CP biotools

TECHNICAL NOTE

Conversion of a 3L Benchtop Glass Bioreactor to a Perfusion Bioreactor for Process Intensification with the Magma™ Advanced Pumping System Controlling the Alternating Tangential Flow (ATF) Filtration

Background on Potential of Process Intensification Through N-1 Perfusion

Process intensification through N-1 perfusion has emerged as a critical strategy for enhancing productivity in biopharmaceutical manufacturing. The biopharmaceutical industry is experiencing a fundamental shift toward process intensification strategies driven by multiple converging factors. For the critical N-1 seed train stage, intensification enables both higher inoculation densities and faster turnaround times. Cell culture intensification through N-1 perfusion enables seed volume ratios of 1:50 to 1:100 (e.g., a 10L bioreactor inoculating a 500-1000L production vessel) compared to traditional 1:5 to 1:10 ratios used in fed-batch seed trains, achieved by maintaining cells in exponential growth phase at high viable cell densities (>50× 10^6 cells/mL), thereby reducing the number of required seed expansion steps, decreasing overall process time by 30-50%, minimizing contamination risk, and substantially lowering facility footprint and capital equipment requirements for seed train operations.[1]

Experiment Objective: Feasibility of Conversion of a 3L Benchtop Glass Bioreactor to a Perfusion Bioreactor for Process Intensification

With the Magma Advance Pumping System (APS), an organization can take advantage of N-1 Process Intensification opportunities by converting an existing bioreactor to a perfusion bioreactor.

The Magma APS offers the following features to operate as a perfusion device connected to a bioreactor (see Figure 1):

- A low shear pump diaphragm that connects to a hollow fiber filter via a sanitary clamp with the filter performing the function of retaining the cells within the bioreactor vessel and the pump creating the alternating tangential flow action (ATF) within the fibers of the hollow fiber to sweep the fiber's inner wall to prevent clogging of the fiber pores.
- The diaphragm pump design and pumping action minimizes cell damage to maximize cell growth and eliminates possibility of tubing failure during a lengthy run.
- Performs the vessel weight control by automating the media feed as spent media is steadily removed via the permeate pump operating at the set vessel volumes per day (VVD) flow rate.
- Has the ability to measure the hollow fiber pressures to monitor filter performance with up to three single use pressure sensors which is particularly useful in monitoring permeate pressure to determine when the filter fiber pores may be clogging.

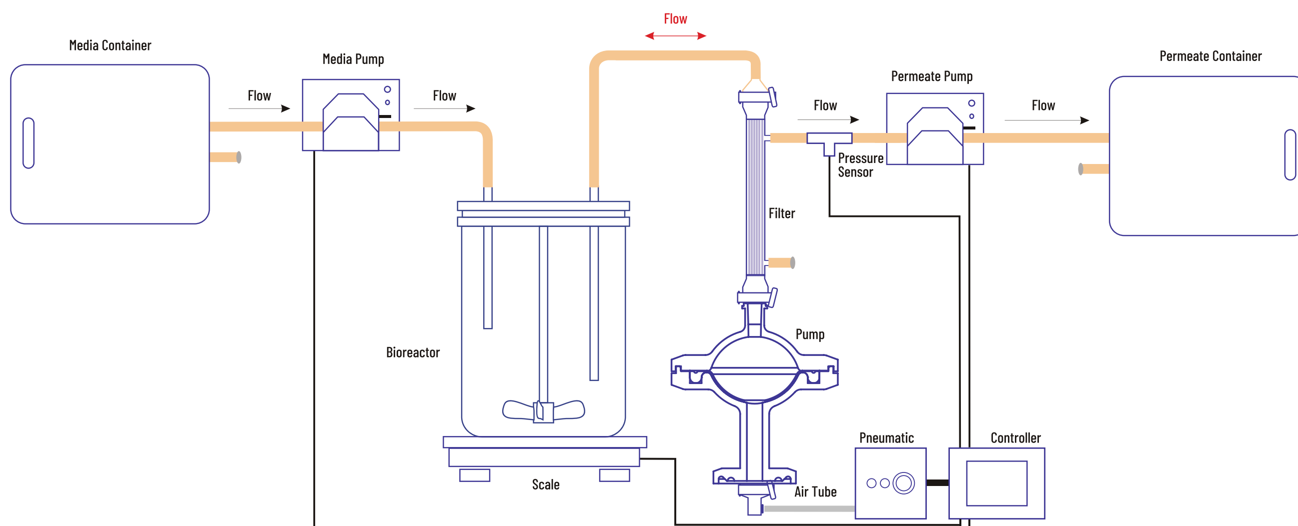

Figure 1- Process Schematic

The bioreactor that is typically used in batch mode, to which the Magma APS was connected is a 3 L Eppendorf BioFlo 320 bioreactor with a 2 L working volume. The BioFlo 320 is a common laboratory workhorse but is not natively designed for perfusion. The Magma APS pump model used was APSP-PH50-SU (Magma APS-50D Dome Pump Head, Polysulfone) with diaphragm APSP-DIA50D (Magma APS-50 Dome Diaphragm, Silicone) and this pump has a displacement volume of 50mL. Hollow fiber filter used was from Cytiva Part number CFP-4-E-4MA Hollow Fiber Cartridge which as a 0.45um pore size, 1mm fiber lumen ID, and 420cm2 of filter area.

The experiment was designed to run 14 days in perfusion mode after the inoculation and initial batch growth of the culture. The objectives for the experiment were three-fold:

1. Increasing cell density to limits that could be achieved without any equipment or media optimization to demonstrate the N-1 Process Intensification potential on a bioreactor typically used by the facility and staff for batch processes.
2. Magma APS robustness for a 14 day perfusion run including beyond the time frame where the maximum bioreactor supported cell density was achieved.
3. Operate without a negative effect on cell growth and viability.

Summary of Work Completed

This study evaluated the performance of the Magma Advanced Pumping System (Magma APS) with ATF filtration in supporting high density CHO cell perfusion culture. Following successful thaw, recovery, and expansion of NISTCHO cells, a 3 L Eppendorf BioFlo 320 bioreactor was inoculated with 2 L at 0.3×10^6 cells/mL and after the initial several days operated in a batch mode, it was then operated in perfusion mode using BalanCD CHO Perfusion medium. The Magma APS controlling ATF filtration was also integrated for automated media feed to control vessel volume and perfusion rate control by the integrated permeate pump. Perfusion was initiated on Day 4 in response to nutrient depletion and increased from 0.5 to 1.5 VVD as cell density rose. Throughout the two-week run, the system maintained stable in and out pump head cycle times (ie, tangential / cross flow rate), controlled the working volume at 2 L, and consistently regulated glucose, glutamine, and lactate concentrations through automated media exchange.

Figure 2 is a photograph the experimental setup and all of the components line up with the Figure 1 process schematic illustration. One exception is the permeate collection scale is under the lab bench with a collection bag placed on it which is filled by the permeate pump.

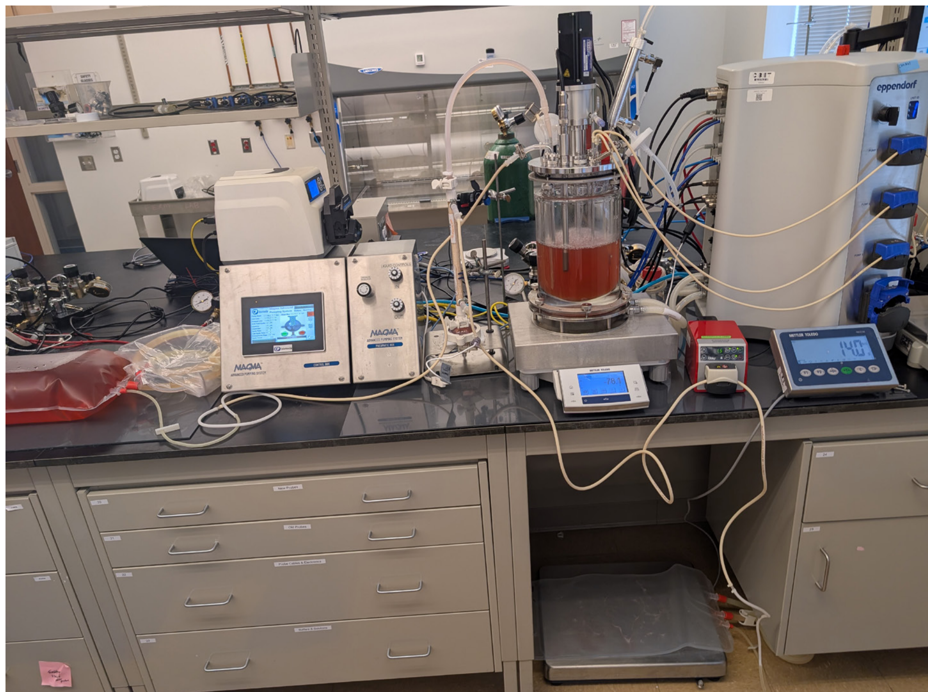

Figure 2. Photograph of Laboratory Setup

The Magma APS enabled cell densities exceeding 40 × 10^6 cells/mL, well beyond typical batch or fed batch processes, demonstrating its robustness and capacity for culture intensification.

As biomass increased on Day 9 to the peak achieved, oxygen transfer limitations required higher sparging rates, increased agitation, and subsequent culture bleed to manage cell density. This shifted to the second objective of the experiment: Equipment Robustness Verification. With many adjustments to maintain the culture during this phase of the experiment for a 14 day perfusion run (modified process parameters and culture bleeds), the Magma APS maintained stable pressures and reliable performance under high cell density conditions including consistent ATF flow rates driven by the Magma APS diaphragm pump and accurate maintenance of the vessel volume. Overall, the trial confirmed Magma APS as a stable and capable perfusion platform, with improved oxygen transfer capacity expected to further enhance culture longevity and perfusion performance in future studies.

Details on Experiment Setup and Results

Seed Culture Preparation

A vial of NISTCHO cells was thawed and seeded into a 125 mL flask containing 30 mL of Ex-Cell Advance Fed-Batch medium, which was the medium used prior to cryopreservation. After three days, cells were passaged again in Ex-Cell Advance Fed-Batch medium to allow for recovery (Passage 1); viable cell density (VCD) and viability at this passage were 2.5 × 10^6 cells/mL and 97.7%, respectively.

Beginning at Passage 2, cells were transitioned into BalanCD CHO Perfusion medium. At Passage 2 (four days after Passage 1), VCD and viability were 5.0 × 10^6 cells/mL and 97.6%, and at Passage 3 (three days later), VCD and viability were 2.5 × 10^6 cells/mL and 96.9%.

At Passage 4, cells were expanded into 200 mL of BalanCD CHO Perfusion medium to generate the bioreactor seed culture, with a VCD of 3.2 × 10^6 cells/mL and viability of 97.0%. Post autoclave, approximately 190 mL of this culture was used to inoculate the bioreactor at a target seeding density of 0.3 × 10^6 cells/mL. The post-inoculation cell count on Day 0 (Sample ID 26001-A) confirmed a VCD of 0.3 × 10^6 cells/mL and viability of 93.9%.

Reactor Set Up and Seeding

An Eppendorf BioFlo 320 3 L jacketed glass reactor was outfitted with the necessary probes, fittings, feed/harvest ports, weldable connections, etc. for CHO cell culture. Bottles containing 50% glucose, antifoam (Sigma Aldrich Antifoam C emulsion), and an empty bottle for 7.5% sodium bicarbonate were also attached via lines connected to the triple addition port. The Magma APS pump head connected to the hollow fiber filter to drive the ATF filtration was assembled next to the reactor. The reactor was placed on a balance for volume control. The retentate line of the hollow fiber filter connected to the diaphragm pump, from the Magma APS, was connected to a harvest dip tube that extended to the bottom of the vessel.

For sterilization, the reactor was filled with 2 L of phosphate buffered saline (PBS). The MAPS was activated to draw PBS into the pump head and wet the membrane. The permeate pump was activated during this time to ensure the shell side of the filter was also wetted. After draining the pump head, filter, retentate line, shell, and permeate line back to the vessel by holding it upside down above the dip tube, a 0.2 um sterile filter was fitted to the permeate line to allow for gas flow during autoclaving. The air line connected to the pump head from the Magma APS control system pneumatic box, was disconnected so the filter and pump could be drained and only the pump head connected to the filter was secured upright to the side of the reactor. The reactor, attached bottles, and the connected filter were sterilized by autoclave (30 minutes at 121°C).

After sterilization, the reactor was allowed to cool for several hours under positive pressure of air supplied through the overlay port. Once cool, the PBS was drained and 2 L of BalanCD CHO Perfusion medium was charged to the reactor through an inline 0.2 um sterile filter. Sodium bicarbonate was added to the appropriate bottle in a biosafety cabinet. The reactor was then connected to the BioFlo 320 control tower, warmed to 37°C, and held overnight with a low flow of overlay air.

The following morning, the media remained clear and pH was in the expected range, so the reactor was seeded with 190 mL of NISTCHO culture at a viable cell density 0.3 x e6 cells/mL and 93.9% viability.

Culture Growth and Perfusion to Peak Density

After seeding, the culture grew normally for the first 4 days. On Day 4 at an elapsed fermentation time (EFT, hh:mm) of 96:50, the cross-flow and volume control of the Magma APS pump were tested successfully. In/out cycle times of the Magma APS pump were set to roughly 6.5 seconds which targets the ATF flow rate at 0.46 LPM (Magma APS-50 pump head with a volume of 50mL). A picture of the pump in a cycle mid point is show in Figure 3 below. According the Cytiva literature, the 0.46 LPM translates to a shear rate of 2300 sec-1. A 10 L bag of sterile BalanCD CHO Perfusion medium was connected to the feed line, and the media feed was started at EFT 98:40 due to a drop in glucose and glutamine concentration to 4.96 g/L and 2.35 mM, respectively. The attached feed pump and balance were used to automatically maintain a 2 L working volume in the vessel. Permeation rate was initiated at 0.5 VVD (0.7 mL/min).

Figure 3. Photo of Pump Cycle at Halfway Point of a Liquid Out Cycle with Cell Culture Visible

Following the start of perfusion, a slight drop in viability (from 96.3% at EFT 98:30 to 93.3% at EFT 116:36) was observed. However, this initially began to increase back up to 94%-95% over the next few days and cell doubling time remained normal. Perfusion rate was increased to 1 VVD (1.4 mL/min) at EFT 141:00. At EFT 164:42, it was observed that the %DO had dropped to 6.8% due to oxygen transfer issues caused by the high cell density (TCD of 19.1e6 cells/mL). To combat this issue, an overlay of pure O2 was applied at 0.5 SLPM, the max O2 sparge rate was raised from 0.3 SLPM to 0.6 SLPM, and agitation was raised from 130 to 140 RPM. Perfusion rate was raised to 1.5 VVD (2.1 mL/min) at EFT 166:00.

At EFT 176:10, %DO began to drop again due to oxygen transfer problems, so max sparge rate and RPM were increased to 0.7 SLPM and 150 RPM, respectively. Despite this, %DO continued to drift down, finally reaching roughly 2% around EFT 186:58, so the max O2 sparge rate was raised again to 0.8 SLPM and agitation was increased to a max of 165 RPM.

At EFT 191:12, viability was measured at 91.6%, which was followed by a sharp drop in %DO to near 0% around EFT 200:82. At EFT 213:58, which is Day 9, agitation and O2 sparge rate were raised to 200 RPM and 0.85 SLPM, respectively. At these conditions of maximum agitation and high sparge rate to support the increased cell density of 42.5e6 cells/mL, there was an additional drop in viability to 87.7% which was observed at EFT 216:05. The culture at this point is shown in a photograph in Figure 4. This triggered the end of the first experiment objective with the peak cell density with the 42.5e6 cells/mL on Day 9 which is 5 days after perfusion was started on Day 4. Without process, equipment, and media optimization, there were persistent challenges in maintaining adequate %DO with the high-density cell culture. The data from the inoculation, equipment, through the achievement of the cell density of 42.5e6 cells/mL is shown in Table 1. The growth curve is shown in Figure 4.

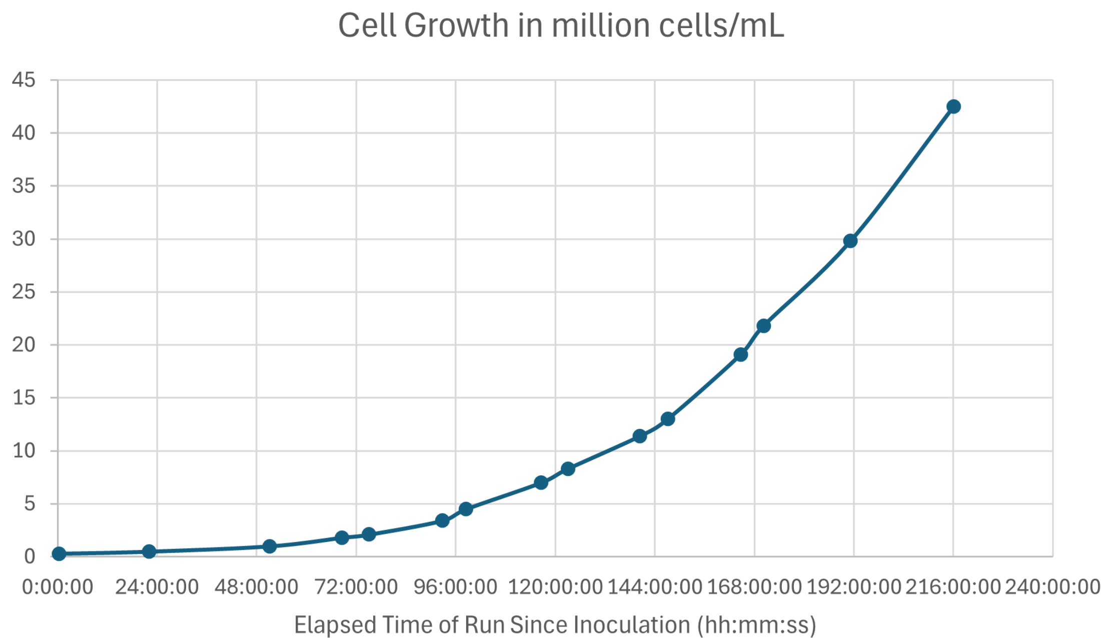

Figure 4. Cell Growth Curve Day 0 Through Day 9

Table 1. Data from Phase 1 of the Experiment

Day #	EFT (hh:mm:ss)	Offline pH	Spent Media Mass (g)	Cell Count			Metabolites				Online Reactor Data			Notes
				Total Cells (x 10^6) cells/mL	VCD (x10^6) cells/mL	% Viability	Glucose (g/L)	Lactate (g/L)	IgG (g/L)	L-Glu (mM)	pH	%DO	%CO2	
0	0:19:00	7.30	N/A	0.3	0.3	93.9	7.63	0.11	0.01	5.5	7.19	80.08	8.5	
1	22:04:00	7.27	N/A	0.5	0.5	95.1	7.75	0.21	0.07	5.12	7.19	55.8	8.3	
2	51:05:00	7.35	N/A	1	1	96.6	7.34	0.51	0.07	4.28	7.19	40	7.8	Restandardized online pH to 7.35
3	68:30:00	7.25	N/A	1.8	1.7	97.1	6.93	0.81	0.03	3.63	7.25	39.5	9.3	
3	75:04:00	7.21	N/A	2.1	2	96.6	6.55	0.96	0.04	3.35	7.22	41.5	9.8	
4	92:43:00	7.21	N/A	3.4	3.3	97	5.4	1.45	0.06	2.64	7.2	38.4	8.4	
4	98:30:00	N/A	98.5	4.5	4.3	96.3	4.96	1.58	0.07	2.35	7.18	40.2	7.7	
5	116:36:00	7.23	767	7	6.6	93.3	4.37	1.69*	0.09	2.58	7.17	40	6.6	*Lactate reading a bit above the testing range
5	123:00:00	7.21	1007.5	8.3	7.7	93.9	4.06	1.85	0.1	2.51	7.17	39.6	6	
6	140:26:00	7.15	1641	11.4	10.8	94.7	2.66	2.19	0.16*	2.03	7.13	40.7	3.6	*IgG reading a bit above the testing range
6	147:06:00	7.13	2118	13	12.3	94.9	2.63	2.09	0.15	2.16	7.12	39.6	4.2	
7	164:42:00	7.05	3467.5	19.1	18	94.3	1.84	1.9	0.2	1.91	7.09	6.8	5.9	DO too low. Changed overlay to 100% O2 0.5 SLPM, max sparged O2 value in cascade set to 0.6 SLPM, agitation to 140 RPM
7	170:13:00	7.1	88.5	21.8	20.6	94.7	1.82	1.81	0.2	1.98	7.14	42	5.4	Waste bag emptied, see general notes
8	191:12:00	7.01	2405.5	29.8	27.2	91.6	0.53	1.72	0.26	1.73	7.16	20.1	5.9	Waste bag emptied, balance tared, spend media stored at 4C
9	216:05:00	7.16	3750	42.5	37.2	87.7	0.05	0.41	0.35	1.41	7.26	19.1	11.1	421g of culture removed.

Equipment Robustness Verification Phase of Project

To start the next phase of the experiment to maintain a 14 day perfusion run, with the high cell density of 42.5e6 cells/mL causing high oxygen demand, this prompted a bleed of 421 g of culture (20% vessel volume). The %DO setpoint was also lowered from 40% to 20% to reduce the demand for a high rate of O2 gas flow.

For the remaining 9 days, to maintain the culture cell density <20e6 cells/mL, agitation was also gradually reduced to 170 RPM to reduce shear and daily 600 g (30% vessel volume) bleed and the %DO setpoint was lowered to 15% with the aim to reduce sheer stress by decreasing total O2 sparge rate. These conditions were maintained throughout the remainder of the growth until the termination of the experiment on Day 18. This amounts to 14 days in a perfusion mode with the Magma APS operationally controlling the ATF filtration rate of the diaphragm pump, controlling the vessel level, and measuring the permeate pressure mainly to monitor the filter performance.

Figure 5. Close-up Photograph of Bioreactor at Peak Cell Density at EFT 216:00

Magma APS Performance During the 18 Days with 14 Days in Perfusion Mode

During the 14 days in perfusion mode, The Magma APS system performed continuously as designed with no failures or alarms. The following observations can be noted:

1. The Magma APS air diaphragm pumped continuously at the same targeted flow rate of 0.46 LPM with no adjustments for the entire run even with the varying culture conditions and biomass changes.
2. The vessel weight was controlled at the targeted 2 liters accurately and this includes automatic replenishment of media after numerous bleeds of the vessel.
3. The Magma APS with its ability to monitor the filter performance via the single use pressure sensors connected to the hollow fiber filter, indicated during the run, even with the high cell densities, that there was no evidence of filter fouling during the run because the permeate line pressure did not drop. If the filter started to foul the permeate pressure would drop because there be more resistance as the permeate pump draws liquid through the filter at the VVD setpoint and the permeate pressure sensor would move to a more negative value and this was not observed.
4. Additionally, not seeing a drop in the permeate pressure indicates the ATF action was effective in sweeping the inner surface of the fibers which prevents fouling during the 14 days of perfusion.

Conclusions

The experimental objectives were as follows:

1. Increasing cell density to limits of process without any equipment or media optimization to demonstrate the N-1 Process Intensification potential on a bioreactor typically used by the facility and staff for batch processes by connecting the Magma APS connected to a hollow fiber filter to the bioreactor.
2. Magma APS robustness for a 14 day perfusion run including beyond the time frame where the maximum bioreactor supported cell density was achieved.
3. Operate without a negative effect on cell growth and viability.

- Regarding the first objective, the Magma APS demonstrated the ability to "unlock" the potential of using existing hardware for process intensification through N-1 perfusion. Using the 3 L Eppendorf BioFlo 320 bioreactor with a 2 L working volume typically used in a batch or fed-batch mode, outfitted with the Magma APS connected to the hollow fiber filter, it was successful in increasing the cell density to 42.5e6 cells/mL in 5 days in perfusion mode (day 9 of the run) from an inoculation density of 0.3×10^6 cells/mL. Oxygen demand exceeded the KLa of the bioreactor overnight between days 7 and 8 and on day 9, a bleed was commenced at the peak density so the culture could be maintained for the 19 days total.

- With the wide range of Magma APS pump sizes available it can be used across a wide range of bioreactor sizes and production hardware to run process intensification at different scales. Additionally, single-use pump heads enable rapid turn-around in process campaigns.

- For the second objective, the 14 days in perfusion mode, this experiment demonstrated that the MAPS is a robust system that can successfully maintain a CHO cell culture and drive cell density far beyond what is achievable in a batch or fed-batch culture in 9 days total (5 days of perfusion). With the automated addition of media as the permeate pump removed media at the desired VVD flow rate, the lactate, glucose, and glutamine levels were maintained smoothly within acceptable limits, and controlled accurately to the 2 L target controlled by the integrated feed and permeate pumps. The Magma APS diaphragm pump flow rate and system pressure remained consistent over the two weeks of perfusion despite the dense culture and changing culture conditions.

- The Magma APS performed well and drove the NISTCHO culture to a density that exceeded the physical oxygen-transfer capabilities of a standard benchtop bioreactor.

- As the culture became extremely dense and the bioreactor struggled with oxygen transfer, the Magma APS filtration system remained perfectly stable for the entire 14 days.

- For the third objective, the viability was stable at 94+/-1% across twice-daily readings from the start of perfusion on day 4 through the end of day 7. There was steady cell growth and high viability in perfusion until days 8 and 9 when the cell density increased significantly and the KLa limitations for the equipment seemed to be reached as the oxygen dropped close to zero and then aggressive gas sparging and agitation was required to try to drive the oxygen up to adequate levels. A subsequent experiment using upgraded bioreactor hardware for highly density mammalian cell cultures would help further shed light on the potential of the Magma APS integrated into a bioreactor in terms of any viability impact.

- This part of the experiment demonstrated equipment reliability- the vessel weight control was maintained during the entire run, and when the bleeds were done, media was replenished automatically. There were no alarms or equipment failures occurred during the entire run.

- And the Magma System with its ability to monitor the filter performance via the single use pressure sensors connected to the hollow fiber filter indicated during the run, even with the high cell densities, there was no evidence of filter fouling during the 19 day run. This indicates the ATF action was effective in sweeping the inner walls of the fibers and prevent then from fouling.

[1] White Paper: "Process Intensification Using the Magma™ Advanced Pumping System for Alternating Tangential Flow (ATF) Filtration: A Comparative Case Study of N-1 Perfusion at 500mL Scale, Authored by bioX Process Development, December 29, 2025;