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Pushing the Limits

Maximizing Scale and Speed with Intensified Single-Use Tangential Flow Filtration (SUTFF)

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Abstract

Biologic manufacturers are operating at increased speeds, volumes, and concentrations to meet the demands of the industry. Faster processing of larger volumes puts pressure on bioprocessing equipment and consumables. In this application note, we assessed the potentially demanding effects of rapid circulation flow rates on 50 L and 200 L Flexsafe® Pro Mixer bag designs when used as the recirculation tank in large-scale, single-use tangential flow filtration (SUTFF) systems, such as the Sartoflow® 4500. We tested three use cases to map the potential of surface fountaining, tube collapse, and bag deformation occurring in the Flexsafe® Pro Mixer bags in different flow path designs.

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Introduction

The growing push to make drugs affordable puts pressure on biomanufacturers to maximize their production efficiency. This might include handling increasing yields, operating at larger scales, and meeting targets more quickly in a small footprint facility.

Bioprocessing scientists commonly use Flexsafe® Pro Mixer bags as single-use (SU) containers throughout upstream and downstream process steps for volumes between 50 L and 3,000 L. This includes tangential flow filtration (TFF) — also known as crossflow filtration (CF) — applications that are important for downstream purification. In this unit operation, the Flexsafe® Pro Mixer bags act as the recirculation tank where the protein can be concentrated and diafiltrated to a target excipient buffer formulation using a product retaining filter, such as the Sartocon® Hydrosart® cassette.

Flexsafe® Pro Mixer bags are preassembled and deliver low shear, high torque mixing. Regardless, there are growing needs for increased filtration rates and scales to keep up with market demands. Comprehensive testing has yet to be carried out to assess the physical demands of increased fluid rate and fill volume on our Flexsafe® Pro Mixer bags and the corresponding setups.

To understand the potentially demanding effects of the required high recirculation flow rates (up to 5,000 L/hr), we carried out application testing across three use cases. We employed 50 L and 200 L Flexsafe® Pro Mixer bags as recirculation tanks in mock single-use TFF | CF system loops capable of covering 7 m² and 14 m² filter (self-contained and cube format) surface areas.

Materials and Methods

Materials
The items and hardware used in these applications are characterized in Tables 1 and 2, respectively.

Single-Use Consumables	Part Number
Sterile filter transfer set with Sartopore® Air sizes 7, 8, 9, and 0	FILTER 5195307AXG-SO X = 7, 8, 9, 0
Flexsafe® Cubical for Pro Mixer 50 L	FMS308237
Flexsafe® Cubical for Pro Mixer 200 L	FMS308518
1" Reinforced SU-loop 1" ID × 1 ⅜" OD without cassette	

Table 1: *SU Items Used in the Study*

Equipment Item	Part Number
Pro Mixer drive unit	FMD 300001
Palletank® for Mixing 50 L	FXC 301951
Palletank® for Mixing 200 L	FXC 301953
PSG Dover; Quattroflow™ QF5050SU; diaphragm pump	
▪ 1" ID BioPAT® Flow (F1) 1" ▪ 1" ID BioPAT® Pressure (P1) 0 – 4 bar ▪ WIKA negative pressure (P2) 1 – 3 bar transmitters ▪ Floor scale	
Flexact® 2.0 for process control and data acquisition	

Table 2: *Equipment Used in the Study*

Image 1: *Flexsafe® Pro Mixer Palletank for Mixing With Drive Unit & Flexact® Modular Tangential Flow | Crossflow Filtration*

Procedure

We pre-inflated the bag with air under aseptic conditions to assure the correct shape, which is cubical without any bulging or signs of collapse. The bag was then filled with the test solution (water or glycerin) and inspected to ensure the impeller was completely covered with liquid. We determined the maximum impeller speed without recirculation by starting the impeller and increasing the speed slowly until a vortex was created. The recirculation loop with a fill or drain pump head was installed, and the loop or line was formed with a tubing-based SU flow kit assembly. The pump was then set to the required flow rate and the determined the maximum impeller speed with recirculation.

Return inlet fountaining was defined by a visual breakage of the surface of the liquid interface (wave crash) and bubble | foam formation. We defined outlet vortex forming by a visual observation of an air funnel tip from the liquid surface reaching the outlet port. Three viscosity levels were used to mimic increasing protein concentration and its effects on liquid surface tension and density. Both 50 L and 200 L bag sizes were tested (50 L is limited with available space on the side and bottom of the bag, and 200 L represents all larger volume mixing bags with equivalent or increased port availability).

Use Case 1

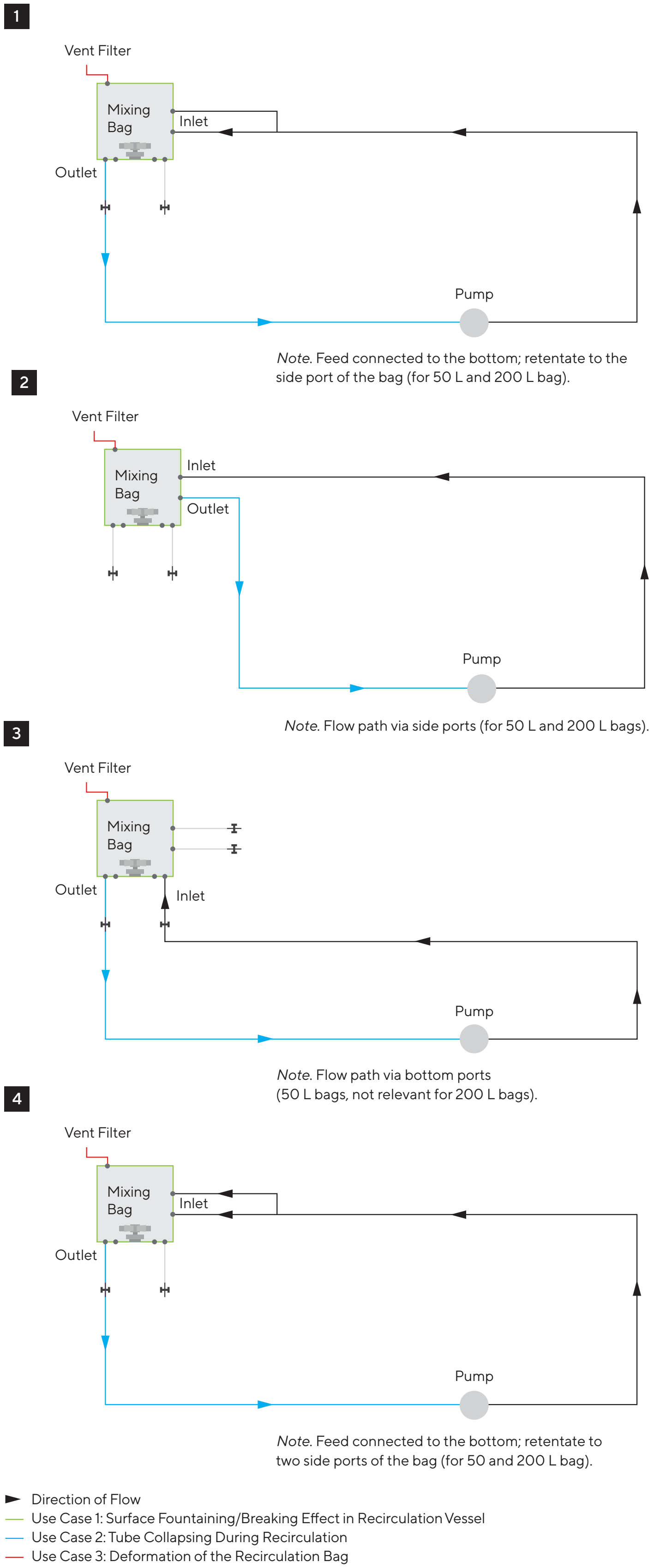
A visual check was conducted to establish if the recirculation rate created a critical fountain (liquid surface breakage), introducing air to the liquid. The impeller speed was then reduced to avoid vortex formation. The bag fill volume and fluid viscosity were also addressed.

Use Case 2

The suction tube was visually inspected to determine if there were any signs of tubing collapse, and the pressure in the suction line (P2) was logged. The procedure was repeated with higher flow rates until 5,000 L/hr was achieved or a tubing collapse event occurred.

Use Case 3

The pressure equalization capability of the vent filter was tested against the theoretical maximum fluid filling | draining rate of the bag to observe any signs of bag collapse or overexpansion.



Note. Feed connected to the bottom; retentate to the side port of the bag (for 50 L and 200 L bag).

Note. Flow path via side ports (for 50 L and 200 L bags).

Note. Flow path via bottom ports (50 L bags, not relevant for 200 L bags).

Note. Feed connected to the bottom; retentate to two side ports of the bag (for 50 and 200 L bag).

Figure 1: *Four Flow Path Setups Tested Across the Use Cases*

Use Case 1: Surface Fountaining | Breaking Effect in Recirculation Vessel

Introduction

Jetting or surface breakage from return lines (from either the bottom or side ports) can lead to protein damage and yield loss due to foaming (1, 2). We compared surface fountaining in Flexsafe® Pro Mixer bag designs from return lines in different flow path routes.

Methods

We recirculated water, a 3cP glycerin | water solution, and a 30 cP glycerin | water solution through a TFF system and compared four different flow path designs (Figure 1). We also tested two bag sizes (50 L and 200 L) and various fluid fill levels (Table 3). For flow paths 1 and 2, various tank fill levels, mixer drive speeds, and retentate return positions were evaluated.

Results and Discussion

With the return line entering the side of the bag, options 1, 2, and 4 showed no fountaining effect. However, option 3 showed a strong fountaining effect, which creates filling levels below those defined in options 1, 2, and 4. Therefore, option 3 is impractical to use. For option 2, it was not possible to reduce the working volume during final concentration, meaning it is also not a viable option. As a result, only options 1 and 4 were used for subsequent testing.

With both options 1 and 4, the minimum feasible working volume is 15 L (50 L bag size) and the full flow range of the QF-5050 pump can be used (Figure 3, Table 3). There appears to be no clear advantage to using two ports simultaneously for the retentate return. As expected, with fluid surface elevation levels and higher viscosity values, the mixer speed can be increased (Figure 3). The mixing efficiency was sufficient, but this was only inspected visually.

The danger of a fluid path short circuit is much higher with the 50 L bags due to the shorter distance between the two ports. For the 200 L bag, the risk is lower. Still, in theory, the Pro Mixer rotation direction is transporting the retentate (with the diafiltration buffer in Sartoflow® 4500) directly toward the feed outlet (Figure 4). The effect on diafiltration was investigated, and ideally an inverted configuration is recommended.

These results demonstrate that the optimum setup is to connect the feed out to the bottom port of the bag and the retentate return inlet to the side port of the bag (Figure 4). Our guidance for use is that the distance between both connector ports should be as large as possible to prevent short circuits, which will negatively influence the diafiltration efficiency.

We also recommend incorporating a tube holder for the retentate return tubes to guide and support the weight of the connection components. Two retentate ports are possible and will enable slightly higher retentate flow rates. However, this limits opportunities for the use of in-bag sensors. Theoretically, it is possible to further reduce the total hold up volume during the final concentration phase (estimation for Flexsafe® Pro Mixer | Sartoflow® 4500 combination: 2.5 L).

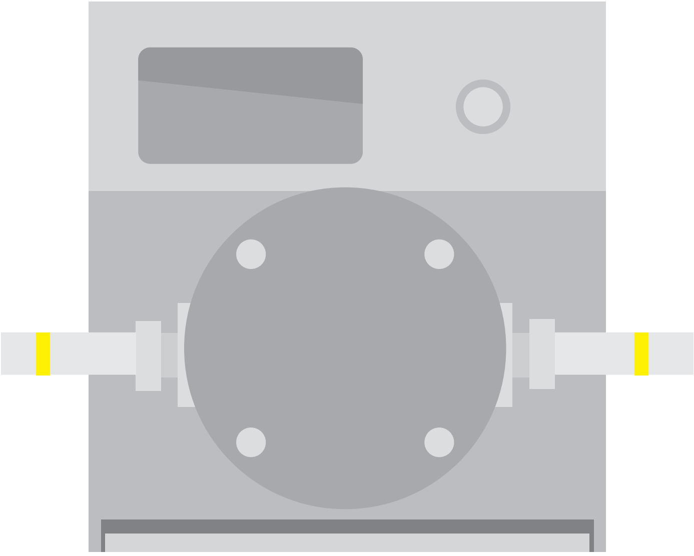


Figure 2: *Illustration of Diaphragm Pump*

50 L Option 1 Design — Max Retentate Flow

Volume	1 cP	3 cP	30 cP
15 L	2520 L/hr	2520 L/hr	2520 L/hr
20 L	4020 L/hr	4020 L/hr	3400 L/hr
25 L	4020 L/hr	4020 L/hr	3400 L/hr
55 L	4020 L/hr	4020 L/hr	3500 L/hr

50 L Option 4 Design — Max Retentate Flow

Volume	1 cP	3 cP	30 cP
15 L	4020 L/hr	4020 L/hr	3600 L/hr
20 L	4020 L/hr	4020 L/hr	3800 L/hr
25 L	4020 L/hr	4020 L/hr	3800 L/hr
55 L	4020 L/hr	4020 L/hr	3800 L/hr

200 L Option 1 Design — Max Retentate Flow

Volume	1 cP	3 cP	30 cP
25 L	4500 L/hr	2520 L/hr	2520 L/hr
32 L	4500 L/hr	3120 L/hr	3400 L/hr
50 L	4500 L/hr	4500 L/hr	3400 L/hr
125 L	4500 L/hr	4500 L/hr	3500 L/hr
205 L	4500 L/hr	4500 L/hr	3500 L/hr

200 L Option 4 Design — Max Retentate Flow

Volume	1 cP	3 cP	30 cP
25 L	4500 L/hr	2520 L/hr	3600 L/hr
32 L	4500 L/hr	3120 L/hr	3800 L/hr
50 L	4500 L/hr	4500 L/hr	3800 L/hr
125 L	4500 L/hr	4500 L/hr	3800 L/hr
205 L	4500 L/hr	4500 L/hr	3800 L/hr

Table 3: *Filling Levels and Flow Rates for Flow Path Options 1 and 4*

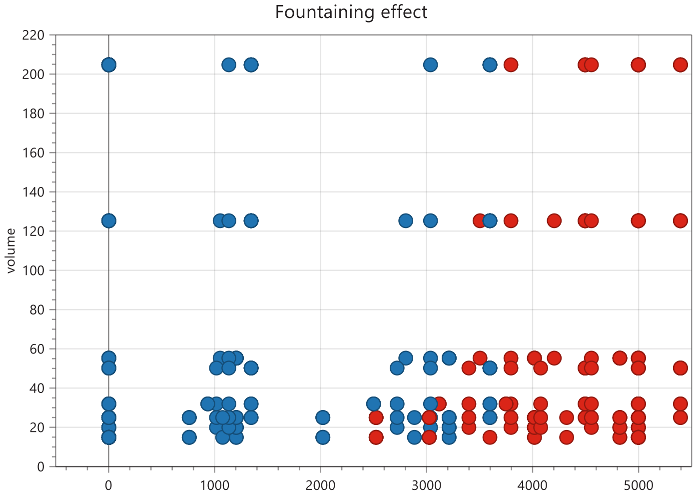


Figure 3: *A 2D Dot Plot of the Principle Factors (Volume in the 3D Mixing Bag on the Y-Axis and Max Retentate Circulation Flowrate on the X-Axis) Resulting in Surface Breakage or Fountaining*

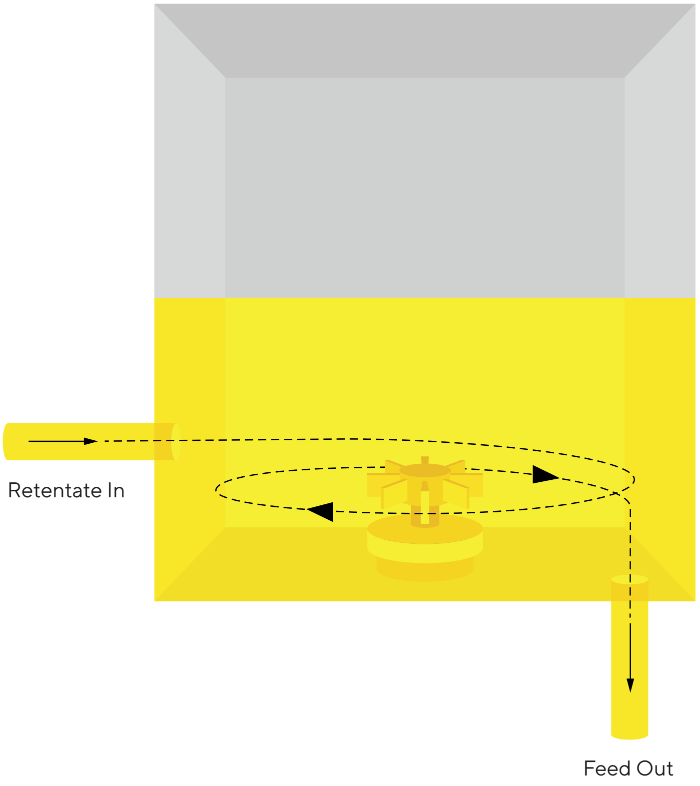


Figure 4: *Ideal Set Up for Mixer and Retentate | Feed Bag Position*

Use Case 2: Tube Collapsing During Recirculation

Introduction

High flow rates between the recirculation bag and the draining | recirculation pump can result in vacuum (suction) pressures up to −0.4 bar (Figure 1). This can cause deformation of the feed tubes when the tubing material is not vacuum-resistant. We tested the possibility of the tube collapsing between the recirculation bag (the suction side) and the recirculation pump by measuring how vacuum pressure correlates with feed flow and tank filling volume | viscosity. We used single-braided tubing as the control and double-braided vacuum-resistant tubing as the proof of concept, which showed to be resistant to collapse.

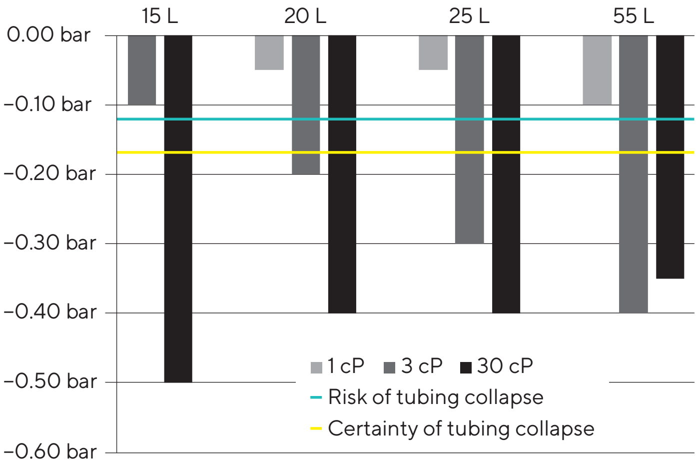
Methods

We recirculated water, a 3cP glycerin | water solution, and a 30 cP glycerin | water solution through a TFF system with a 50 L and 200 L recirculation tank. The suction side was visually inspected for any deformation of the flow kit at each flow rate setpoint. Additionally, the pressure at L2 was logged.

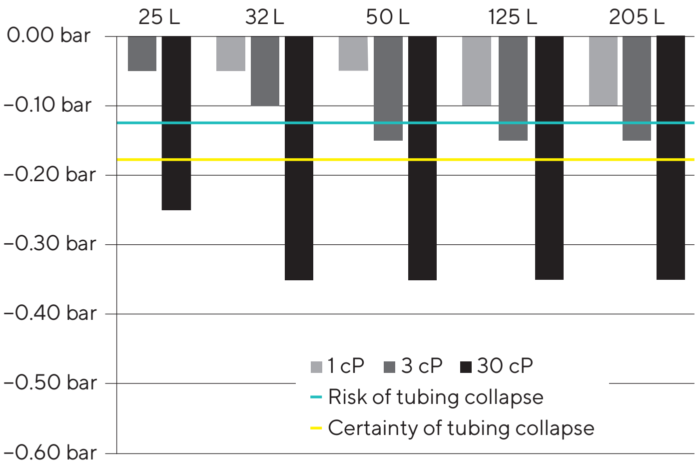
Results and Discussion

Our results confirm that high flow rates caused increased vacuum pressure. Elevation (height aspect ratio, or the difference of height from the outlet to the pump) influenced vacuum pressure only the first 1,500 – 2,000 L/hr or 0 – 100 mbarg. At flow rates above 4,000 L/hr, vacuum resistant | double-braided tubing is necessary for operation. Otherwise, there is a significant risk | certainty of tubing collapse when using single-braided tubing. With both tank sizes, suction pressure elevates with increasing viscosity, leading to the collapse of the feed tube. This increased viscosity correlates with the higher surface tension forces, increasing vacuum pressure effects. Elevation and higher fill volume increase hydrostatic positive pressure at the base of the tank | bottom port of the feed | suction line.

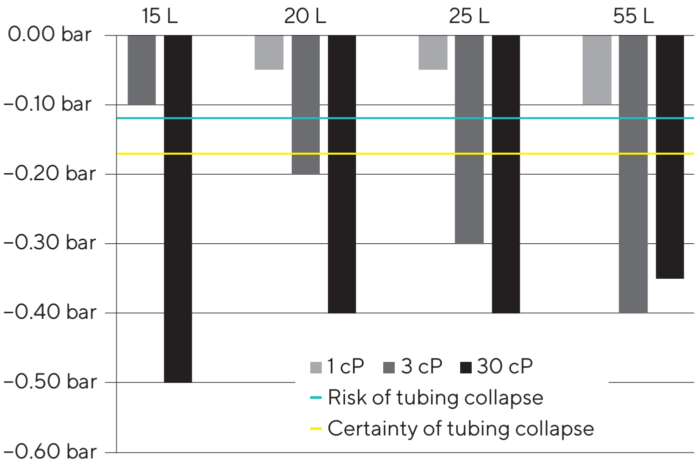
50 L Flow Path Setup Option 4 — Average Suction Pressure



200 L Flow Path Setup Option 1 — Average Suction Pressure



50 L Flow Path Setup Option 1 — Average Suction Pressure



200 L Flow Path Setup Option 4 — Average Suction Pressure

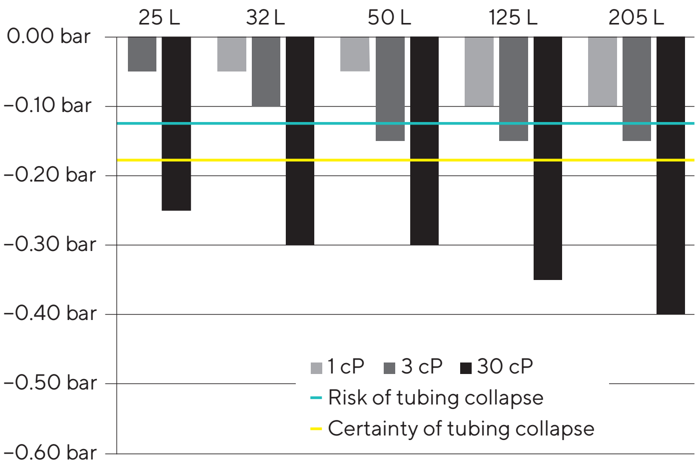


Figure 5: *Correlation of Feed Flow and Vacuum Pressure*

Therefore, the recirculation tank's total volume and fill volume is a factor to consider in the risk of feed tubing collapse.

We recommend using a double-braided silicone tube which is extremely rigid toward vacuum pressures. The connections at the bag should also ideally consist of double-braided tubing. If using single-braided tubing, we recommend that the length does not exceed 100 mm.

Use Case 3: Deformation of the Recirculation Bag

Introduction

Changes in recirculation tank fill volume due to the high permeate flow rates present in large-scale TFF systems during (final) concentration steps could lead to deformation of the recirculation bag. Such deformations can lead to dead zones, folds, and creases in the mixing bag and inefficient mixing. It is also important how the sizing of the recirculation vent filter affects the draining of the bag compared to draining under non-aseptic conditions with an open top port.

Methods

We tested four recirculation tank vent filter sizes (Sartopore® Air), from 0.05 m² – 0.45 m² (Table 4) and used two types of feed pumps to fill and drain the recirculation tank:

1. Watson Marlow 730 series pump — Flexact® Modular Crossflow setup
2. Almatech PSG Quattroflow 1200 pump — Sartoflow® 4500 setup.

We used both pumps at their maximum output, utilizing ½" ID tubing.

Image 2: *Sartoflow® 4500 With Installed 1" Flowkit*

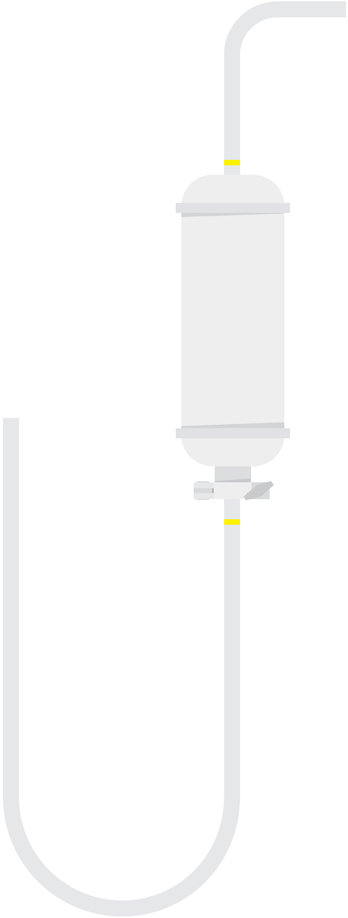


Figure 6: *Illustration of Normal Flow Filtration Line*

Results and Discussion

Our results indicate that for filling flow rates up to 800 L/hr (i.e., WM 730 with ½" ID in Flexact® Modular), a size 9 (0.2 m²) Sartopore® Air is required; for filling flow rates up to 1,200 L/hr (QF 1200 in Sartoflow® 4500), a size 0 (0.45 m²) Sartopore® Air is required. The vent filter retained its diameter. Therefore, the available Flexact® filter holders can be used.

Size	m² Filter Area	Observation
7	0.05 m²	Strong inflation | collapsing failed
8	0.10 m²	Strong inflation | collapsing failed
9	0.20 m²	Strong inflation | collapsing failed OK up to 12 L/min: suitable for WM 730 feed pump
0	0.45 m²	OK up to 20 L/min: suitable for WM 730 and Almatech Quattroflow 1200 feed pump

Table 4: *Recirculation Tank Vent Filter Sizing*

As neither overfilling (expansion) nor collapsing of the recirculation bag is desired within the TFF process, selecting an appropriate size for the vent filter is necessary. The displaced air (from when the bag is filled with fluid) must be let out. When we drained the bag, the flexible shape was not compressed inwards; therefore, mixing was unimpeded and the fluid dynamics of the container were unaltered.

The Palletank® design does not physically contain the top of the bag (with the 8" port). Unchecked expansion or inflation results in the Flexsafe® film stretching the bag upwards to the free space. As a result, both the nominal bag volume and the overall pressure inside the bag increase. Thus, the maximum flow rate of fill and drain versus the time needed to achieve the nominal bag volume is considered the worst case.

Conclusions & Recommendations

Biomanufacturers are always searching for new ways to intensify their production processes, maximizing overall productivity. This typically includes faster processing and dealing with larger volumes and higher concentrations. An important consideration when taking steps towards maximizing production efficiency is the limits of equipment and consumables employed in the process. This is particularly important for next-generation facilities that use process intensification principles coupled with single-use (SU) solutions to purify biomolecules.

There is a growing need for data to support SU solutions for the downstream purification of biomolecules at increasingly large scales. This includes testing the maximum capacity of equipment and consumables and defining ideal setups to ensure their optimum performance.

We performed functional testing to examine potential inlet | outlet feed | return orientations of the Flexsafe® Pro Mixer bag in a large-scale SU TFF system. To test the process parameter limits of the recirculation tank, we examined three cases:

1. The potential for surface fountaining.
2. The possibility of tube collapse.
3. How the size of the vent filter can affect bag deformation.

Surface fountaining introduces air into the sample, which can cause protein damage. This phenomenon occurs when the distance from the bulk liquid surface and the return port outlet is too short and the returning liquid velocity breaks the liquid surface.

We tested this effect in four orientations and found that the optimum setup is to connect the feed out to the bottom port of the bag and the retentate return inlet to the side port of the bag, which corresponds to options 1 and 4 (Figures 1 and 4). There appears to be no clear advantage of using two ports simultaneously for the retentate return. Therefore, we recommend option 1. We also suggest the distance between both connector ports should be as large as possible to prevent short circuits and that a tube holder should be used to support the retentate return tubes.

The clamping support of the retentate return point on the Flexsafe® Pro Mixer bag ensures the re-entry of the return flow at 90 degree to the side of the bag. This prevents an upwards return flow angle which would increase the degree of fountaining and surface breakage, and reduce the minimum working volume space. The exit (feed) and entry (retentate) ports on the Flexsafe® Pro Mixer bag also took into consideration the rotational direction of the impeller. Testing the retentate flow both against and with the impeller rotation ensured the turbulence and mixing effects were within acceptable levels.

Tube collapse can occur under high vacuum pressures due to rapid flow rates and viscous solution. We examined tube deformity with increasing flow rates, filling volumes, and viscosity. Our results revealed a significant risk of tube collapse at flow rates above 4,000 L/hr, and we recommend using vacuum-resistant, double-braided tubing at flow speeds any higher than this.

The circulation bag itself can become over-inflated or creased due to changing fill volumes during draining or filling. We tested the effect of tank vent filter sizes and flow rates on the structure of the Flexsafe® Pro Mixer bag. For filling flow rates up to 800 L/hr, we recommend using a 0.2 m^2 filter. For filling flow rates up to 1,200 L/hr, we recommend a 0.45 m^2.

In conclusion, our observations suggest that commonly used single-use TFF setups remain powerful tools, even at the high speeds, volumes, and concentrations demanded by the biopharmaceutical market today.

References

(1) Clarkson, J. R., Cui, Z. F., & Darton, R. C. (1999).
Protein denaturation in foam: I. Mechanism study.
Journal of Colloid and Interface Science, 215(2), 323–332.
https://doi.org/10.1006/jcis.1999.6255

(2) Maa, Y. F., & Hsu, C. C. (1997).
Protein denaturation by combined effect of shear and air-liquid interface. Biotechnology and Bioengineering, 54(6), 503–512.
https://doi.org/10.1002/(SICI)10970290(19970620)54:6<503::AID-BIT1>3.0.CO;2-N

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