulate to the same extent as in membrane-based systems. By continuously removing not only product but also cellular byproducts, the system helps maintain a healthier and more stable culture environment, which can enhance process robustness and product consistency in the long term. To enable its use in a production setting, a downstream clarification step would be required, using a small-area depth filtration followed by a 0.2 µm filtration. A potential production process incorporating the kytero® system is outlined schematically in Figure 4-7.

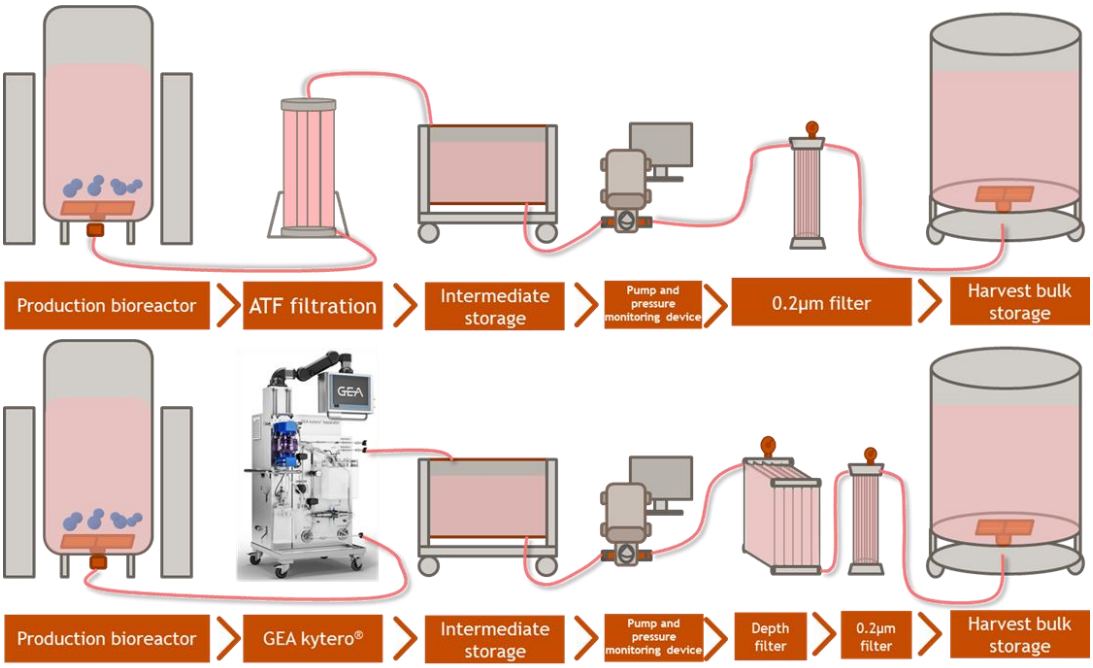

Figure 4-7: Comparison of two perfusion processes: traditional ATF filtration (top) and the GEA kytero® single-use centrifuge (bottom). Both workflows include intermediate storage, pressure monitoring, and final sterile filtration (0.2 µm) before bulk storage, with the kytero® setup integrating an additional depth filtration step for improved clarification.

The ATF perfusion strategy and the GEA Kytero® strategy are two approaches used for perfusion processes, each with distinct process designs and operational advantages.

In the ATF perfusion strategy, the cell culture is processed using an ATF filtration system, which retains cells in the bioreactor while allowing the cell-free harvest to pass through. After ATF filtration, the harvested product is collected in an intermediate storage container. It is then pumped through a 0.2 µm filter before it reaches the final harvest bulk storage. In contrast, the GEA Kytero® strategy integrates a dedicated, single-use filtration system designed specifically for the gentle clarification of biological products. The process also begins at the production bioreactor, but instead of ATF, the product is processed through the kytero® system. After intermediate storage, the harvest is passed through a depth filter with small filter area before undergoing 0.2 µm filtration and finally being transferred to bulk storage. A key difference lies in the filtration steps and system design. While the ATF strategy includes a single filtration step before sterile filtration, the kytero® approach introduces an additional depth filtration stage to improve clarity before 0.2 µm filtration.

5 Conclusion

The GEA kytero® 500 single-use pharma separator was intensively evaluated as part of a 15-day, 100 L perfusion process utilizing a CHO cell line expressing a monoclonal IgG1 antibody. The system ensured consistent and continuous cell retention as well as the harvest of clarified, product-rich supernatant. Throughout the process, high cell viability and stable antibody productivity were maintained. Overall, the GEA kytero® 500 demonstrated robustness and efficiency, supporting a reliable and productive long-term perfusion operation.

The process setup with the kytero® 10 system also indicated promising results, with comparable growth phase and peak densities. While the final configuration of the bowl and device is still being optimized for perfusion, the system shows strong potential for robust performance and a high degree of comparability and scalability in terms of cell growth and viability.

Comparability to other perfusion scales and systems has been observed, in terms of process control, cell growth and product yield, suggesting that kytero® technology can be seamlessly integrated into existing workflows. In addition to this comparability, the kytero® platform offers notable advantages over conventional perfusion systems, particularly regarding an operator friendly handling, an easy and fast plug and play setup, a high process safety and a dual use for perfusion and cell separation are major advantages of the single-use centrifuge.

For a fully integrated perfusion production process, an additional downstream setup is required. A combination with a depth filtration would lead to significant lower turbidity values and small 0.2 µm filter areas. The most suitable setup must be identified individually in dependence on the cell line characteristics and product.

6 References

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7 Abbreviations

Abbreviation	Description
ATF	Alternating Tangential Flow
CHO	Chinese Hamster Ovary
CIP	Cleaning-In-Place
CRF	Cell Retention Filter
CSPR	Cell-specific perfusion rate
DNA	Deoxyribonucleic acid
HCP	Host Cell Protein
IgG	Immunoglobulin G
LMW	Low Molecular Weight
mAb	Monoclonal Antibody
NTU	Nephelometric Turbidity Unit
RCF	Relative Centrifugal Force
TCD	Total Cell Density
USP	Upstream Processing
VCD	Viable Cell Density
vvd	Vessel volume per day
WCW	Wet Cell Weight
WFI	Water for Injection