APPLICATION NOTE

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

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Short Abstract:

The GEA kytero® 500 single-use pharma cell separator is an innovative single-use disk stack separator for the Upstream Processing Clarification step. The device has been intensively tested in a cell harvest setup subsequently of a 200L CHO cell fed-batch process. The GEA kytero® 500 showed a high separation efficiency and capacity as well as an easy plug & produce operation. Data for the primary clarification evaluation (pressure, turbidity, DNA, HCP, LDH, product titer and quality) was obtained for different flow and centrifugation rates. Furthermore, the effect on 0.2 µm filter capacity as a single-clarification step as well as in combination with a secondary depth filtration was gained to evaluate the complete cell harvest procedure. It was possible to identify the optimum setting for the investigated IgG4 producing CHO cell line to ensure low turbidity as well as high step yield and 0.2 µm filter capacity. Furthermore, the newly released GEA kytero 10®, the world's smallest single-use disk-stack centrifuge, was tested for its cell separation performance and comparable clarification results were obtained.

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.

Inhalt

1 Introduction

State of the art Upstream Processing (USP) production is often closely linked to intensified process types, which are characterized by high cell densities to increase cell mass and consequently product titer (e.g. intensified fed-batch processes). However, process intensification also comes together with new challenges for each process step with regard to process control, limitations and complexity. One major challenge in the context of intensified bioproduction is the cell separation process step to generate a cell-free harvest bulk which contains the active biopharmaceutical ingredient (API) for subsequent Downstream Processing. Due to high cell densities, more cell mass with partly lower viability and higher turbidity has to be separated, which means that classical platform approaches such as depth filtration reach their capacity limits or large filter areas are required. For the installation of large depth filter areas, a large-area production facility and high amounts of flush-medium are required, which is not suitable for small-area production facilities, could lead to yield loss and increases costs and complexity [1,2]. Furthermore, the use of classical stainless-steel separation centrifuges does not fit to the concept of CDMO multi-product facilities which are mainly based entirely on the use of single-use materials to avoid any risk of cross contamination or complex cleaning and decontamination [2].

Still, especially during cell separation, a high process reliability and safety is desired in order to harvest bioreactors completely, otherwise high product losses may occur. This is exactly where the GEA kytero® single-use pharma cell separator could offer significant advantages. The innovative single-use centrifuge design in combination with a small-footprint, an easy operation and a high primary clarification capacity could save time, labor as well as costs and ensures a high process safety.

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 cell separation

performance. To this end, a 14 days 200L CHO cell fed-batch process was run to produce a representative raw bulk. The raw bulk was subsequently harvested with different centrifuge settings to generate several harvest bulk fractions which were extensively analyzed. Data for turbidity, product titer and quality, concentrations for DNA, HCP and LDH, the residual solid ratio as well as the 0.2 µm filter capacity were determined to ensure a comprehensive performance evaluation of the GEA kytero®. In addition, a combination of the GEA kytero® with a secondary depth filtration for turbidity reduction was discussed to provide a complete assessment of a USP cell harvest setup.

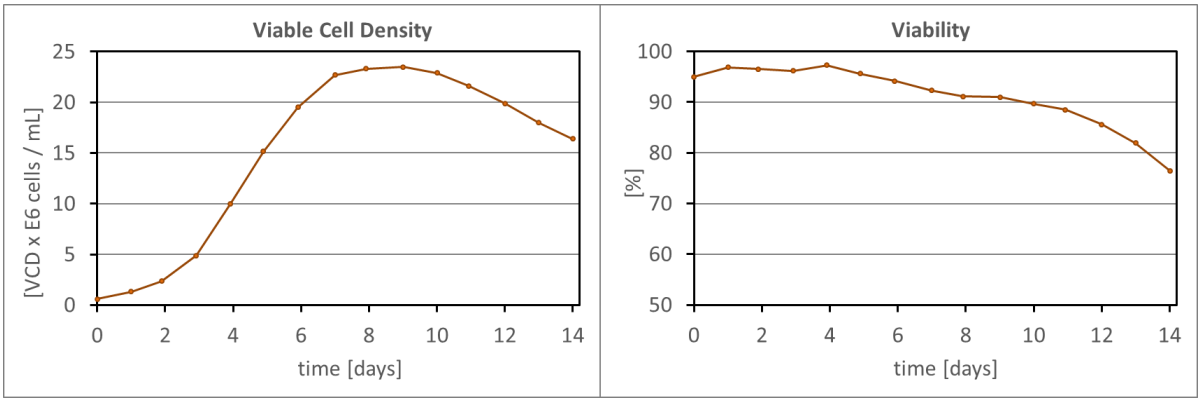
Additionally, a 14 days 10L CHO cell fed-batch process was run to produce a raw bulk to test the kytero® 10 as a down-scale instrument for lab-scale processes. Similar to the tests with the kytero® 500, the raw bulk was harvested with different centrifuge settings to generate several harvest bulk fractions, from which the turbidity, product titer as well as DNA and HCP concentrations were generated to evaluate the clarification performance of the kytero® 10 and the comparability to the kytero® 500.

2 Materials and methods

2.1 Raw Bulk parameters GEA kytero® 500

The raw bulk data is summarized in Table 2-1. A 14 days state of the art fed-batch process was executed in a 200L single-use stirred-tank bioreactor to produce a monoclonal IgG4 antibody. The fed-batch process was characterized by a seeding density of 0.6 E6 cells/mL and feed addition between day 3 and 14. This led to a peak VCD of 23.5 E6 cells/mL, a final VCD of 16 E6 cells/mL, a final viability of 76.5 % and a final product titer of 3.6 g/L within a final cell suspension mass of 200 kg. The wet cell weight ratio in the raw bulk was 5.0%.

Table 2-1: Raw Bulk parameter

General Raw Bulk parameter	Process value
Process mode	Fed-Batch
Product type	IgG4
Process duration [days]	14
Seed VCD day 0 [cells/mL]	0.6 E6
Maximum VCD during process [cells/mL]	23.5 E6
Final (day 14) Raw Bulk parameter	**Process value**
Viable cell density (VCD) [cells/mL]	16.4 E6
Total cell density (TCD) [cells/mL]	21.4 E6
Integral of VCD (IVCD) [cells*day/mL]	216 E6
Viability [%]	76.5
	
Wet cell weight (WCW) [%]	5.0

Bulk weight [kg]		200
pH [-]		7.23
Turbidity [NTU]		1405
Titer [g/L]		3.6 ± 0.04
HCP concentration [ppm]		223,377 ± 12,565
DNA concentration [µg/mL]		12.5 ± 1
LDH concentration [mU/mL]		2,816 ± 1027
Product quality evaluation by SE-HPLC [%]	LMW species	0.05
	Main IgG	98.8
	HMW species	1.15
Product quality evaluation by CE-SDS (non-reduced) [%]	LMW species	0.8
	Non-glycosylated IgG	2.5
	Main IgG	96.7

2.2 Raw Bulk parameters GEA kytero® 10

The raw bulk data is summarized in Table 2-2. A 15-day continuous process was executed in a 10L single-use stirred-tank bioreactor to produce a monoclonal antibody. The hybrid process was characterized by a seeding density of 4 E6 cells/mL perfusion was started on day 2. This led to a peak VCD of 93 E6 cells/mL, a final VCD of 37 E6 cells/mL, a final viability of 85.4 % and a final product titer of 1.9 g/L within a final cell suspension mass of 9 kg. The wet cell weight ratio in the raw bulk was 7.5%.

Table 2-2: Raw Bulk parameters

General Raw Bulk parameter	Process value
Process mode	hybrid (batch & perfusion)
Product type	IgG1
Process duration [days]	15
Final (day 15) Raw Bulk parameter	**Process value**
Viable cell density (VCD) [cells/mL]	36.9 E6
Total cell density (TCD) [cells/mL]	43.2 E6
Viability [%]	85.4
Turbidity [NTU]	1334
Wet cell weight (WCW) [%]	9
Bulk weight [kg]	9
Titer [g/L]	0.7 ± 0.1

2.3 Cell separation setup

2.3.1 GEA kytero® 500

An overview of the GEA kytero® 500 pharma cell separator is given in Figure 2-1 and the main technical parameters are summarized in Table 2-2.

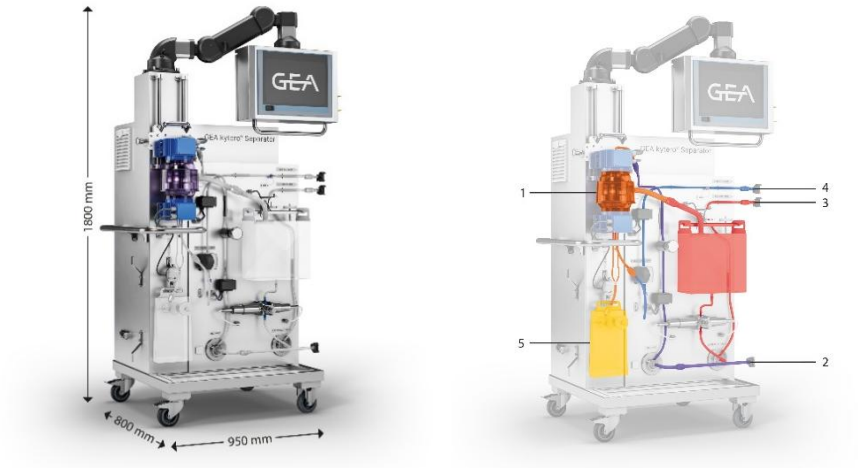


Figure 2-1: GEA kytero® 500 picture and schematic layout: (1) Separating unit (bowl); (2) Cell suspension feed line; (3) effluent line of clear phase to harvest bulk; (4) effluent line of solid phase / cell mass; (5) post production drain bag.

The GEA kytero® cell Separator consists of the proven GEA disk stack technology which for decades has been a mainstay in safe, reliable and efficient biopharma manufacturing processes of all global manufacturers. The compact and movable skid comes with fully integrated turbidity and flow meters in addition to pumps, tubes, connections and 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. kytero® is available in two sizes for the processing of 500L and 2000L.

Table 2-3: GEA kytero® 500 main technical parameter

Instrument parameter	Specification
Dimensions (LxWxH) [mm]	950 × 800 × 1.800
Expected flow rate [L/h]	50 - 150
Weight (net) [kg]	280
Power demand	110 V/60 Hz or 230 V/50 Hz
Material of kytero® single-use cartridge (housing and bowl)	Polycarbonate
Material of kytero® single-use path	Silicon, TPE, PE, PP, PSU, PPSU, PC
Integrated sensors	Pressure, feed flow rate, turbidity, weight

2.3.1.1 Experimental setup GEA kytero® 500

The experimental setup is shown in Table 2-3 and Figure 2-2. Five different bowl speed (centrifugation) rates (3500, 4000, 5000, 6000 and 7000 RPM) and three different cell suspension feed flow rates (50, 100, 120 L/h) were tested. The effluent of each setting was collected and analyzed separately. Thus, seven harvest bulk fractions were generated and each harvest bulk fraction was analyzed as shown in Figure 2-2.

Table 2-4: GEA kytero® 500 instrument settings for the generation of different harvest bulk fractions

Harvest bulk fraction no.	Feed flow rate [L/h]	Bowl speed rate [RPM]	Concentrate target concentration [%]
1	50	3500	75
2		4000	
3		5000	
4		6000	
5		7000	
6	100	5000	
7	120	5000	

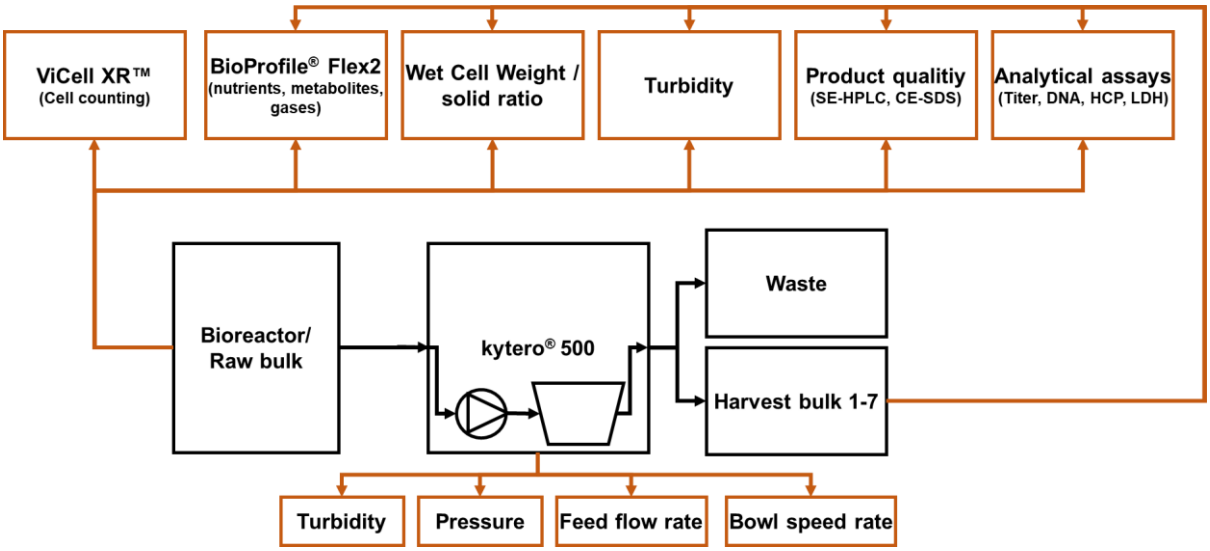


Figure 2-2: Schematic cell harvest experimental setup

2.3.2 GEA kytero® 10

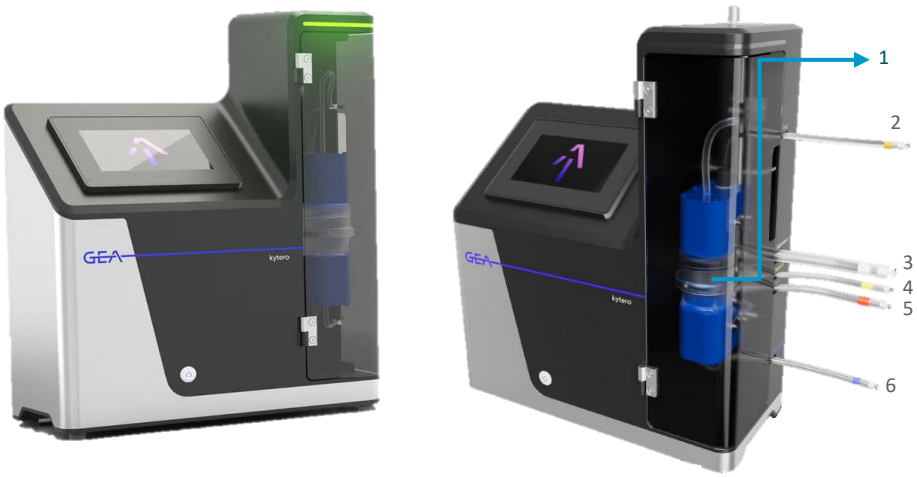


Figure 2-3: GEA kytero® 10 picture and schematic layout: (1) Separating unit (bowl); (2) Cell suspension feed line; (3) effluent line of clear phase to harvest bulk; (4) sterile process air; (5) post production drain bag; (6) concentrate.

The GEA kytero® 10 is the world's smallest single-use disk-stack centrifuge. It is designed for processing volumes from 1 to 10 L. It is particularly well suited for fed-batch bioprocessing with CHO cells, where maintaining cell integrity and culture productivity is critical. It offers gentle, low-shear separation with a non-contact Breezedrive® system. This ensures high cell viability, rapid setup, and secure biocontainment. As a compact tabletop unit that requires only power and compressed air, the need for CIP/SIP cleaning is eliminated and therefore workflows are accelerated. The single-use, gamma-treated assemblies reduce contamination risks and support seamless scale-up and process transfer to larger GEA models.

Table 2-5: GEA kytero® 10 main technical parameters

Instrument parameter	Specification
Dimensions (LxWxH) [mm]	450 x 200 x 575
Expected flow rate [L/h]	2 - 6
Weight (net) [kg]	15
Power demand	100 V/50 Hz or 240 V/60 Hz
Material of kytero® single-use cartridge (housing and bowl)	Somos Bioclear, BASF Ultracur 3D RG35
Material of kytero® single-use path	C-Flex
Integrated sensors	No integrated sensors
Centrifugation rate technical range [rpm]	5200 - 13000
Used centrifugation rate for CHO perfusion setup [rpm]	6500

2.3.2.1 Experimental setup GEA kytero® 10

The experimental setup is shown in Figure 2-4. Three different bowl speed (centrifugation) rates (6500, 7500, 8500RPM) and three different cell suspension feed flow rates (2, 3, 4 L/h) were tested. The effluent of each setting was collected and analyzed separately. Thus, seven harvest bulk fractions were generated and each harvest bulk fraction was analyzed as shown in

Figure 2-4.

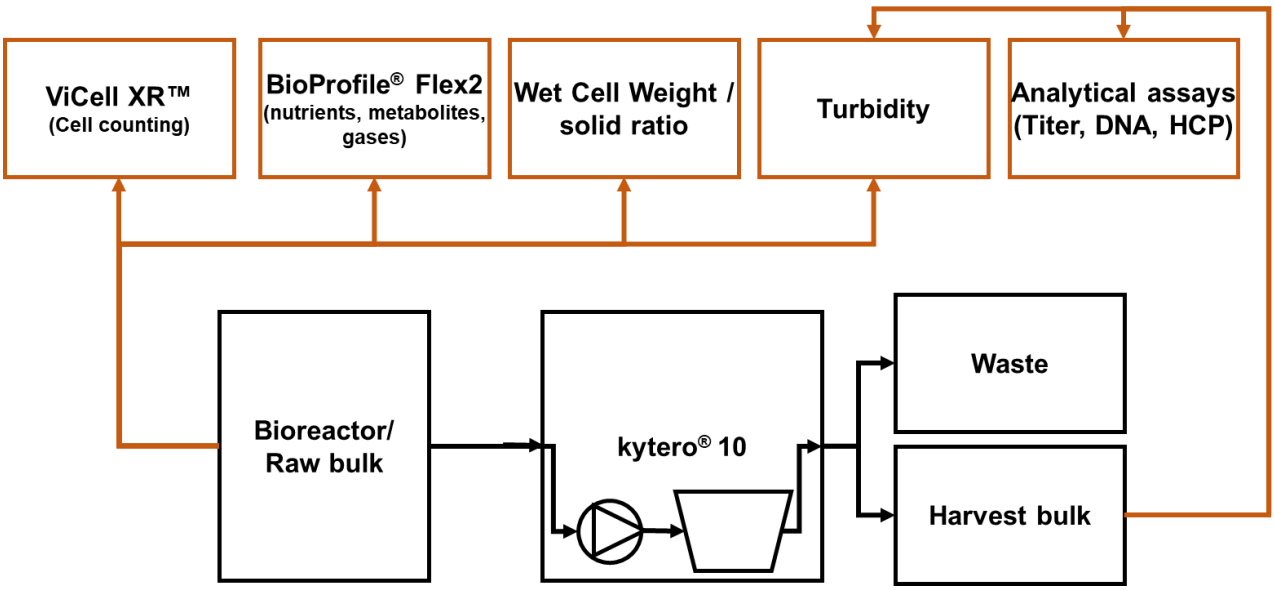


Figure 2-4: Schematic cell harvest experimental setup

2.4 Equipment & methods

Table 2-6 summarizes the main equipment including the application and methods.

Table 2-6: 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
Turbidity Meter	TB350 IR / Lovibond	Offline turbidity measurement of samples	infrared turbidity meter [IR] is used for measurement by the intensity of the scattered light at an angle of 90 degrees
Protein A-measurement device	Octet K2 / Sartorius Stedim Biotech	Measurement of protein 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
	ROTOFIX 32 A / Hettich	Concentrate discovery and spin tests of cell suspension	Setpoint of 2000g/min with different spin times
Single-use stirred-tank bioreactor	XDR-200/ Cytiva with control unit from ISE	Production bioreactor	14 days fed-batch process
	XDR-10/ Cytiva with control unit from ISE [(1)]	Production bioreactor	15 days hybrid process
Plate Reader	Tecan Infinite M1000 / Tecan	HCP-Assay	New CHO HCP ELISA Kit F550-1 / Cygnus
		LDH-Assay	LDH Activity Assay (MAK066) / Sigma
		DNA-Assay	Picogreen dsDNA-Assay / Quant-iTä
HPLC	Prominence UFLC Fast LC / Shimadzu	Product quality Investigation. Samples were prepared with Protein A HP Spin Trap™ Columns (Cytiva) before analysis	SE-HPLC with TSKgel G3000 column
Capilar Electrophoresis	BioPhase 8800/ SCIEX		CE-SDS with Protein Analysis Kit (Sciex C30085)
Pressure monitor	SciPress Monitor / Parker Hannifin	Pressure Monitoring during filter capacity determination	0.2µm filter: Sartopore XLG 2 / Sartorius 0.8µm / 0.2µm pore size 0.27 m² filter area pressure maximum: 1.8 bar
Peristaltic pump	Masterflex L/S / Cole Palmer	Pump for filter capacity determination	Depth filter types: Millistak+® HC Pro D0SP, 20cm² Millistak+® HC Pro C0SP, 20cm² Millistak+® HC Pro X0SP, 20cm²

1) Equipment/ methods used for Kyero 10 harvest study.

3 Results and discussion

3.1 Pre-tests for the GEA kytero® 500 setup

Prior to the main cell centrifugation test, a pre-investigation spin test of cell concentration and turbidity was performed using the Hettich ROTOFIX centrifuge. The centrifuge was used at a setpoint of 2000 xg and the cell suspension was centrifuged with 5 different spin times. At 2000 xg, the g-force is in a similar range to that of the GEA kytero® 500, allowing the initial parametrization (bowl speed rate, feed flow rate) to be carried out based on the required spinning time and relative centrifugal force. The data is shown in Table 3-1.

Table 3-1: Pre-Centrifugation spin tests

Spin Time [sec]	30	60	120	240	480
Concentrate [vol%]	4.5	4.0	3.2	3.0	3.0
Turbidity [NTU]	>100	>100	99	83	82
Centrifuge tube					

Conclusion of the pre-spin-test was that a high cell concentration (=Concentrate [vol%] in Table 3-1, means cell pellet to total volume ratio) and the lowest turbidity can be achieved with longer spin times (correlates with lower feed flow rates for the GEA kytero® 500). However, many small particles were part of the raw bulk which can only be reduced to a certain level by higher spinning times. Thus, a target turbidity limit of ~80 NTU was determined.

3.2 GEA kytero® 500 cell separation performance evaluation

During the centrifugation with the GEA kytero®500 the online values for turbidity, feed flow rate and bowl speed were monitored and are shown in Figure 3-1. The online process values for turbidity reveal a higher fluctuation at the fraction switchover points. Indeed, the new setpoint changes the liquid height in the harvest line intermediate venting bag (red bag, Figure 2-1) thereby leading to bubble input and sensor signal disturbance. With a constant setting during centrifugation and a constant liquid height in the harvest line intermediate venting bag, a smoother signal can be achieved (e.g. for 2nd half of harvest bulk fraction 3).

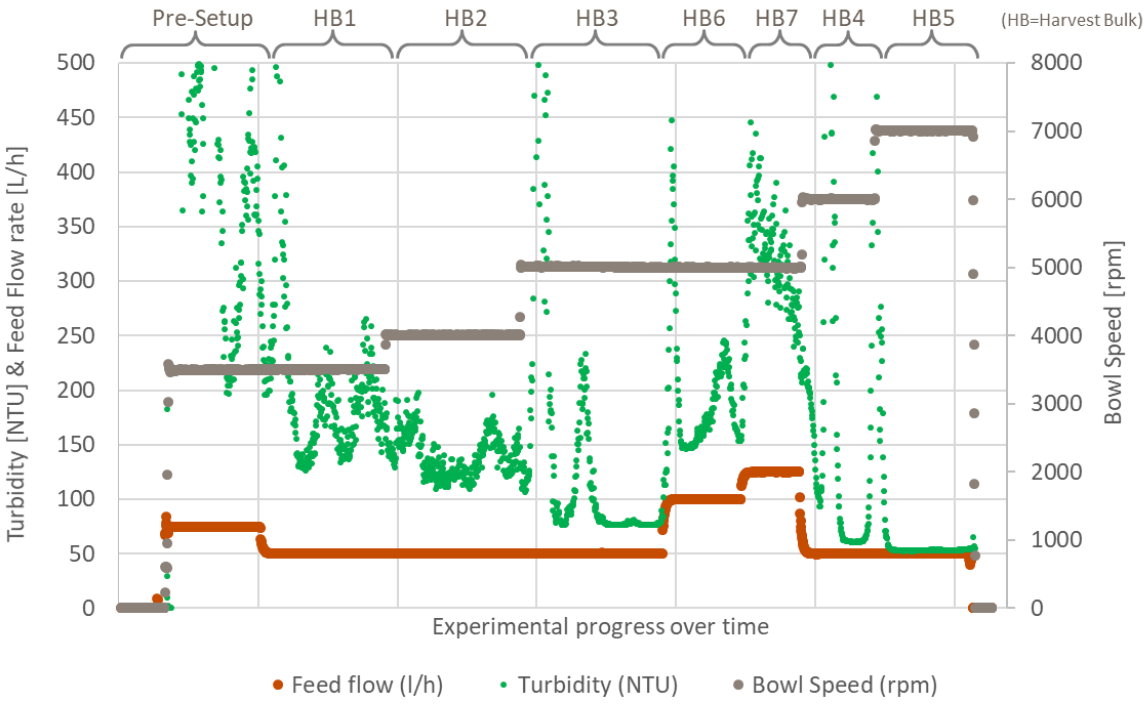


Figure 3-1: GEA kytero® 500 online data with different settings for harvest bulk generation.

Table 3-2 gives an overview of each harvest bulk fraction regarding harvest mass, turbidity, lactate and pH. Table 3-3 shows the spin tube pictures of the residual solid ratio determination.

The residual solid ratio steadily decreased with increasing bowl speed. For harvest bulk fractions 4 (50L/h and 6000 RPM) and 5 (50L/h and 7000 RPM) no solid phase could be detected anymore. The highest residual solid ratio was detected for harvest bulk fraction 7 (120L/h and 5000 RPM) with 0.08% at the highest feed flow rate and therefore lowest bowl holding time. However, in general a sufficient solid separation was achieved without any product loss related to residual liquid in the solid waste phase. Figure 3-2 shows an exemplary solid waste phase of harvest bulk fraction 5. A stable cell pellet without residual liquid was generated.

Correlative to the residual solid ratio data, the offline measured turbidity was reduced with increasing bowl speed and increased by higher feed flow rates. The lowest turbidity was achieved for harvest bulk fraction 5 (50L/h and 7000 RPM) with 83 NTU and the highest turbidity was observed for harvest bulk fraction 7 (120L/h and 5000 RPM) with 113 NTU.

Table 3-2: Filtration results (mass of harvest fractions, turbidity, lactate, pH) for each harvest bulk fraction.

Harvest Bulk fraction	Mass of harvest fraction [kg]	Average online turbidity [NTU]	Offline turbidity [NTU]	Lactate [mM]	pH [-]
HB1) 50 L/h - 3500 RPM	17.3	172	106	33	7.36
HB2) 50 L/h - 4000 RPM	22.9	150	96	35	7.34
HB3) 50 L/h - 5000 RPM	28.5	138	91	36	7.34
HB4) 50 L/h - 6000 RPM	15.4	63	87	35	7.36
HB5) 50 L/h - 7000 RPM	18.2	54	83	35	7.36
HB6) 100 L/h - 5000 RPM	23.7	201	105	35	7.36
HB7) 120 L/h - 5000 RPM	25.9	307	113	36	7.39

Table 3-3: Pictures of supernatant with residual cell pellet to total volume ratio of each harvest bulk fraction after additional centrifugation at 2000 xg for 3 minutes.

HB1) 50 L/h – 3500 RPM	HB2) 50 L/h – 4000 RPM	HB3) 50 L/h – 5000 RPM	HB4) 50 L/h – 6000 RPM	HB5) 50 L/h – 7000 RPM	HB6) 100 L/h – 5000 RPM	HB7) 120 L/h – 5000 RPM
						
						

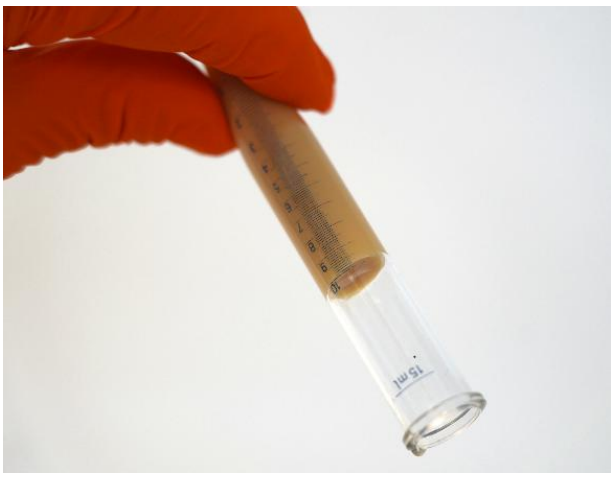


Figure 3-2: Exemplary picture of concentrate sample at setting of 50L/h and 7000 RPM with a stable cell pellet and no detectable residual fluid.

Table 3-4 summarizes the product, DNA, HCP and LDH concentration data. The product quality data can be found in Table 3-5. All data sets are shown in Figure 3-4 to Figure 3-10.

No effects of the different settings were observed for pH (Table 3-2), titer, DNA-concentration and product quality (as determined by SE-HPLC and CE-SDS). A mean titer of 3.5 ± 0.1 g/L, a mean pH of 7.36 ± 0.02 and a mean DNA-concentration of 13.3 ± 0.3 µg/mL was measured for all harvest bulk fractions. In contrast to a depth filtration based cell separation, the titer is not diluted by pre and post filter flushing, which minimizes the total harvest bulk mass and potential product loss. In addition, the steady harvest bulk pH could benefit product quality.

A trend was possibly identified for the HCP-concentration, whereby a higher bowl speed could lead to an increased release of HCP, especially for cells with partially damaged cell membranes. Indeed, the lowest HCP-concentration was measured for harvest bulk fraction 1 (50L/h and 3500 RPM) and the highest for harvest bulk fraction 5 (50L/h and 7000 RPM). A similar trend was observed for the LDH concentration. However, the HCP and LDH assays are subject to greater variations at raw bulk level and thus both observed trends are not considered significant.

Table 3-4: Analytical results for each harvest bulk fraction.

Harvest Bulk fraction	Titer [g/L]	DNA concentration [µg/mL]	HCP concentration [ppm]	LDH concentration [mU/mL]
HB1) 50 L/h - 3500 RPM	3.7 ± 0.15	13.4 ± 0.9	196.967 ± 18.081	2.516 ± 86
HB2) 50 L/h - 4000 RPM	3.6 ± 0.10	13.2 ± 0.9	229.550 ± 8.759	2.724 ± 63
HB3) 50 L/h - 5000 RPM	3.7 ± 0.10	13.1 ± 0.8	237.440 ± 18.052	2.950 ± 226
HB4) 50 L/h - 6000 RPM	3.4 ± 0.08	12.9 ± 0.9	252.824 ± 6.388	3.343 ± 93
HB5) 50 L/h - 7000 RPM	3.5 ± 0.09	13.2 ± 0.2	249.672 ± 748	3.362 ± 364
HB6) 100 L/h - 5000 RPM	3.5 ± 0.15	13.0 ± 0.6	234.988 ± 4.447	2.392 ± 567
HB7) 120 L/h - 5000 RPM	3.6 ± 0.13	14.0 ± 0.9	221.298 ± 1.301	3.290 ± 141

Table 3-5: Product quality results for each harvest bulk fraction.

Harvest Bulk fraction	SE-HPLC [%]			CE-SDS [%]		
	LMW	Main IgG	HMW	LMW	NG-IgG	Main IgG
HB1) 50 L/h - 3500 RPM	0.1	98.8	1.1	0.7	2.5	96.6
HB2) 50 L/h - 4000 RPM	0.1	98.9	1.0	0.8	2.5	96.8
HB3) 50 L/h - 5000 RPM	0.0	99.0	1.0	1.5	3.0	95.6
HB4) 50 L/h - 6000 RPM	0.0	99.2	0.8	1.2	2.8	96.1
HB5) 50 L/h - 7000 RPM	0.0	99.0	1.0	0.8	2.6	96.6
HB6) 100 L/h - 5000 RPM	0.0	99.0	1.0	1.3	3.0	95.7
HB7) 120 L/h - 5000 RPM	0.0	98.9	1.1	1.1	2.8	96.1

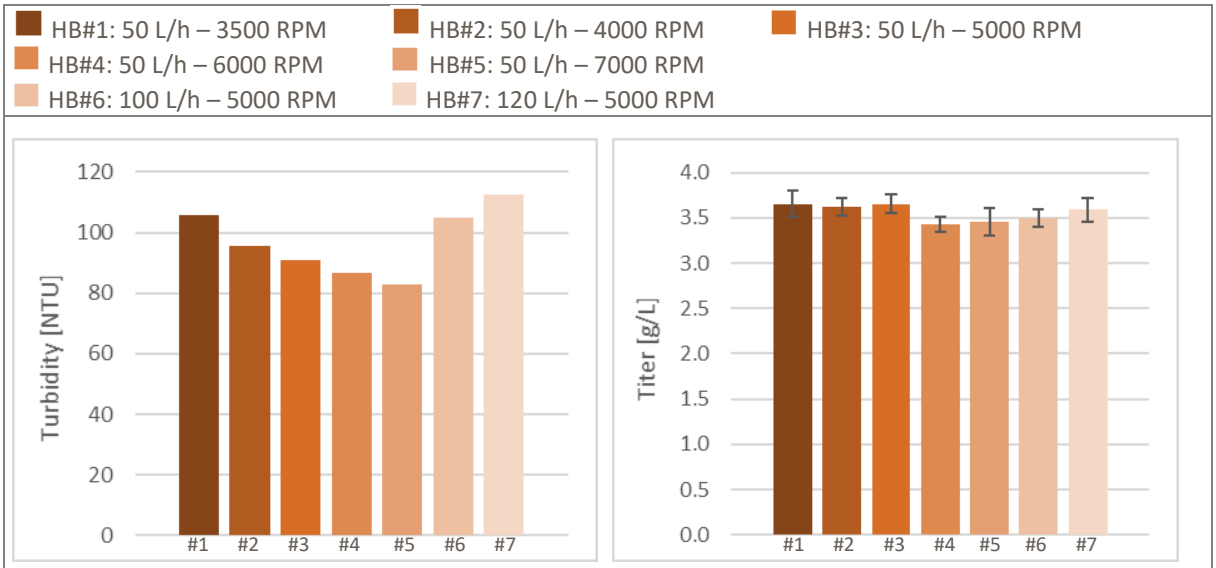


Figure 3-3: Turbidity data for each harvest bulk fraction.

Figure 3-4: Titer data for each harvest bulk fraction.

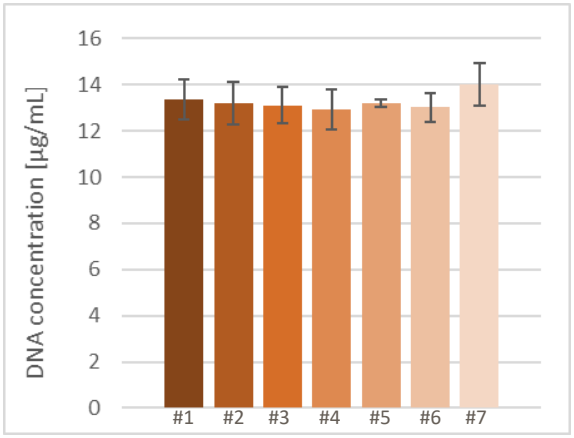


Figure 3-5: DNA concentrations for each harvest bulk fraction.

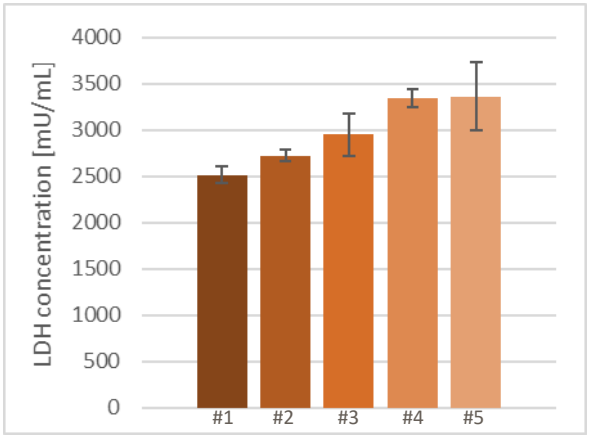


Figure 3-6: LDH concentrations for all harvest bulk fractions at 50 L/h flow rate and different centrifugation rates.

HB#1: 50 L/h – 3500 RPM
HB#2: 50 L/h – 4000 RPM
HB#3: 50 L/h – 5000 RPM
HB#4: 50 L/h – 6000 RPM
HB#5: 50 L/h – 7000 RPM
HB#6: 100 L/h – 5000 RPM
HB#7: 120 L/h – 5000 RPM

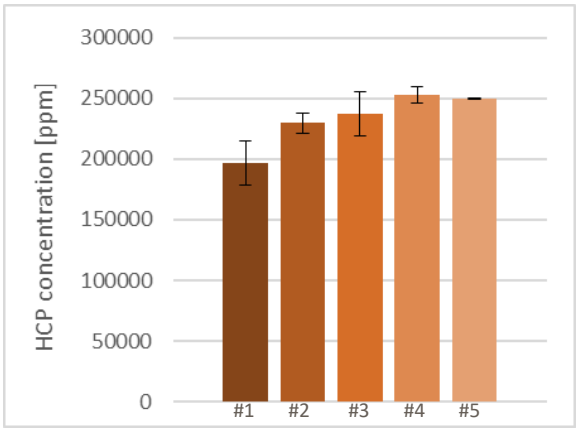


Figure 3-7: HCP concentrations for all harvest bulk fractions at 50 L/h flow rate and different centrifugation rates.

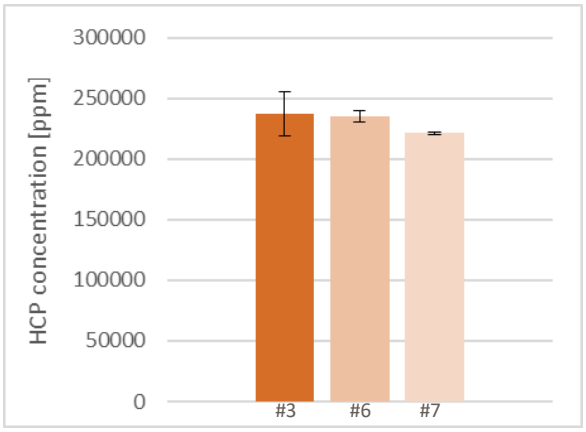


Figure 3-8: HCP concentrations for all harvest bulk fractions at 5000 RPM and different flow rates.

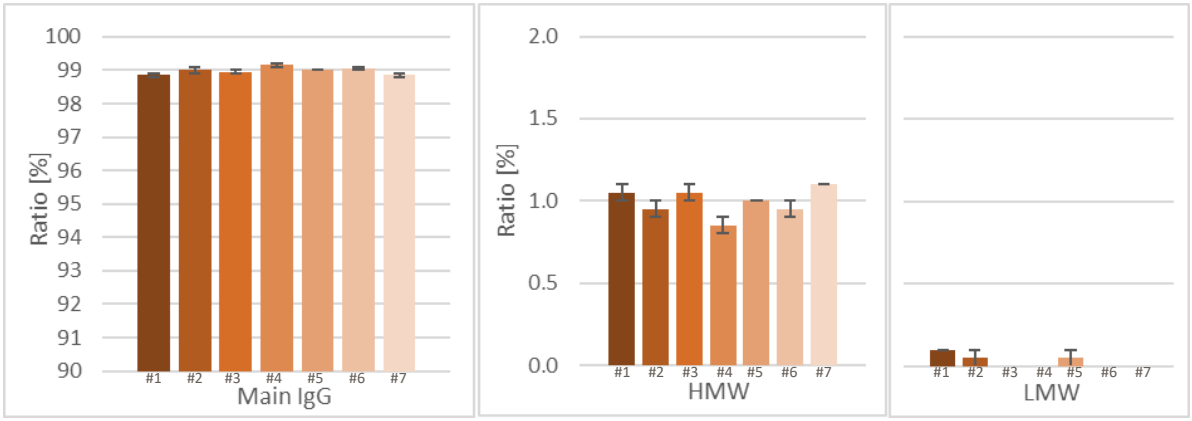


Figure 3-9: SE-HPLC data for each harvest bulk fraction.

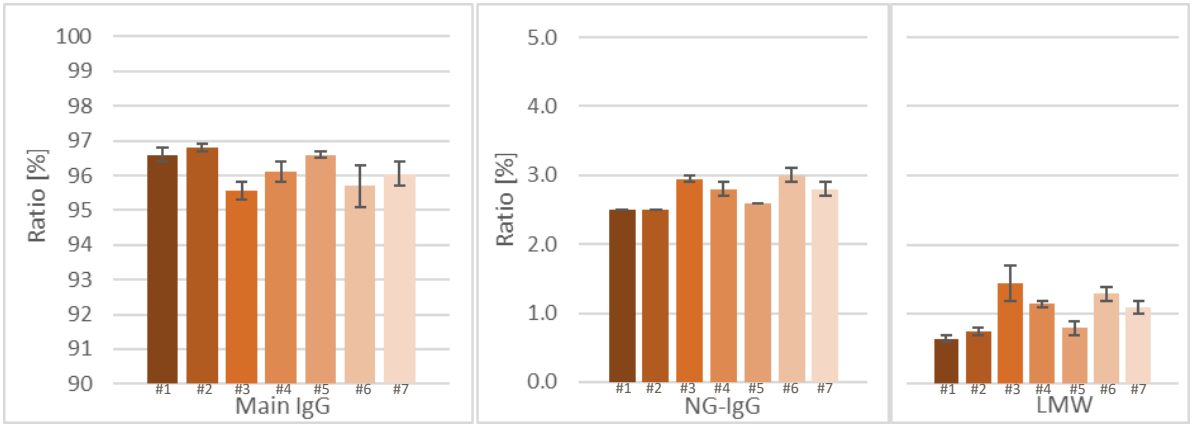


Figure 3-10: CE-SDS (non-reduced) data for each harvest bulk fraction.

3.3 Evaluation of complete cell harvest setup including 0.2 µm filter capacity determination

To evaluate the GEA kytero® 500 performance as a primary clarification system, in the overall cell harvest setup including the 0.2µm filtration, a subsequent experiment was carried out. Each harvest bulk fraction was filtered with a Sartopore 2 XLG (0.8/0.2µm) filter until a maximum pressure of 1.8 bar was reached. The maximum achieved capacities can be found in Table 3-6.

The highest 0.2 µm filter capacity was achieved for harvest bulk fraction 3 (50 L/h - 5000 RPM) with 56 L/m², although this fraction did not have the lowest turbidity. However, fraction 4 (50 L/h – 6000 RPM) and 5 (50 L/h - 7000 RPM) also had higher HCP and LDH concentrations, which could result in lower 0.2 µm filter capacities.

Table 3-6: Results for determination of 0.2 µm filter capacity post centrifugation.

Harvest Bulk fraction	Offline turbidity [NTU]	Maximum 0.2 µm filter capacity [L/m²]
HB1) 50 L/h - 3500 RPM	106	35
HB2) 50 L/h - 4000 RPM	96	42
HB3) 50 L/h - 5000 RPM	91	56
HB4) 50 L/h - 6000 RPM	87	47
HB5) 50 L/h - 7000 RPM	83	33
HB6) 100 L/h - 5000 RPM	105	34
HB7) 120 L/h - 5000 RPM	113	22

In general, the determined turbidities and filter capacities for the evaluated cell line and product would be critical for a direct 0.2 µm filtration, as there would be a high risk of filter clogging. Therefore, three different synthetic depth filter types with varying pore sizes were evaluated for their turbidity reduction effect. Smaller pore sizes led to improved turbidity reduction but also faster pre-filter pressure build-up and lower filter capacities. The data as well as pros and cons are summarized in Table 3-7 and Table 3-8. Depending on the cell line, product, growth profile and basic turbidity, the most suitable setup must be selected to define the cell separation process flow scheme (Figure 3-4).

Table 3-7: Results for evaluation of turbidity reduction by implementation of a secondary depth filter post centrifugation and pre 0.2µm filtration.

Secondary depth filter	Mean turbidity reduction by secondary depth filtration [NTU]	Theoretical mean turbidity before 0.2µm filtration based on centrifugation result for 50 L/h at 5000 RPM (91 NTU) [NTU]	Experimental or theoretical[1)] max. 0.2µm filter capacity [L/m²]	Min. required 0.2µm filter area for 1000L scale [m²]
---	---	91	56	>17.9
Millistak HC Pro D0SP (pore size 9-1 µm)	-28	63	81[1)]	>12.3
Millistak HC Pro C0SP (pore size 3-0.2 µm)	-43	48	106[1)]	>9.4
Millistak HC Pro X0SP (pore size ~0.1 µm)	-67	24	209[1)]	>4.8

Table 3-8: Pros and Cons of different methods for turbidity reduction by implementation of a secondary depth filter post centrifugation and pre 0.2µm filtration.

Clarification setup (according Figure 3-12)		**Pros**	**Cons**
B	Only centrifugation	- Most simple, operator-friendly setup - No additional depth filter setup - No filter pre-flushing	- Highest turbidity - Large 0.2 µm filter necessary with potential 0.2 µm filter clogging
A	Additional Millistak HC Pro D0SP (pore size 9-1 µm)	- Best filter capacity - No pressure build-up pre-D0SP	- Additional depth filter setup/flushing - Only moderate turbidity reduction
	Additional Millistak HC Pro C0SP (pore size 3-0.2 µm)	- Good turbidity reduction - Good filter capacity - Only slight pressure build-up pre-C0SP	- Additional depth filter setup/flushing
	Additional Millistak HC Pro X0SP (pore size ~0.1 µm)	- Best turbidity reduction	- Additional depth filter setup/flushing - Fast pressure build-up pre-X0SP - Low filter capacity

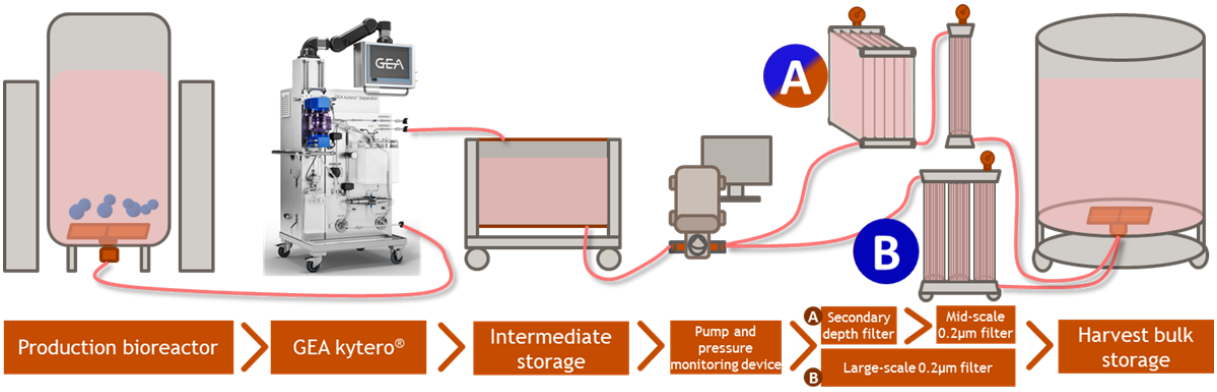


Figure 3-11: Exemplary process flow scheme from Raw Bulk to Harvest Bulk with two options for cell separation. GEA kytero® pharma cell separator as primary clarification system. Intermediate storage of centrifugate in storage tank before optional secondary depth filtration or direct 0.2µm filtration for final harvest bulk generation. The intermediate storage tank is used for pressure relief, as the GEA kytero® single-use manifold is only pressure-stable up to 1.2 bar and higher pressures can occur during 0.2µm filtration.

Regardless of the final setup, the centrifuge has numerous advantages over classic depth filtration. Figure 3-12 & Table 3-9 compares the main economic factors of the different process types for a 1000L scale harvest.

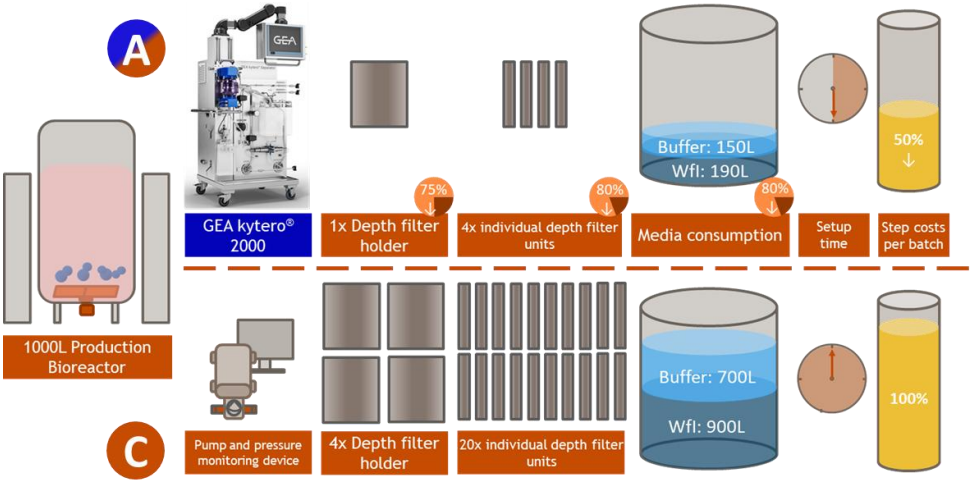


Figure 3-12: Comparison of economic and environmental factors between different cell harvest setups in 1000L scale.

Table 3-9: General assessment of different setup options for cell separation procedure in 1000L scale.

	C **Depth filtration + 0.2µm filtration**	B **GEA kytero® + direct 0.2µm filtration** (with large-scale 0.2µm filter)	A **GEA kytero® + secondary depth filtration + 0.2µm filtration** (with medium-scale 0.2µm filter)
Costs per batch	High	Medium (~25%↓)	Low (~50%↓)
Setup time	High	Low (~90%↓)	Medium (~50%↓)
Foot print	High	Low	Medium
Depth filter area [%]	100	0	20
Filter pre-flushing	Yes	No	Yes
Liquid waste management [L]	1100	0	200
Solid (material) waste [x 200L waste container]	9	2	4
Amount of variable single-use components [piece]	~50	~10	~20

The use of the GEA kytero® reduces the costs per batch (up to 50%), setup time (up to 90%), foot print, flush volume/ liquid waste management (up to 100%), solid (plastic material) waste management, the number of single-use components (up to 80%) as well as the depth filter area (up to 100%) for the cell separation process step in comparison to depth filtration.

3.4 GEA kytero® 10 cell separation performance evaluation as primary clarification step

During the centrifugation with GEA kytero® 10, several combinations of different feed flow rates and bowl speed rates were tested, to determine the optimal clarification settings. The turbidity values of the material obtained were measured offline immediately after the collection. Turbidity measurements were used as a primary indicator of separation efficiency, with lower NTU values reflecting superior clarification performance.

Table 3-10 summarizes the measured turbidity values.

Table 3-10: Turbidity results as a preliminary assessment of suitable settings

Feed flow rate	Bowl speed rate	Turbidity
[L/h]	[RPM]	[NTU]
2	6500	123 ± 1
	7500	116 ± 0
	8500	109 ± 1
3	6500	144 ± 0
	7500	135 ± 3
	8500	124 ± 1
4	6500-8500	477 ± 113

The combination of feed flow: 2 L/h and bowl speed: 8500 rpm delivered the best clarification performance with a turbidity of 109 ± 1 NTU.

The kytero® 10 data confirms the trends observed with the kytero® 500 very well. An increasing centrifugation rate led to reduced turbidity values, while an increase in feed flow rate led to an elevated turbidity. At a feed flow rate of 4 L/h, a significant decline in clarification efficiency was observed, as the bowl was overflowing due to excessive inlet liquid flow.

An additional assessment of host cell protein (HCP) and residual DNA content was performed, providing a more precise evaluation of the kytero® 10's cell separation capability. These results are shown in Table 3-11.

Table 3-11: Results of HCP, DNA and Titer measurements

Feed flow rate	Bowl speed rate	HCP concentration	DNA concentration	Product Concentration
[L/h]	[RPM]	[ng/ml]	[µg/mL]	[µg/mL]
2	6500	26.1 ± 0.83	13.2 ± 0.22	619 ± 0.02
	7500	27.8 ± 0.30	13.5 ± 0.03	617 ± 0.01
	8500	29.0 ± 0.36	13.6 ± 0.01	632 ± 0.01
3	6500	27.3 ± 0.39	13.8 ± 0.31	618 ± 0.00
	7500	32.0 ± 0.21	13.4 ± 0.15	644 ± 0.03
	8500	32.4 ± 0.16	13.8 ± 0.29	645 ± 0.02
4	6500	32.9 ± 0.16	16.2 ± 0.19	656 ± 0.03
	7500	31.8 ± 0.81	19.8 ± 0.98	674 ± 0.02
	8500	29.5 ± 0.75	16.4 ± 0.25	675 ± 0.00

As expected, and again similar to the results generated with the kytero® 500 experiments, as the bowl speed increased, the HCP concentration of the harvest fractions also increased, while no significant product loss or DNA concentration fluctuations were observed for the different bowl speed and feed flow rates.

Figure 3-13, Figure 3-14, Figure 3-15, and Figure 3-16 summarize the kytero® 10 data set.

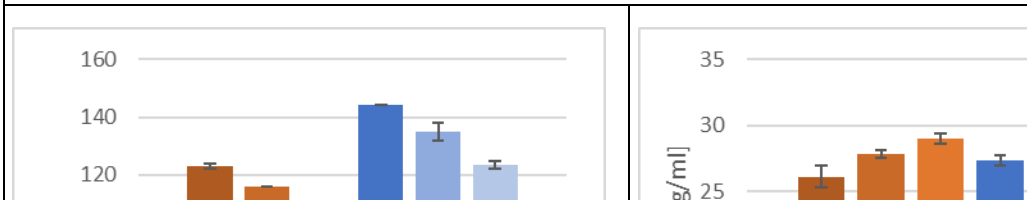


Figure 3-13: Comparison of turbidity values.

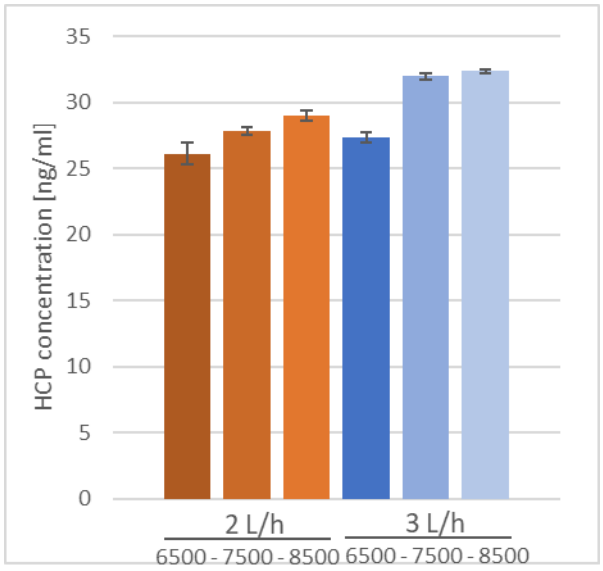


Figure 3-14: Comparison of HCP concentration values.

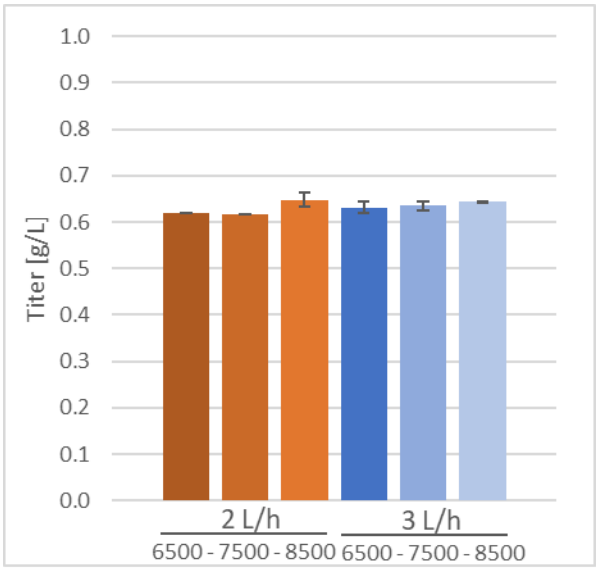


Figure 3-15: Comparison of titer values.

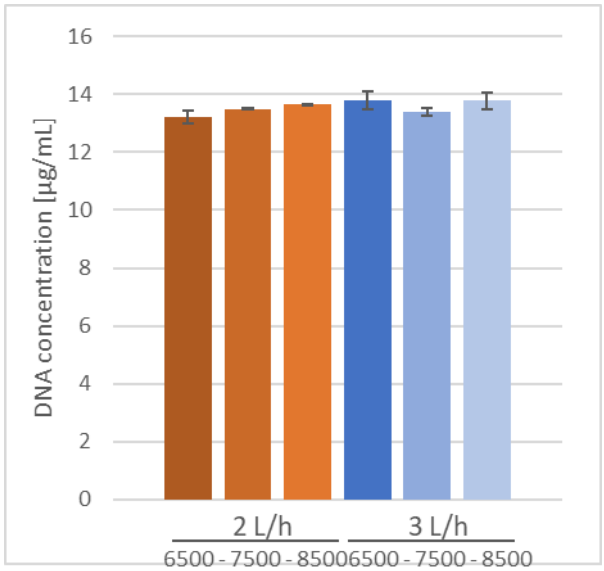


Figure 3-16: Comparison of DNA concentration values.

4 Conclusion

The GEA kytero® 500 single-use pharma separator was intensively evaluated as part of a 200L CHO cell fed-batch process. Five different bowl speed rates and three flow rates were tested and the cell harvest performance for each setting was investigated.

The sweet spot of the instrument, for the selected IgG4 producing CHO cell line, was identified at a bowl speed rate of 5000 RPM in combination with a feed flow rate of 50 L/h. Lower bowl speed rates as well as higher feed flow rates led to an increased turbidity, higher residual solid ratios and consequently to lower maximum 0.2 µm filter capacities. Although higher speed bowl rates of up to 7000 rpm led to even lower turbidity values, the maximum 0.2 µm filter capacity for these fractions was lower than for the 5000 RPM setpoint, for which the highest capacity of 56 L/m² was determined. Elevated HCP and LDH concentrations were determined for increased speed bowl rates which could accelerate 0.2 µm filter clogging.

No product loss was observed during centrifugation and no negative effects on harvest bulk pH, DNA-concentration or product quality were observed for the tested settings.

With the optimal centrifuge setting of 5000 RPM and 50 L/h, a direct 0.2 µm filtration post-centrifugation might be possible for the selected cell line, even up to 1000L production-scale. However, it was shown that a combination with a secondary depth filtration would lead to significant lower turbidity values and 0.2 µm filter areas. Especially for intensified high cell density fed-batch processes or processes with low viability profiles, a further turbidity reduction might be beneficial to ensure process safety and an economic cell harvest setup. The most suitable setup must be identified individually in dependence on the cell line characteristics and product.

Similarly to GEA kytero® 500, three different bowl speed rates and three different feed flow rates were evaluated for the GEA kytero® 10 and comparable trends were observed for all parameters. Lower bowl speed rates as well as higher feed flow rates led to an increased turbidity. HCP values increased with higher bowl speed rates whereas no product loss or effects on harvest bulk DNA-concentration were observed. The experiments resulted in the identification of 2L/h feed flow rate at 8500 RPM bowl speed rate for the best clarification performance. The best turbidity values achieved with the kytero® 10 (109 ± 1 NTU) were 30% higher than those of the kytero® 500. However, a direct comparison is not meaningful, as a different process type as well as a different cell line and product were used for the kytero® 10 evaluation, which had for example a 2.3 times higher final VCD.

In general, the GEA kytero® single-use pharma separator showed many advantages over a classical depth filtration based cell harvest approach. A high and almost unlimited capacity as a primary clarification system in combination with an operator friendly handling, an easy and fast plug and play setup, no need of pre- or post-flushing, a high process safety and a small footprint are major advantages of the single-use centrifuge. Furthermore, the system is directly equipped with all sensors for a sufficient in-line process monitoring of pressure, flow and turbidity with only a few necessary single-use components.

The GEA kytero® single-use pharma separator ensures a high cell harvest step yield and process safety without any cell mass limitations and is therefore an excellent instrument for high quality GMP compliant harvest bulk generation in biotechnology and biopharmaceutical industry.

5 References

[1] Dryden WA, Larsen LM, Britt DQ, Smith MT (2021) Technical and economic considerations of cell culture harvest and clarification technologies. Biochemical Engineering Journal, Volume 167

[2] Gupta A, Amara JP, Gousseinov E, Cacace B (2020) Recent advances in harvest clarification for antibodies and related products. Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics, Elsevier, pp. 117-136

6 Abbreviations

Abbreviation	Description
CE-SDS	Capillary Electrophoresis with sodium dodecyl sulfate
CHO	Chinese Hamster Ovary
CIP	Cleaning-In-Place
DNA	Deoxyribonucleic acid
HCP	Host Cell Protein
HPLC	High performance liquid chromatography
HMW	High Molecular Weight
IgG	Immunoglobulin G
LDH	Lactate dehydrogenase
LMW	Low Molecular Weight
NG-IgG	Non-Glycosylated Immunoglobulin G
RCF	Relative Centrifugal Force
TCD	Total Cell Density
USP	Upstream Processing
VCD	Viable Cell Density
WCW	Wet Cell Weight
WFI	Water for Injection