Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Performance Guide

Mobius® FlexReady Solution with Smart Flexware® Assemblies for Tangential Flow Filtration

How to Use This Guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® FlexReady Solution with Smart Flexware® Assemblies for Tangential Flow Filtration (TFF). This guide includes information from a number of applications and case studies that were designed and/or selected to provide a diverse overview of the system performance under a range of expected processing conditions.

The results included in this guide summarize outcomes and observations obtained in studies conducted using particular model feed streams and experimental conditions. Therefore, all test results should be confirmed by the end user using feed stream and process conditions representative of the user's application. It is important to note that results are intended as general examples and should not be construed as product claims or specifications.

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Introduction

The Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF is a fully automated system designed to enable the development and clinical-scale operation of TFF processes for the concentration and diafiltration of mAbs, vaccines, plasma and therapeutic proteins. The system has the same functionality as traditional TFF systems, and by incorporating a completely single-use flow path it provides operational flexibility while eliminating concerns of carryover or cross-contamination.

The Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF is available in two different scales: the TF2S and the TF3S. Both solutions use the same base carts and instrumentation and simply switch clamshells, pumps, and membrane holders to accommodate a wide range of concentration and diafiltration processes. In addition to feed pump, retentate valve, and transfer pump control loops, both systems also include an automated flow control valve on the filtrate line to enable open UF and MF operations. The maximum reachable flow rate during processing may change, mainly due to the membrane area and product viscosity.

A variety of recycle vessel configurations are available to provide maximum process capability. Vessel volumes of 50 L and 100 L are available for the TF2S system and a vessel volume of 200 L is also available for the TF3S system. In addition, all sizes are available with a plastic carrier or a double-jacketed carrier, depending on whether or not temperature control is required. All recycle vessels come with integrated load cells, bottom-mounted mixer, and temperature sensor.

A 0-600 kg capacity floor scale has been specified that connects easily to both systems and allows for tracking of filtrate volume as well as calculation of filtrate flow rate, flux, concentration factor, diavolumes, and membrane permeability. This is a highly recommended option.

Conductivity, UV and pH instrumentation is available as an option for the TFF filtrate line. The UV cell measures dual wavelengths of 280 nm and 300 nm (as standard) with an optical path length of 1, 2.5, or 10 mm. The instrumentation can be selected in Multi-use or Single-use format.

The specific system size and configuration of components that was used to generate the performance data included in this guide will be noted in the Methods section for each study.

Summary of Studies

1. TF2S feed flow calculation accuracy

Background and Objective

Feed flow rate is a key control parameter for TFF processes. The Mobius® FlexReady Solution for TFF uses an algorithm to correlate feed pump speed and pump discharge pressure to a calculated flow rate, as opposed to directly measuring flow rate via a flow meter. Excluding the flow meter reduces the system holdup volume and allows higher final protein concentration targets. The objective of this test is to confirm that the Quattroflow™ 1200 feed pump (P001) delivers flow that matches the calculated flow displayed on the system HMI. Conditions from 0.2 to 20 L/min against discharge pressures from 0 - 4 barg (0 - 58 psig) with solution viscosities of 1 cP, 20 cP, and 35 cP will be tested. The accuracy claim for the system is +/- 10% over a flow range of 2 – 18 L/min against pump discharge pressure from 0 – 4 bar and viscosities up to 20 cP. Conditions outside of this range were tested for information only to characterize performance.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. A flexible line was connected from the feed pump inlet port to a 100 L container filled with RO water. The retentate flushing assembly was connected at port M on the Smart Flexware® assembly and directed back to the container of water. A Pellicon® SIP plate (Part #XX42STMPL) was installed between the TFF liner plates instead of a membrane cassette.

All equipment was set to AUTO mode. The retentate control valve (PCV-001) was set to 0% Closed. The filtrate control valve (PCV-101) was set to 0% Open. The feed pump (P-001) was set to speed control mode. The "Single Pass Flush from Recycle Bag" was opened.

Manual flow rate measurements were collected from the retentate line using a graduated pitcher and stopwatch at pump speed settings from 5% to 100%. In addition, the calculated feed flow rate displayed on the HMI was recorded at each setpoint. The measurements were repeated with the retentate pressure control valve set to a % closed position that resulted in a feed pump discharge pressure (PT-001) of approximately 4 barg (58 psig).

All water was pumped out of the flow path and the container was emptied. A solution of 70% w/w glycerin/water was prepared to achieve a viscosity of approximately 20 cP. The viscosity of the glycerin solution was measured using a rotating spindle viscometer (Brookfield Engineering Labs) both prior to testing and after the testing was complete. The glycerin solution was added to the 100 L container. All flow rate measurements were repeated, both with the retentate valve fully open and with the retentate valve set to apply approximately 4 barg (58 psig) of pump discharge pressure.

Finally, a solution of 77% w/w glycerin/water was prepared to achieve a viscosity of approximately 35 cP and all of the measurements were repeated a third time.

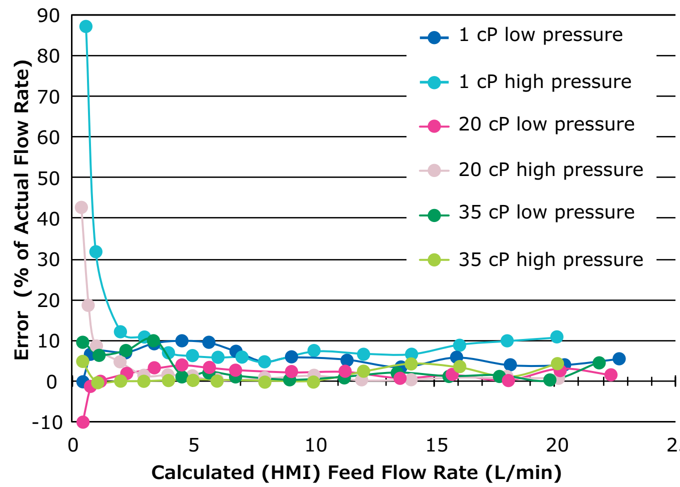

Figure 1A. Accuracy of Feed Flow Calculation for the TF2S System at low and high pump discharge pressures and viscosities from 1 – 35 cP

Results and Conclusions

As shown in Figure 1A, the calculated feed flow rate met the +/- 10% accuracy claim for flow rates between 2 – 18 L/min at viscosities of 1 – 35 cP and pump discharge pressures of 0 – 4 bar. The one exception was 2 – 3 L/min at 1 cP and 4 bar, where the error was as high as 12%. In most cases, the accuracy was significantly better than 10%. Flow rates between 18 – 20 L/min also met the accuracy target. While accuracy does degrade at low flow rates, in many cases the flow calculation accuracy remained acceptable even as low as 0.5 L/min – especially at low pump discharge pressure.

2. TF3S feed flow calculation accuracy

Background and Objective

Feed flow rate is a key control parameter for TFF processes. The Mobius® FlexReady Solution for TFF uses an algorithm to correlate feed pump speed and pump discharge pressure to a calculated flow rate, as opposed to directly measuring flow rate via a flow meter. Excluding the flow meter reduces the system holdup volume and allows higher final protein concentration targets. The objective of studying the feed flow accuracy for the TF3S is to confirm that the QuattroFlow™ 4400S feed pump (P-001) delivers flow that matches the calculated flow displayed on the system HMI. Conditions from 5 to 41 L/min against discharge pressures from 0 - 4 barg (0 - 58 psig) with a solution viscosity of 1cP were tested. The accuracy claim for the TF3S system is +/- 10% over a flow range of 4 - 40 L/min for Process and 4 – 20 L/min for Pre-Use Flush, against pump discharge pressure from 0 – 4 bar and 1 cP viscosity.

Materials and Methods

Several tests confirm the capability of the QuattroFlow™ 4400S pump integrated into the TF3S system. These include an analysis of the minimum and maximum flow rates (described in 2.1), a characterization test for flow vs. pressure with several pump chambers/drive combinations (2.2), and a flow accuracy verification test (2.3), as for the TF2S. All tests used two configurations or operation modes: Process and Pre-Use Flush.

All Flexware® assemblies were installed into the system per the User Guide. In the process configuration, the 200 L recycle bag was filled with RO water. The retentate flushing assembly was connected at port M on the Smart Flexware® assembly and directed back to the container of water. In the Pre-Use Flush configuration, a flexible line was connected from one inlet port of the transfer assembly to a container filled with RO water. The transfer manifold outlet was then connected to the flush assembly at port J, and the flush assembly was further connected to the inlet of the feed pump (P001). A Pellicon® SIP plate (Part #XX42STMPL) was installed between the TFF liner plates instead of a membrane cassette. Prior to running any tests, a priming procedure described in the User Guide is required.

All equipment was set to AUTO mode. The retentate control valve (PCV-001) was set to 0% Closed. The filtrate control valve (PCV-101) was set to 0% Open. The feed pump (P-001) was set to speed control mode. The "Single Pass Flush from Recycle Bag" flow path was opened. All tests are performed using water at ambient room temperature.

Several additional tests are described briefly herein. Pressure pulsations were monitored using various pump chambers and met the acceptance criteria of ≤ 0.6 barg (9 psig). A duration test was conducted where the pump delivered 35 L/min at 4 barg (58 psig) for over 14 hours. An estimate of hold-up volume for the 4400S pump head was measured with an average volume of 178 (n = 2).

2.1. Minimum and Maximum Flow Rates

This test challenged the pump to deliver minimum and maximum flow rate at 4 barg (58 psig) discharge pressure. A calibrated pressure sensor, reference flow meter and diaphragm (backpressure) valve are installed downstream of the pump before the cassette holder. To initiate the test in the Process configuration, the pump control speed was set to a certain percentage to achieve a flow rate ≤4 l/min at 4 +/- 0.2 barg (58 +/- 3 psig) discharge pressure (PI-001). This value is recorded as the minimum flow rate. The speed was then adjusted to achieve a flow rate ≥40 L/min at 4 +/- 0.2 barg (58 +/- 3 psig) discharge pressure. This value is recorded as the maximum flow rate. In the Pre-Use Flush configuration, the pump control speed is set to a certain percentage to achieve a flow rate ≥ 20 L/min at 4 +/- 0.2 barg (58 +/- 3 psig) discharge pressure.

Using the Process configuration, a pump speed set point of 5% and 60% achieved the minimum and maximum flow rates, respectively. A pump speed set point of 40.6% during the Pre-Use Flush configuration achieved the required flow rate of ≥ 20 L/min.

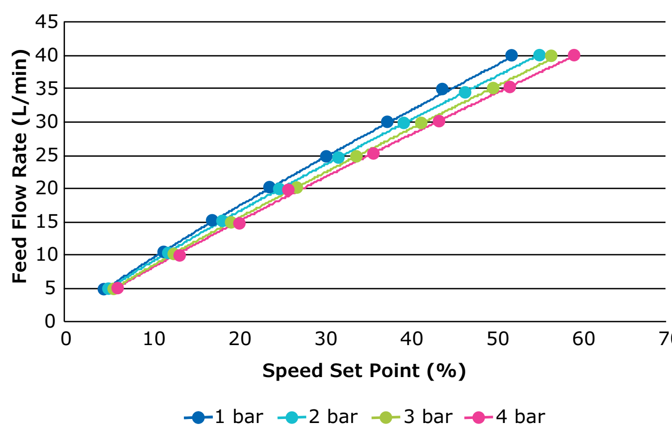

Figure 2A. Flow Rate Analysis, Process Configuration

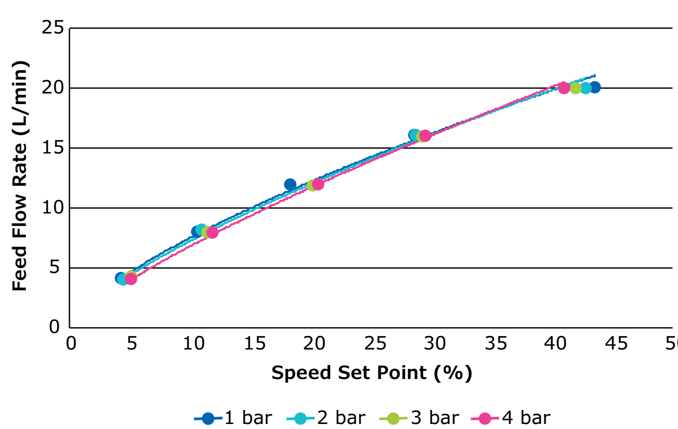

Figure 2B. Flow Rate Analysis, Pre-Use Flush Configuration

2.2. Flow vs. Pressure Characterization

Data from this test built the algorithm to correlate feed pump speed and pump discharge pressure to the calculated (HMI) flow rate for P-001 in both process and Pre-Use Flush configurations. Four pump discharge pressures were tested: 1, 2, 3, and 4 barg (15, 29, 44, and 58 psig). At a particular pressure, the series of target flow rates were achieved by increasing the pump speed %. The value was recorded for each target flow rate. Inlet/outlet connections were the same as in 2.1.

Results and Conclusions

Figure 2A presents the speed % set point versus feed flow rate for each of the 4 discharge pressures in the Process configuration. Figure 2B presents the speed % set point versus feed flow rate for each of the 4 discharge pressures in the Pre-Use Flush configuration. These values are entered into the feed pump flow rate algorithm which underlies the flow rate (FI-001) displayed on the HMI.

2.3. Flow Accuracy Characterization and Verification

The accuracy of the flow calculation algorithm on the TF3S system was then verified. The acceptance criteria for the calculated Flow (FI-001) displayed on the HMI was <10% error compared to the value measured on a calibrated flow meter. The pump control speed (%) was tested between 5% and 60% to achieve 4 – 40 L/min. The diaphragm (backpressure) valve was modulated to achieve various pump discharge pressures (PT-001) from 1 - 4 barg (15 - 58 psig). The resultant flow rate was measured using a reference flow meter. Both the calculated flow rate (FI-001) and the pump discharge pressure (PI-001) displayed on the HMI were recorded.

For the Process configuration, verification was repeated with a.) 3 pump heads on the same pump drive, and b.) 1 pump head on a second pump drive. For the Pre-Use Flush configuration, verification was repeated with 3 pump heads on the same pump drive.

Results and Conclusions

As shown in Figures 2C and 2D, the calculated feed flow rate met the +/- 10% accuracy claim for flow rates between 4 – 42 L/min for the process configuration and 4 – 19 L/min for the Pre-Use Flush configuration, for a viscosity of 1 cP and pump discharge pressures of 1 - 4 barg (15 - 58 psig). The one exception is the 24 L/min 2 bar data point at 10.5% error. In most cases, the accuracy was significantly better than 10%. The lowest pump speed tested was 5% and 4%, respectively.

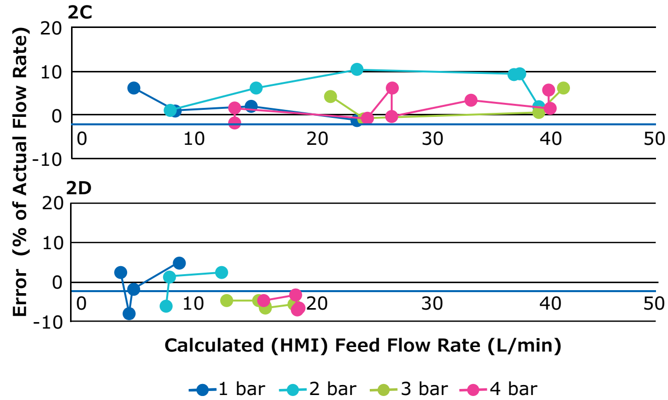

Figure 2C. Flow Accuracy Characterization and Verification, Process Configuration

Figure 2D. Flow Accuracy Characterization and Verification, Pre-Use Flush Configuration

3. TF2S filtrate flow calculation accuracy and delay time to achieve reading

Background and Objective

Filtrate flow rate is a critical parameter to monitor and record for TFF processes, since flux is derived from filtrate flow rate. The Mobius® FlexReady Solution for TFF calculates filtrate flow rate based on change in weight over time of the collected filtrate volume on the floor scale. Since the floor scale that is specified for the system has a 600 kg capacity, in order to achieve a stable reading at low flow rates, an averaging function is included in the software which, in addition to stabilizing the calculation also creates a slight delay time between an actual change in flow rate and the observed change in flow on the HMI readout. The objective of this test is to confirm that the calculated filtrate flow rate that is displayed on the HMI matches the actual measured flow when the system is used with the 600 kg-capacity floor scale that is specified as an option, and also to characterize the delay time required to achieve a stable, accurate flow rate display on the HMI during step changes to the flow rate. Flow rates from 80 mL/min to 8.3 L/min were tested using water (1cP). These flow rates correspond to a flux range of 10 LMH on 0.5m^2 of membrane up to 200 LMH on 2.5m^2 of membrane. To meet the accuracy claim of the system, the flow rate shown on HMI should differ by no more than +/- 5% from the actual flow rate over the range of 0.16 to 6.25 L/min. Flow rates outside of this range were tested for information only. Delay time was collected for information only.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. A flexible line was connected from the feed pump inlet port to a 100 L container filled with RO water. The retentate flushing assembly was connected at port M on the Smart Flexware® assembly and directed back to the container of water. A Pellicon® SIP plate (Part #XX42STMPL) was installed between the TFF liner plates instead of a membrane cassette. The filtrate line was directed to a 50 L container that was placed on the connected floor scale and the scale was tared from the HMI.

All equipment was set to AUTO mode. The “Single Pass Flush from Recycle Bag” flow path was opened. The retentate control valve (PCV-001) was set to 0% Closed. The filtrate control valve (PCV-101) was set to 0% Open. The feed pump (P-001) was set to flow control mode and turned on at a setpoint of approximately 10 L/min.

Once the flow was stabilized, the filtrate control valve was opened very slightly to generate a slow filtrate stream. The time required for the filtrate flow reading to stabilize on the HMI was measured with a stopwatch. Once stabilized, the actual filtrate flow was manually measured using a graduated cylinder/pitcher and stopwatch. The stabilization time, the HMI readout of filtrate flow rate, and the measured filtrate flow rate were recorded. In addition, if the HMI value continued to shift, the high and low readings were recorded over an approximately 1 minute duration in order to calculate variability.

The filtrate control valve was incrementally opened further to achieve a range of higher filtrate flow rates up to 8.3 L/min. The same stabilization and recordings as described above were made at each point.

Results and Conclusions

Figure 3A shows that over the range of 0.4 – 8.3 L/min filtrate flow rate, the calculated value differed by < 5% from the actual value. Below 0.4 L/min, the error increases to 10-35% at the lowest filtrate rates. In addition, Figure 3A also demonstrates that the HMI readouts were very stable at values down to approximately 2 L/min. Below this, there was greater bounce in the readings as the flows decreased. However, the bounce does not impact accuracy.

While not shown on a graph, the time required to reach a stable readout of filtrate flow rate on the HMI was approximately 60 seconds across the full range of flows tested.

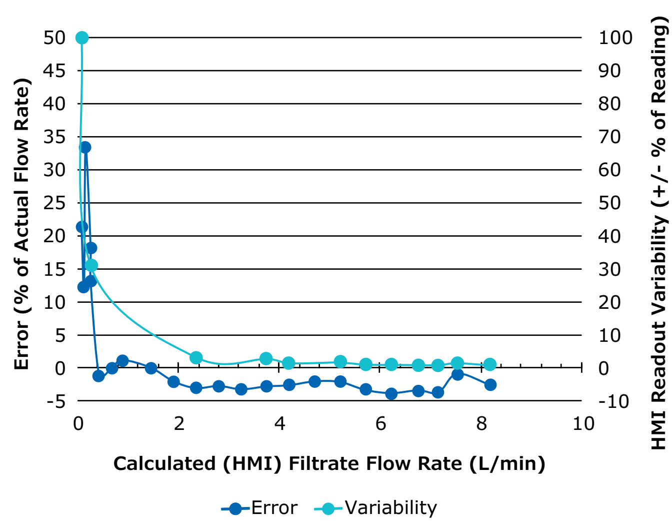

Figure 3A. Accuracy and variability of filtrate flow calculation for the TF2S system

4. TF2S filtrate flow measurement accuracy using a filtrate non-intrusive clamp-on flow meter

Background and Objective

Filtrate flow rate is a critical parameter to monitor and record for TFF processes, since flux is derived from filtrate flow rate. The Mobius® FlexReady Solution for TFF is available with an optional ultrasonic non-intrusive filtrate flow meter which is clamp-on the filtrate line. This flow meter can be easily integrated into any existing Mobius® FlexReady Solution for TFF system without any software update. Only Flexware® assemblies designed with single-use filtrate instrumentation, without any filtrate instrumentation, with filtrate sampling pore or without any filtrate sampling pore can be installed with this clamp-on flow meter.

The objective of studying the filtrate flow accuracy for the TF2S is to confirm that the flow measured by the ultrasonic non-intrusive flow meter matches the expected flow accuracy below 2 L/min and over the flow range of 2-10 L/min. The accuracy claim for the TF2S system is +/- 120 mL/min displayed on the system HMI over the flow range of 0.2-2 L/min and +/- 5% of the measured flow displayed on the system HMI over the range of 2-10 L/min at 20°C and using RO water.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. The flow meter support was attached to the clamshell before installing the ultrasonic non-intrusive filtrate flow meter into it. The flow meter, the converter box, the clamshell and the flow meter support attached to the clamshell were connected following the installation guide for installing the TFF SU Filtrate Flow sensor (Quick Installation Guide for MBSMTTINSSUFMTX2 and MBSMTTINSSUFMTX3). The filtrate flow meter was enabled on the HMI Maintenance faceplate and the FI101 filtrate flow range was set up from 0 to 10 L/min with 2 decimals displayed. A mass flow meter was connected upstream from the non-intrusive filtrate flow meter as reference between the connectors labelled A and C on the Smart Flexware® assembly. No membrane cassette was installed. A flexible line was connected from the feed pump inlet port to a container filled with RO water at 20°C. The feed pump was ramped up to defined flow rates. The HMI value readout of filtrate flow rate and the value readout of the mass flow meter were recorded after feed flow rate stabilization and compared. Repeatability and reproducibility were also assessed as well as temperature effect.

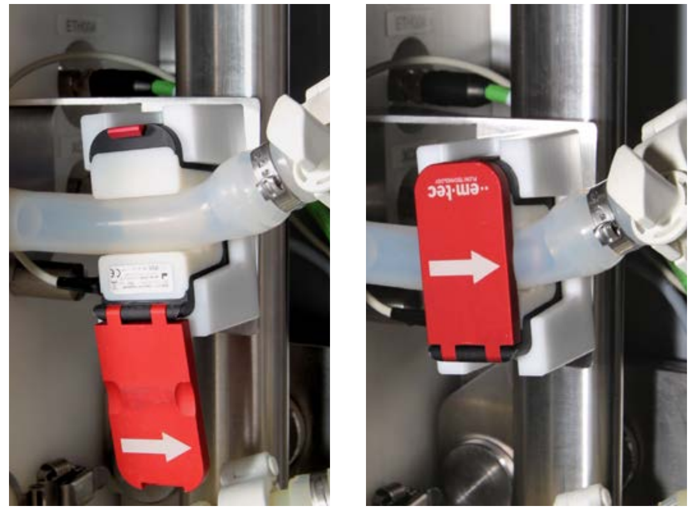

Figure 4A. Non-intrusive flow meter open and closed

Results and Conclusions

Figure 4B demonstrates that over the range of 2-10 L/min filtrate flow rate, the HMI values readout differed by < 5% from the actual value at 20°C and with RO water. In addition, Figure 4C shows that the filtrate flow error met the +/- 120 mL/min accuracy claim below 2 L/min filtrate flow rate at 20°C and with RO water. Figure 4D and 4E show that the HMI readouts still met the +/- 120 mL/min and +/-5% accuracy below 2 L/min and between 2 and 10 L/min filtrate flow, respectively, at 4 and 45°C and using RO water. However, a new sensor calibration is required when the operating fluid temperature doesn't match a temperature of 20°C.

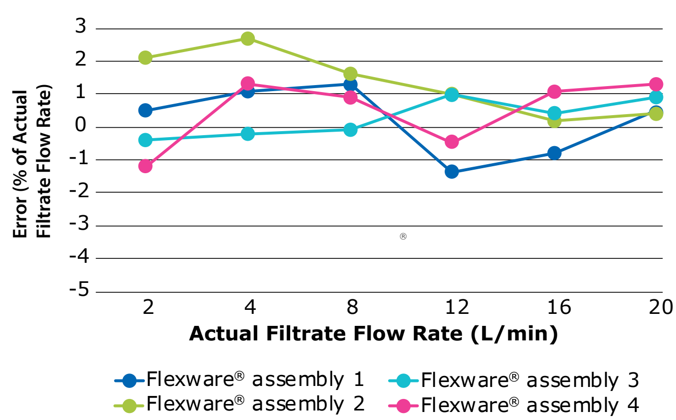

Figure 4B. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 2-10 L/min

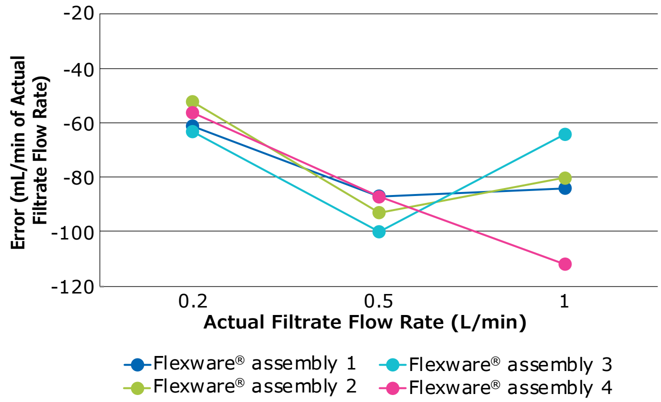

Figure 4C. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 0.2-2 L/min

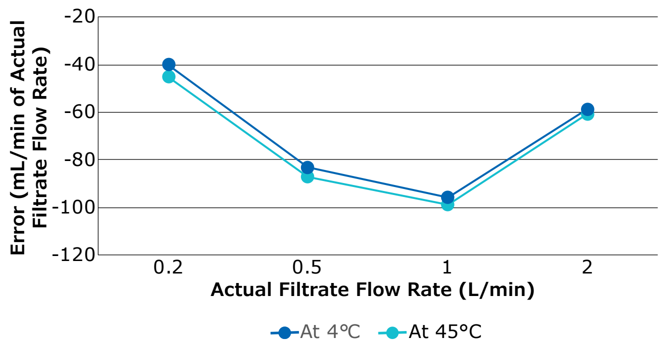

Figure 4D. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 0.2-2 L/min at 4 and 45°C

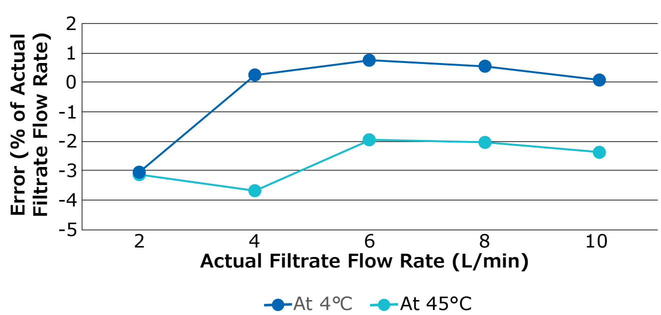

Figure 4E. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 2-10 L/min at 4 and 45°C

5. TF3S filtrate flow measurement accuracy using a filtrate non-intrusive clamp-on flow meter

Background and Objective

Filtrate flow rate is a critical parameter to monitor and record for TFF processes, since flux is derived from filtrate flow rate. The Mobius® FlexReady Solution for TFF is available with an optional ultrasonic non-intrusive filtrate flow meter which is clamp-on the filtrate line. This flow meter can be easily integrated into any existing Mobius® FlexReady Solution for TFF system without any software update. Only Flexware® assemblies designed with single-use filtrate instrumentation, without any filtrate instrumentation, with filtrate sampling pore or without any filtrate sampling pore can be installed with this clamp-on flow meter.

The objective of studying the filtrate flow accuracy for the TF3S is to confirm that the flow measured by the ultrasonic non-intrusive flow meter matches the expected flow accuracy below 2 L/min and over the flow range of 2-20 L/min. The accuracy claim for the TF3S system is +/- 120 mL/min displayed on the system HMI over the flow range of 0.4-2 L/min and +/- 5% of the measured flow displayed on the system HMI over the range of 2-20 L/min at 20°C and using RO water.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. The flow meter support was attached to the clamshell before installing the ultrasonic non-intrusive filtrate flow meter into it. The flow meter, the converter box, the clamshell and the flow meter support attached to the clamshell were connected following the installation guide for installing the TFF SU Filtrate Flow sensor (Quick Installation Guide for MBSMTTINSSUFMTX2 and MBSMTTINSSUFMTX3). The filtrate flow meter was enabled on the HMI Maintenance faceplate and the FI101 filtrate flow range was set up from 0 to 10 L/min with 2 decimals displayed. A mass flow meter was connected upstream from the non-intrusive filtrate flow meter as reference between the connectors labelled A and G on the Smart Flexware® assembly. No membrane cassette was installed. A flexible line was connected from the feed pump inlet port to a container filled with RO water at room temperature. The feed pump was ramped up to defined flow rates. The HMI value readout of filtrate flow rate and the value readout of the mass flow meter were recorded after feed flow rate stabilization and compared. Repeatability and reproducibility were also assessed as well as temperature effect.

Results and Conclusions

Figure 5A demonstrates that over the range of 2–20 L/min filtrate flow rate, the HMI values readout differed by < 5% from the actual value at 20°C and with RO water. In addition, Figure 5B demonstrates that the filtrate flow error met the +/- 120 mL/min accuracy claim below 2 L/min filtrate flow rate at 20°C and with RO water.

Figure 5C and 5D show that the HMI readouts still met the +/- 120 mL/min and +/-5% accuracy below 2 L/min and between 2 and 20 L/min filtrate flow, respectively, at 4 and 45°C and using RO water. However, a new sensor calibration is required when the operating fluid temperature doesn't match a temperature of 20°C.

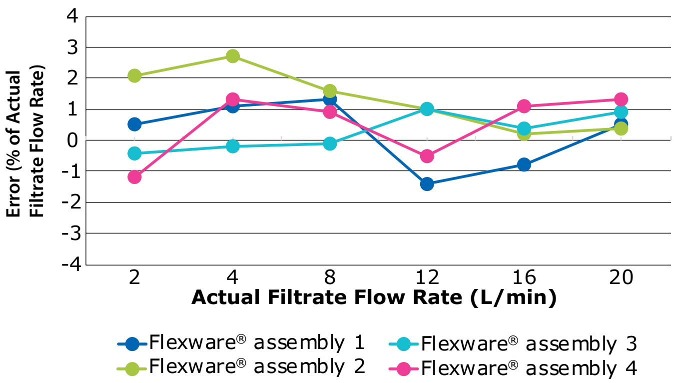

Figure 5A. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 2-20 L/min

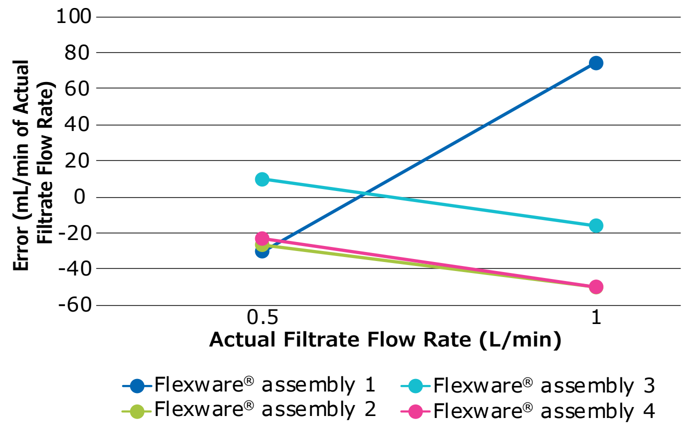

Figure 5B. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 0.4-2 L/min

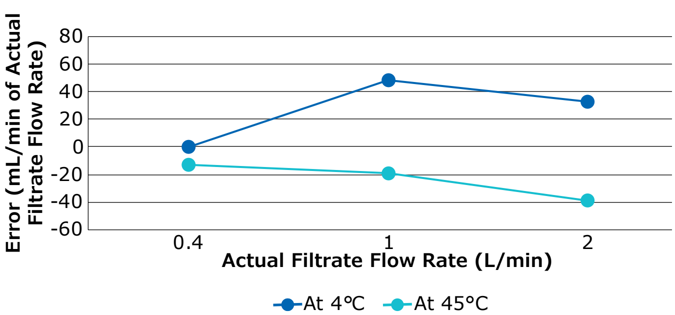

Figure 5C. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 0.4-2 L/min at 4 and 45°C

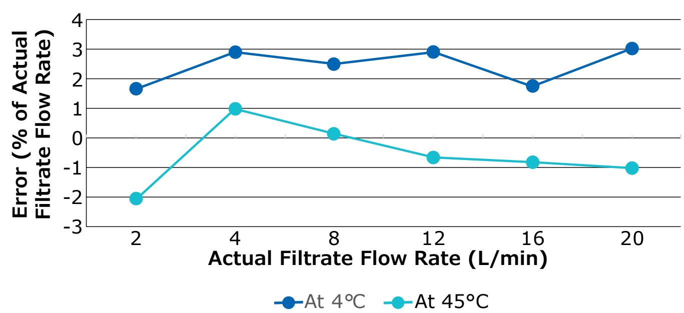

Figure 5D. Accuracy of the filtrate flow measurement (HMI) using the ultrasonic non-intrusive filtrate flow meter from 2-20 L/min at 4 and 45°C

6. TF2S system pressure drop versus flow rate and viscosity

Background and Objective

Selecting an appropriate flow path line diameter for a TFF system involves ensuring that it is not so large as to result in excessive holdup volume, which limits the extent of protein concentration that can be achieved, while also making sure that it is not so small that it results in excessive pressure drop. There are two pressure drops that are of concern for this test. The total system pressure drop is the line drop from the feed pump discharge through the feed lines, membrane holder, and retentate line back to the recycle vessel, which can be measured using the feed pressure sensor (PT-001) since the recycle vessel pressure is zero. The retentate pressure drop is the line drop from the outlet of the membrane holder through the retentate line back to the recycle vessel, which can be measured using the retentate pressure sensor (PT-002). Since the Mobius® FlexReady Solution for TFF has a maximum pressure rating of 4 barg (58 psig), a high total system pressure drop could limit the flow rate that can be driven through the membrane channels to increase mass transfer and drive high flux. This is especially true at high protein viscosities and high concentrations. A high retentate line pressure drop could limit the minimum transmembrane pressure (TMP) that can be achieved even with the retentate control valve fully open. In a worst-case, this could cause the TMP setpoint for a particular process to be unachievable.

The objective of this test was to determine the pressure drop in the feed/retentate flow path as a function of feed flow rate. Since pressure drop is dependent on flow rate and viscosity, as well as line size, conditions from 0.5 to 20 L/min and viscosities of 1 cP, 20 cP, and 35 cP were evaluated.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. The recycle bag outlet was connected to the feed pump inlet. The Smart Flexware® bag retentate port was connected to the recycle bag return via the retentate sampling assembly. A Pellicon® SIP plate (Part #XX42STMPL) was installed between the TFF liner plates instead of a membrane cassette. The recycle bag was filled with approximately 30 L of RO water using the transfer pump and inlet manifold.

All equipment was set to AUTO mode. The "Membrane Recycle" flow path was opened. The retentate control valve (PCV-001) was set to 0% Closed. The filtrate control valve (PCV-101) was set to 0% Open.

The feed pump (P-001) was set to flow control mode with a setpoint of 0.5 L/min. Once the flow and feed pressures stabilized, the feed flow rate (FI-001), feed pressure (PT-001), and retentate pressure (PT-002) were recorded from the HMI display. The feed pump flow setpoint was sequentially increased in small steps and the stabilization and recordings were repeated at each new flow setpoint.

After the pressure drop at flow rates from 0.5–20 L/min were recorded, water was drained from the system using the "Single Pass Flush from Recycle Bag" flow path. The entire sequence was repeated using an approximately 70% w/w glycerin/water solution to generate data at 20 cP. It was repeated a third time using an approximately 77% w/w glycerin/water solution to generate data at 35 cP. All tests were conducted at ambient temperature (19 - 24°C) and viscosity was verified using a Rheo Sense microVISC™ viscometer with temperature control set to 24°C.

Results and Conclusions

The Pellicon® SIP plate was used for this study in place of actual Pellicon® membrane cassettes so that the full range of achievable flow rates could be evaluated without membrane pressure drop limitations and impact on measurements. The Pellicon® SIP plate is a stainless steel rectangular annular ring with a thickness of 3/8". It has the external dimensions (length and width) of a Pellicon® cassette and an o-ring that allows it to be installed into a membrane holder and torqued into place. When installed, fluid or steam can flow through the holder without any significant pressure drop.

Figure 6A shows the total pressure drop through the TF2S system across the full range of achievable flow rates for viscosities up to 35 cP.

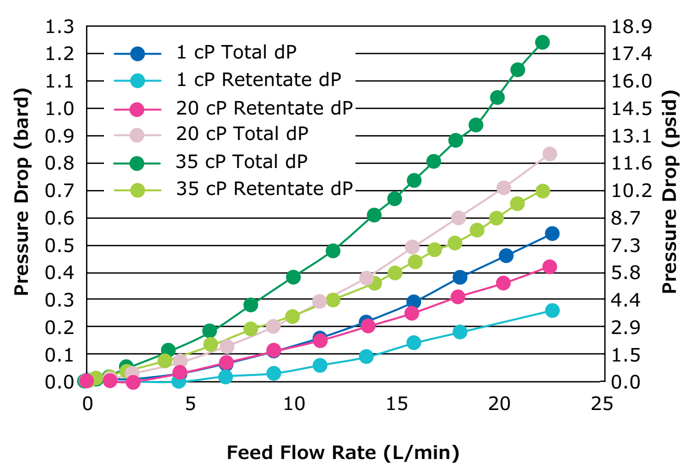

Figure 6A. Total and retentate pressure drop (bar and psi) versus feed flow rate and viscosity for TF2S with Smart Flexware® assembly

7. TF2S & TF3S minimum working volume versus feed flow rate

Background and Objective

Understanding the minimum working volume (MWV) within a system is critical to ensuring that air is not entrained into the outlet of the recycle bag. The system holdup volume is important for recovery, whereas the MWV, which varies with cross flow rate, is important for processing. Testing performed at a range of flow rates will elucidate how low a solution can be concentrated volumetrically at a given flow rate. This is particularly important for high concentration processes.

The objective of this test was to determine the minimum working volume as a function of feed flow rate. Since MWV is dependent on flow rate and viscosity, conditions from 0.5 to 18 L/min and viscosities of 1 cP and 20 cP were evaluated for the TF2S and conditions from 4 to 40 L/min and a viscosity of 1 cP were evaluated for TF3S. For the minimum mixed vessel volume, see section 6.

Materials and Methods

All Flexware® assemblies were installed into the system per the User Guide. The recycle bag outlet was connected to the feed pump inlet. The TF2S and TF3S recycle vessels used in these studies are 100 and 200 L, respectively. The Smart Flexware® bag retentate port was connected to the recycle bag return via the retentate sampling assembly. A Pellicon® SIP plate (Part #XX42STMPL) was installed between the TFF liner plates instead of a membrane cassette. All equipment was set to AUTO mode. The mixer was disabled. The "Membrane Recycle" flow path was opened. The retentate control valve (PCV-001) was set to 100% open. One of two methods was used: additive or subtractive.

MWV at 1 cP – TF2S – (Subtractive method): The filtrate control valve (PCV-101) was set to 100% open. A manual valve was installed on the filtrate outlet. The feed pump was turned on in flow control mode. The recycle bag was inflated with the attached inflator (in 2 of the 4 runs), then was filled with 2.5 L of water. The feed pump was ramped to a flow rate of 18 L/min to fill the flow path with the water from the recycle tank. The manual valve was opened to allow air discharge on the filtrate line. Once the lines were filled, water was removed from the system using the manual filtrate valve until the minimum working volume was reached at 18 L/min, as defined by no air entrainment into the feed line. The volume of water collected was used to calculate the minimum working volume. The volume of water required to fill the filtrate line and the Pellicon® SIP plate was subtracted from the results.

The feed pump output was decreased in increments to a minimum flow rate of 2 L/min. At each increment, the minimum working volume was assessed in the same manner. The entire sequence was repeated 3 more times.

MWV at 20 cP – TF2S – (Additive method): The filtrate control valve (PCV-101) was set to 0% Open. The recycle bag was filled with approximately 0.5 L of a 70% glycerine/water solution using the transfer pump and inlet manifold. The feed pump was turned on in speed control mode with a set point of 2%. The flow path was filled slowly with solution from the recycle tank. If more solution was required to fill the lines it was added in 100 mL aliquots. Once the lines were filled, the volume required for filling at this flow rate was recorded.

The feed pump was slowly ramped to a flow rate of 0.5 L/min and more solution was added to the recycle bag in the same manner as before. When the lines were completely filled and the fluid was recirculating without entraining air, the total volume added and the flow rate were recorded. This was then repeated for each flow rate set point.

After the MWV at flow rates from 0.5 – 20 L/min was recorded, solution was drained from the system using the "Single Pass Flush from Recycle Bag" flow path.

MWV at 1 cP – TF3S – (Subtractive method): The same setup procedure was followed as for the TF2S. The feed pump was ramped to a flow rate of 40 L/min to fill the flow path with the water from the recycle tank. The manual valve was opened to allow air discharge on the filtrate line. Once the lines were filled, the water was removed from the system using the manual filtrate valve until the minimum working volume was reached, as defined by no air entrainment into the feed line. The volume of water collected was used to calculate the minimum working volume. The volume of water required to fill the filtrate line and the Pellicon® SIP plate was subtracted from the results.

The feed pump output was decreased in increments to a minimum flow rate of 4 L/min. At each increment, the minimum working volume was assessed in the same manner.

Results and Conclusions

The Pellicon® SIP plate was used for this study in place of actual Pellicon® membrane cassettes so that the full range of achievable flow rates could be evaluated without membrane pressure drop limitations and its impact on measurements. The two different methods show consistent results across the Mobius® FlexReady Solution with Smart Flexware® Assemblies platform.

MWV for TF2S – Figure 7A shows the minimum working volume for the TF2S system across the full range of achievable flow rates for viscosities up to 20 cP. The 1 cP data is an average of 4 runs. Error bars show the standard deviation. Variability is due in part to user/procedure and the bag inflation practice used: inflating before filling is best case whereas not inflating is worst case (highest MWV).

MWV at 1cP – TF3S – Figure 7B shows the minimum working volume for the TF3S system across the full range of achievable flow rates for 1 cP viscosity.

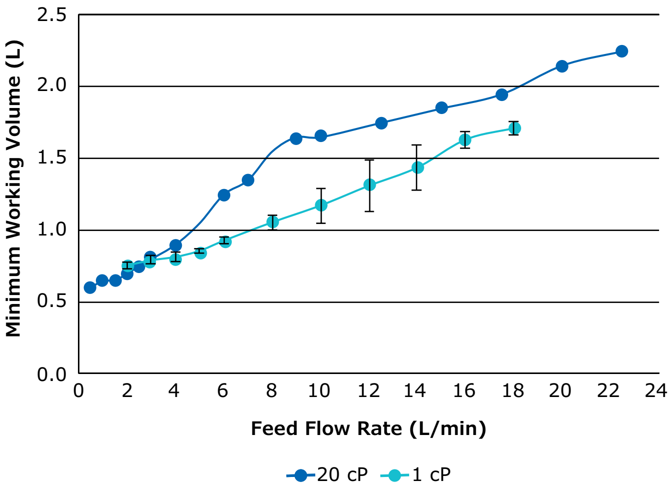

Figure 7A. Minimum Working Volume versus Flow Rate and Viscosity for TF2S

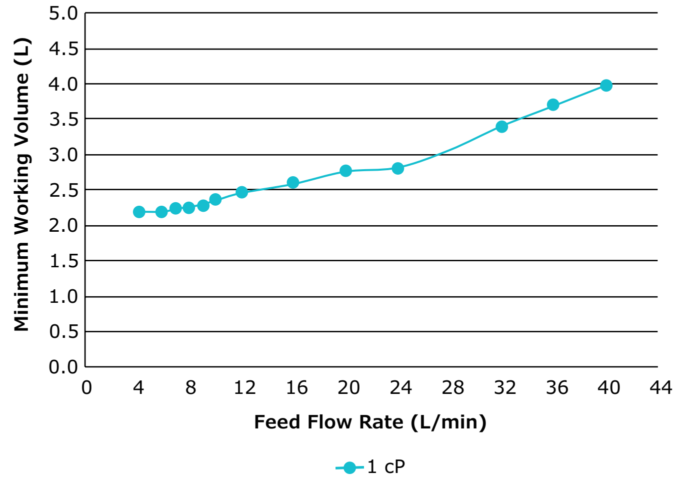

Figure 7B. Minimum Working Volume versus Flow Rate and Viscosity for TF3S

8. Min/max effective mixer speed versus 50 L and 100 L recycle vessel volume at 1 cP

Background and Objective

Mixing in a TFF system is important for keeping product homogeneous throughout the process. Too vigorous mixing may be detrimental to shear sensitive solutes. It is therefore useful to characterize the vessel mixing across the mixer speed and working volume range to understand surface turbulence, as an indicator of mixing vigor.

Materials and Methods

The recycle bag is installed into the TF2S recycle vessel following the instructions provided in the User Guide. The mixer motor is engaged with the impeller cup on the bottom of the bag. The load cells are tared prior to adding 1 cP solution via the transfer pump and inlet manifold to a starting volume of 0.5 L. Mixing speed (0–100%) is adjusted to 5% using the attached handheld controller. The liquid surface is observed at each mixing speed increment, up to the maximum tested. More volume (up to 95 L) is added to the recycle bag and the mixing speed excursion is repeated, starting again at 5% mixing speed. Table 1 shows the mixing conditions.

Type of Mixing	Description/Observation
Calm Surface	No surface motion
Gentle Mixing	Slight movement at the surface
Mixing	Rapid movement; no dimples
Mixing w/ Starting Turbulence	Dimples begin to form
Turbulence	Churning, peaks and troughs

Table 1. Mixing Criteria

Results and Conclusions

Figure 8A and Figure 8B show the onset of surface turbulence at 1 cP and potential areas of concern for shear sensitive mixing operations in the 50 L and 100 L vessels, respectively. The impeller becomes submerged around 2.5 L in the 50 L vessel and 6 L in the 100 L vessel. As volume increases above this level, the mixer speed at which turbulence sets in also increases. At 12 L and 30 L in the 50 L and 100 L vessels, respectively, the full mixer speed no longer generates surface turbulence. To minimize the subjectivity of this test, more than one operator agreed to the observations.

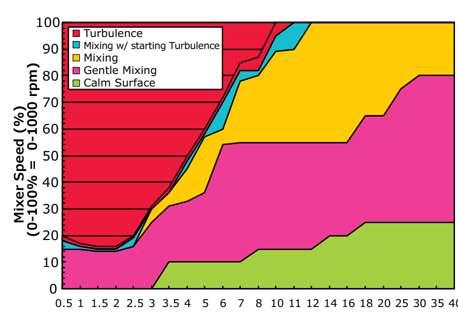

Figure 8A. Mixer Speed [%] vs. Water Volume (L) in the 50 L recycle vessel.

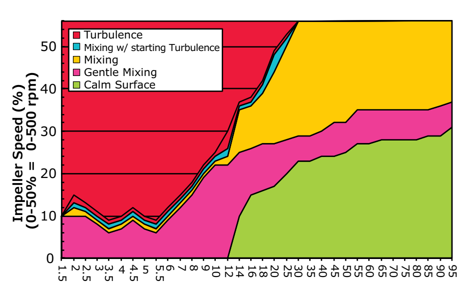

Figure 8B. Mixer Speed [%] vs. Water Volume [L] in the 100 L recycle vessel. Mixer speed is limited to ~50%, or 500 RPM

9. Demonstration of protein UF/DF step: Feed flow control and retentate TMP control

Background and Objective

Ultrafiltration (UF) is used to purify and concentrate a retained solute. Diafiltration (DF) is used to exchange the solvent/buffer. UF/DF steps are used throughout the biomanufacturing process, particularly for final formulation. Achieving a high product yield is a primary metric for UF/DF, along with product purity and a correct buffer formulation. Towards this end, a UF/DF process with model protein ran on the TF2S with Smart Flexware® assemblies to demonstrate automation control features - ability to execute each process step, accuracy of step-to-step transitions based on programmed transition criteria, and ability of all control loops to achieve and maintain their process setpoints during ramp up, intentional hold/resume activation, and/or step changes to the setpoints during processing - and prove high recovery. Feed flow control and retentate pressure control loops controlled the UF/DF.

Materials and Methods

Feed Flow Control and TMP Control

All Flexware® assemblies were installed into the system per the User Guide and configured for Pre-Use flushing. A single Pellicon® 3 cassette with 1.14m^2 30kD Ultracel® membrane (Part #P3C030C10) was installed between the TFF liner plates. The cassette was flushed with water, according to the Pellicon® 3 Cassettes Installation & User Guide (AN1065EN00) and using the following system procedures.

The transfer manifold was connected to the flushing assembly at port J then to the feed pump inlet. The flush assembly on the retentate outlet and the filtrate outlet was directed to drain. Caustic solution and water were connected to the TF2S via XV-431 and XV-441 transfer manifold valves, respectively. Using the HMI controls, the "Water Source" and "Single-Pass Flush from Inlet Manifold" flow paths were engaged. The retentate control valve (PCV-001) and filtrate control valve (PCV-101) were fully opened.

Flow control mode was engaged on the feed pump and set to 5.7 L/min. Retentate pressure control was engaged on the PCV-001 and set to 0.3 barg (5 psig), to target a conversion ratio of 30-50%. The product recovery valve (XV-003) and lower filtrate drain valve (XV-102) are both pulsed to rinse these lines. After 23 L of water was flushed through the membrane to drain, offline conductivity measurements confirmed both retentate and filtrate outlets achieved < 2 µS/cm. Removing the feed line from the source of water allowed the lines to be pumped empty before switching off the pump.

The process configuration was set up by connecting the recycle bag outlet to the feed pump inlet and the retentate outlet to the recycle bag return via the retentate sampling assembly. The transfer manifold was connected to the transfer pump inlet and the transfer pump outlet to the Smart Flexware® bag transfer port. 5% SeraCare™ Human Gamma Globulin was diluted to 5 g/L with 50 mM Sodium Acetate pH 5.5 to a volume of 50 L in a Mobius® MIX 50 L mixer, which was connected to the XV-411 inlet. An additional 100 L of sodium acetate (50 mM, pH 5.6) was prepared as DF buffer in the Mobius® MIX 100 L mixer, which was connected to the XV-421 inlet. The "Feed Source Open" and the "Recycle Bag Fill" flow paths were engaged to transfer 30 L of protein feedstock into the recycle bag using the transfer pump at a speed setpoint of approximately 25%. The mixer is enabled in Auto mode.

Using a pre-programmed recipe, the following processes were executed: a fed-batch concentration with a 30 L tank volume setpoint, a batch concentration to 10 L product volume, a 3-diavolume diafiltration, and a final batch concentration to 2.5 L protein volume. The feed pump flow control was set at 5.7 L/min, and the retentate pressure control was set at 1 bar. The filtrate was collected on the integrated floor scale for totalizing. Critical alarms were tested to verify correct process resume functionality.

After the final concentration step is complete, a recommended depolarization step engaged the "Membrane Recirculation" flow path, fully closed the filtrate control valve, opened the retentate control valve, and set a feed pump delta P control of 0.3 bard (5 psid) pressure drop across the cassette feed channel to recirculate for 10 minutes, with active mixing and a final UV sample. Product recovery commenced via air blow down (see Section 8. Optimization of Protein Product Recovery from TF2S system) with a bulk sample for UV/yield calculation. An additional buffer flush was used to recover any remaining protein from the system and complete the protein mass balance (load versus recovery). Yields were calculated for both recovery procedures. Cleaning commenced using 0.1 N NaOH connected to XV-431 inlet and standard procedures.

Results and Conclusions

All the steps of a protein UF/DF process were executed in this trial, from system setup and membrane flushing through to product recovery, and including a 20x VCF and 3x DF. Automation features such as flow paths, feed flow and delta P control loops, retentate pressure control loop, and recipe control including step transition criteria were employed. The mass balance of the process is the primary metric for this UF/DF trial and is shown in Figure 9A. The bulk recovery from the vessel and using air blow down for the tubing and cassette yielded 98.4% of the protein added. Another 5.0% came from the buffer recovery and only 0.1% appeared in the filtrate. The mass balance returned 103.5%.

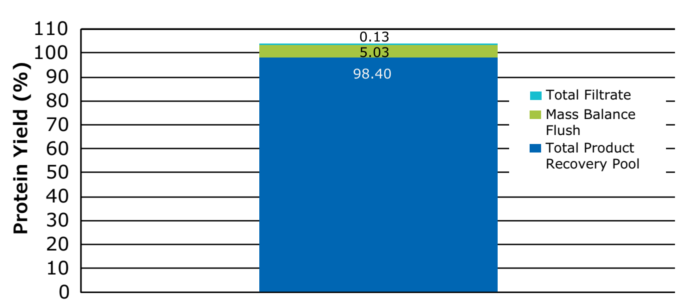

Figure 9A. Yield of a Protein UF/DF Process on TF2S with Smart Flexware® Assemblies

10. Optimization of protein product recovery from TF2S system

Background and Objective

Minimizing product loss within the TF2S with Smart Flexware® assemblies maximizes process economics. Product recovery from the system must therefore be optimized to maximize yield with minimum dilution. While the majority of the product can be recovered via a low drain point, there will still be some product left in the system lines and filter cassette. Two methods to recover this remaining product are: air "blow down" and buffer displacement. The achievable yield will vary between processes based on factors such as product viscosity, dilution tolerance, and how close the final protein pool volume is to the minimum working volume (making recovery more difficult). For the purpose of this testing a yield of ≥98%, with a dilution of ≤20%, would be considered excellent and a recovered protein yield of ≥95%, with a dilution of ≤30%, would be considered acceptable. The objective of this test was to demonstrate the steps for both recovery methods and compare overall yields and protein dilution.

10.1. Preparation for Product Recovery – Choice of Product Recovery Assembly

The flow path of the Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF is designed such that a product recovery assembly should be attached at the outlet of valve XV-003. The typical assembly consists of a 2D bag with an optional sterile filter. Because the recovery will occur in several different steps where the protein concentration is not homogenous, it can be useful to have a way of actively mixing the pool once recovery is complete (i.e. recovering into a Mobius® MIX bag). Having mixing available also simplifies any post-recovery concentration adjustment or pool conditioning. If air will be used to blow down any of the residual liquid in the lines or membranes, recovering directly from the system through a sterile filter into the collection vessel/bag can be challenging because the sterile filter housing may need to be vented multiple times. In this case, it may be easier to sterile filter the product pool after recovery, mixing, and any conditioning is complete.

Prior to starting product recovery, connect an appropriate product recovery assembly/container to the outlet of valve XV-003.

10.2. Preparation for Product Recovery – Choice of Buffer or Air for Product Displacement

While the product in the recycle vessel is relatively easy to recover, the product in the recirculation path and the membrane channels will need to either be displaced with diafiltration buffer or blown down with air to achieve highest product yield. The benefit of using buffer is that the product is not subjected to potentially damaging air/liquid interfaces. However, the product will end up diluted to some extent by the buffer. If a specific final product concentration must be met and buffer will be used for product displacement during product recovery, it is recommended to slightly over-concentrate the product during UF. If overconcentration is not possible, air blow down should be considered instead of buffer.

If diafiltration buffer will be used to help displace product out of the system tubing and membrane channels, ensure that the transfer line is filled with buffer from buffer source valve XV-401 up to transfer valve XV-005. Also ensure that additional buffer is connected at the buffer source valve.

If air will be used instead of buffer to help blow down the system, it can be helpful to fully drain the transfer line from the inlet manifold up to the connection at transfer valve XV-005 in order to avoid any product dilution from residual buffer in the line. However, draining the line does require breaking connections (opening the process flow path to the atmosphere), so it may be preferable to leave the transfer line filled with buffer and account for any dilution it causes when this volume is added to the product pool. To empty the transfer line, ensure that the flow path is set to "Default". Disconnect the transfer line at the outlet of valve XV-005 and direct the transfer tubing (the section coming from the transfer pump) to waste/drain. Select the "Air Source Open" flow path, set the transfer pump to speed control mode with a setpoint of 10%, and turn on the transfer pump. Ensure that the upstream side of the transfer manifold air filter is open. Continue to pull air through the transfer line with the pump until it is empty. Then, reconnect the transfer line to the outlet of valve XV-005.

10.3. Membrane Depolarization:

Unless the protein is at very high concentration, it is recommended to run a low pressure recirculation for 5 - 10 minutes at the end of the UF/DF operation prior to product recovery in order to depolarize the membrane. For the low pressure recirculation, select the flow paths "Batch UF or Membrane Recycle" and "All Source Valves Closed". Set the retentate pressure control valve (PCV-001) to 100% open and set the filtrate control valve (PCV-101) to 100% closed. Set the feed pump (P-001) either to dP control with a setpoint of 5 psid or to % speed control with a setpoint of 2-5%. Turn on the pump and recirculate at the low pressure conditions for 5 – 10 minutes, then turn off the pump. When all flow has stopped, select the "Default" flow path and set the PCV-001 to 100% closed.

10.4. Product Recovery

Case Study 1 – Our method:

Recover product that is in the recycle vessel by opening the flow path "Product Recovery 1 & 3 – Empty Recycle Bag." Also, set filtrate control valve (PCV-101) to 0% Open. Using a feed pump speed setpoint of 3–5%, slowly pump product from the recycle bag into the product recovery container until the bag is empty and the outlet is still filled with fluid.

Recover product that is contained within the retentate line between PCV-001 and the recycle bag by opening the flow path "Product Recovery 2 – Empty Retentate Line" and also opening either "Air Source Open" (if air blow down will be used) or "Buffer Source Open" (if buffer displacement will be used). Set retentate pressure control valve PCV-001 to 100% closed. Set the transfer pump to speed control mode with a setpoint of 3-5% and run the pump just long enough to displace product in the line back into the bag. It may be helpful to quickly install a manual clamp as close as possible to the recycle bag retentate return just before shutting off the transfer pump. This helps keep the fluid that was pushed into the bag from draining back down into the retentate line once the pump is stopped.

Recover any product that was pushed into the recycle bag by repeating the first recovery step. This time, however, continue to run the feed pump until both the bag and the feed line are empty and product has been pumped to the juncture point of valve XV-003. Once the pump is stopped, remove the manual clamp from the recycle bag retentate return (if it was used) to allow any remaining fluid to flow back into the retentate line for recovery in the following step.

Recover product that is held up in the recycle tubing, membrane channels and holder by opening the flow path "Product Recovery 4 – Empty Membranes" and setting the retentate pressure control valve to 100% open. The source flow path ("Air Source Open" or "Buffer Source Open") should still be selected from the previous step. Using a transfer pump speed setpoint of 3–5%, slowly displace product from the retentate line through the membranes into the product recovery container.

If air is being used to displace product, run the pump just long enough to effect product recovery but do not continue pumping if foaming starts. If buffer is being used to displace product, a volume of approximately the membrane holdup plus an additional 100–200 mLs should be sufficient to recover most of the remaining protein. If possible, it can be helpful to collect sequential aliquots of 50–100 mLs throughout the buffer displacement, analyze each aliquot for protein concentration, and only pool the fractions having sufficient protein quantity with the final recovered pool. This volume can then be set as the buffer displacement volume target for subsequent runs. Alternatively, a separate in-line UV flow cell could be attached to the product recovery line during the first run to determine the volume at which the protein absorbance drops below the point of interest for collection.

During all product recovery steps, manual manipulation of the tubing to enhance draining can be helpful.

Case Study 2 – Alternative method:

Recover product that is in the recycle vessel by opening the flow path "Product Recovery 1 & 3 – Empty Recycle Bag." Using a feed pump speed setpoint of 3-5%, slowly pump product from the recycle bag into the product recovery container just until the bag is empty.

Recover product that is contained within the retentate line between the recycle bag and the retentate sampling port or XV-004 using gravity by closing first PCV-101 and XV-001 and opening the valves XV-004, PCV-001, XV-002 and XV-003 until the Flexware® assembly is empty. The recycle bag inlet and the retentate sampling port can be leaned to help the draining of the fluid.

Recover product that is held up in the recycle tubing, membrane channels and holder by keeping the previous flow path, disconnecting the Flexware® assembly between the recycle bag and the retentate sampling port and connecting air source. A pressure gauge and a pressure regulator should be connected as well. Apply a pressure up to 0.3 bar (read on the pressure gauge or on the HMI) to displace product from the retentate line through the membranes into the product recovery container. After recovery, disconnect the air source and reconnect the Flexware® assembly between the recycle bag and the retentate sampling port.

Buffer can be added directly inside the recycle bag or by using the transfer pump and the source flow path ("Buffer Source Open") to enhance the yield based on the minimum working volume.

A few minutes of buffer recirculation will boost the membrane recovery and close the mass balance. Recover buffer by following the previous steps.

10.5. Mass Balance Verification

As a final verification of process performance, it is recommended (at least during process development) to execute a mass balance step to characterize all remaining product left in the system. To do this, transfer approximately 1 minimum working volume of buffer to the recycle bag, using the "Recycle Bag Fill" and the "Buffer Source Open" flow paths, with the retentate pressure control valve 100% closed and a transfer pump speed setpoint of approximately 20%. Once the buffer has been added to the bag, follow all of the above steps for membrane depolarization and product recovery, collecting this buffer mass balance pool into a separate container collected process filtrate, and the mass balance buffer pool should very closely sum to the amount of protein in the pre-UF/DF feedstock. If there is a significant discrepancy, process troubleshooting is warranted. If the buffer mass balance pool indicates a significant amount of protein, it indicates that further optimization of the product recovery methodology is needed.

10.6. Data from Application Testing

Case Study 1 – Our method:

Product Recovery evaluation using both the air blow down and buffer displacement methods was performed on the TF2S system. The system included a 50 L non-jacketed recycle container. Commercially-available polyclonal IgG at 50 g/L was used as the protein feedstock. One Pellicon® 3 cassette with 1.14 m^2 30kD Ultracel® membrane (P3C030C10) was installed in the system and cleaned and flushed prior to introducing the protein. After a 10-minute protein recirculation, the procedure described above was followed (membrane depolarization, product recovery, mass balance verification) once using air blow down and then a second time using buffer displacement. The product pool was collected into a clean graduated pitcher directly from the outlet of the product recovery port (XV-003). No bag or sterile filter was attached.

Figure 8A shows that the recovery of protein in the pool (product recovery steps 1 – 4) was similar for both methods; 96.4% yield was obtained using air blow down and 97.1% yield was obtained using buffer displacement. Mass balance for both tests was between 100 – 103%.

Figure 8B shows how the protein yield increases as volume of buffer used during displacement increases. However, this is offset by the fact that protein concentration decreases with increasing buffer displacement volume. For processes where a specific final protein concentration is required, significant dilution during product recovery is not practical. Therefore, a buffer displacement volume that nearly maximizes yield while allowing the protein concentration to remain above the threshold limit is desired. In our specific test case, we found that using a buffer displacement volume of approximately 370 mL allowed for a 96.8% yield (versus a plateau yield of 97.1%) while only resulting in 10% product dilution. Since the feed channel holdup volume of a Pellicon® 3 cassette with 1.14 m^2 30kD Ultracel® membrane is 170 mL, this means that approximately 200 mL of buffer over and above the cassette holdup was needed for adequate flushing of the lines and holder. This target (200 mL plus membrane holdup) would be a reasonable starting point for buffer displacement product recovery using other installed membrane areas in the TF2S, since the system tubing and TFF liner plate volume does not change as membrane area is modified.

Finally, it is very unlikely that use of a different size recycle container would change any of the product recovery results since recovery of product from the recycle vessel (Step 1) is very straightforward with no real areas for yield loss.

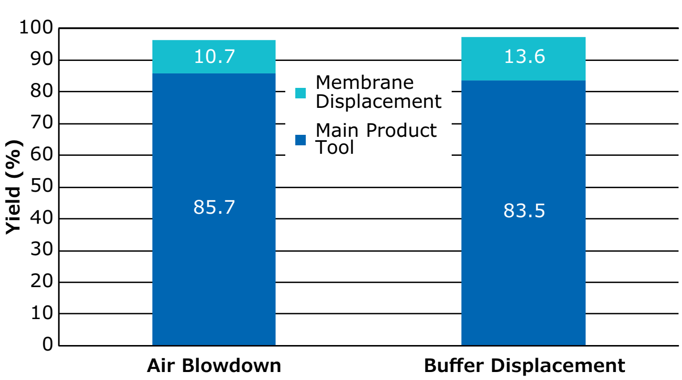

Figure 8A. Protein Yield during Product Recovery from Mobius® FlexReady system (TF2S) with 50 L recycle container, Pellicon® 3 cassette with 1.14 m^2 30kD Ultracel® membrane 50 g/L IgG

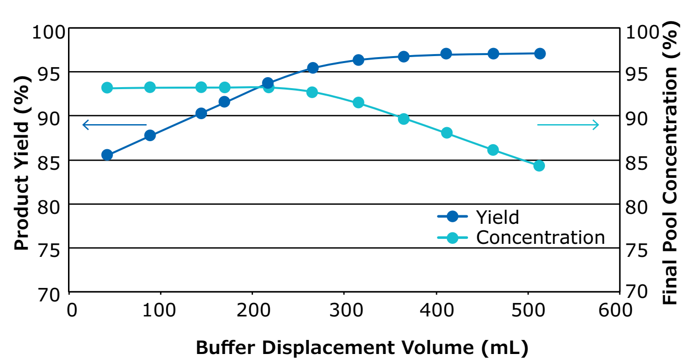

Figure 8B. Product Yield and Dilutions versus Buffer Displacement Volume

11. Temperature control performance of jacketed recycle vessel

Objective

Jacketed recycle vessels are available as an option for the Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF in 50 L, 100 L, and 200 L volumes for temperature maintenance during process operations. These vessels are essentially the Mobius® MIX 50, 100, and 200 L jacketed vessels, sharing the same geometry, MOC, mix bag, impeller, motor and controller. The jacket covers the side wall and part of the bottom of the vessel. Connections include jacket inlet, outlet, vent, and over-pressure rupture valve including a pressure gauge. Active temperature control must be provided by an off-skid temperature control unit (TCU), provided by the end-user. Some cooling and heating trends illustrate basic performance of the jacketed mixers.

Materials and Methods

The TCU was connected to the jacketed vessel, filled with heat transfer fluid (glycol) and the TCU/jacket system was vented of air. The mixer bag was installed, the mixer was engaged, and the feed/retentate lines were connected. The mixer bag was inflated using the on-board pressure regulator to ensure correct seating of bag. The mixer bag was filled with respective amount of water to simulate product volume. Two volumes were tested for each vessel, approximately ½ and full volume. For each volume, 1 cooling and 2 heating tests were run.

Mixer	Volume (L)	Test No.	Heat/Cool	Tini (°C)	Tfin (°C)
50 L	25/45	1	C	20	4
100 L	50/95	2	H	4	20
200 L	100/200	3	H	20	45

Table 2. Temperature control performance test plan

At the start of each test, the TCU temperature was set and the TCU pump was turned on. The vessel mixer was turned on. Thermocouples were used to monitor temperatures. The time to achieve the target end point was recorded.

The TCU parameters for the tests during operation were as follows:

	Chiller	Heater
Utility inlet pressure (barg)	0.5	0.5-0.7
Utility inlet flow rate (L/min)	8	8
Utility temperature (°C)	< 2	50-55
Ambient temperature (°C)	25	25
Vessel mixer (RPM)	310 (50 L) or 340 (100 L & 200 L)	

Table 3. Temperature control unit operating parameters for performance test

Results and Conclusions

The results for each of the 3 vessels are given in the tables below. The temperature trends are shown in the figures.

Note: End-user performance will vary depending on the capacity of the TCU used and other parameters. These results are provided for information only.

Vessel	Volume (L)	Test No.	Time (h:m)
50 L (Mobius® MIX 50 L mixer)	25	1	1:12
		2	0:15
		3	1:08
	45	1	2:30
		2	0:21
		3	0:58

Table 4. Temperature control performance test results for 50 L vessel (Mobius® MIX 50 L mixer)

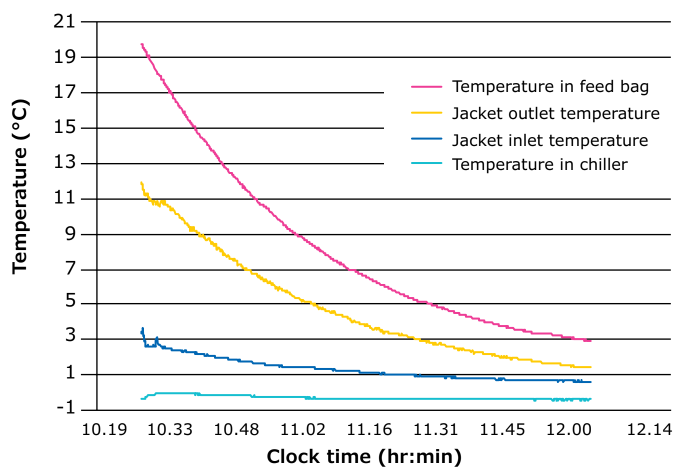

Figure 9A. 50 L (Mobius® MIX 50 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 25 L

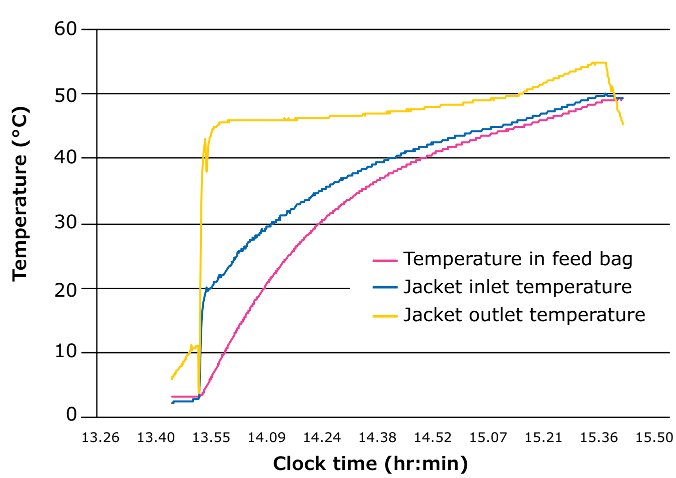

Figure 9B. 50 L (Mobius® MIX 50 L mixer) jacketed vessel heating time from 4°C to 45°C; volume in feed bag was 25 L

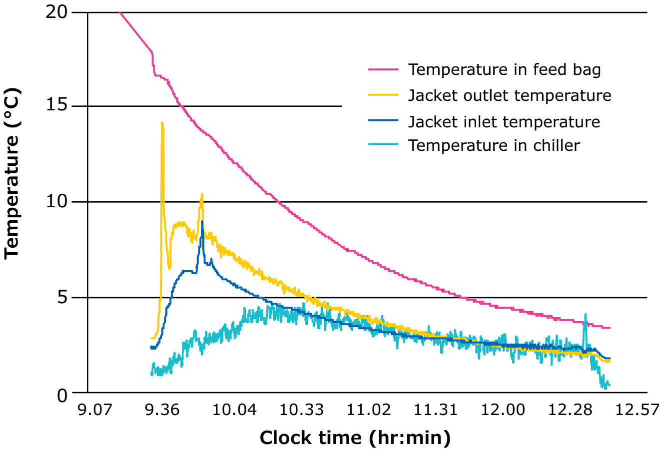

Figure 9C. 50 L (Mobius® MIX 50 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 45 L

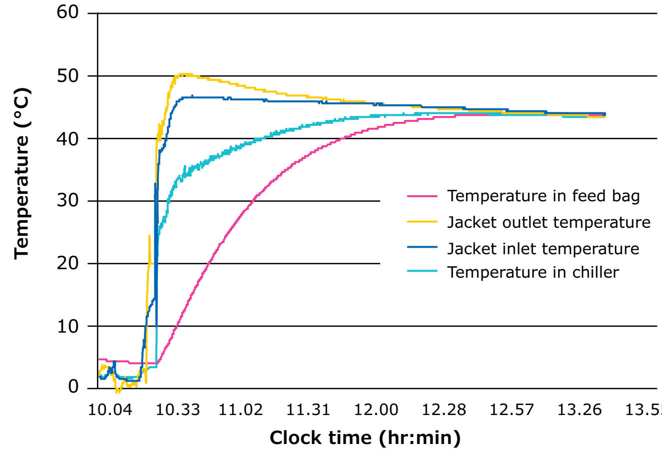

Figure 9D. 50 L (Mobius® MIX 50 L mixer) jacketed vessel heating time from 4°C to 45°C; volume in feed bag was 45 L

Vessel	Volume (L)	Test No.	Time
100 L (Mobius® MIX 100 L mixer)	50	1	2:21
		2	0:16
		3	0:59
	95	1	2:34
		2	0:19
		3	0:50

Table 5. Temperature control performance test results for 100 L vessel (Mobius® MIX 100 L mixer)

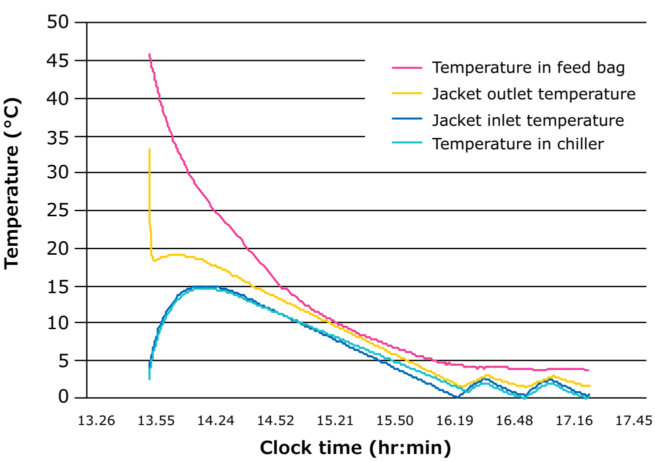

Figure 9E. 100 L (Mobius® MIX 100 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 50 L

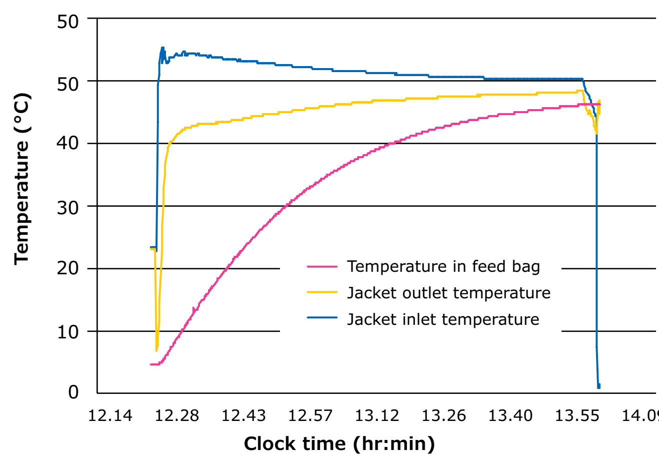

Figure 9F. 100 L (Mobius® MIX 100 L mixer) jacketed vessel heating time from 4°C to 45°C; volume in feed bag was 50 L

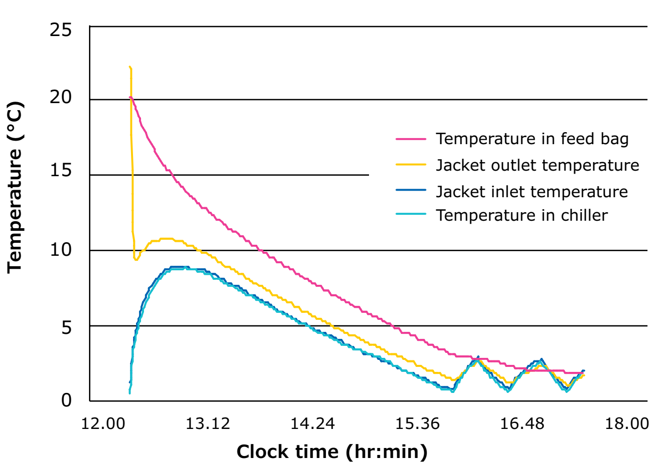

Figure 9G. 100 L (Mobius® MIX 100 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 95 L

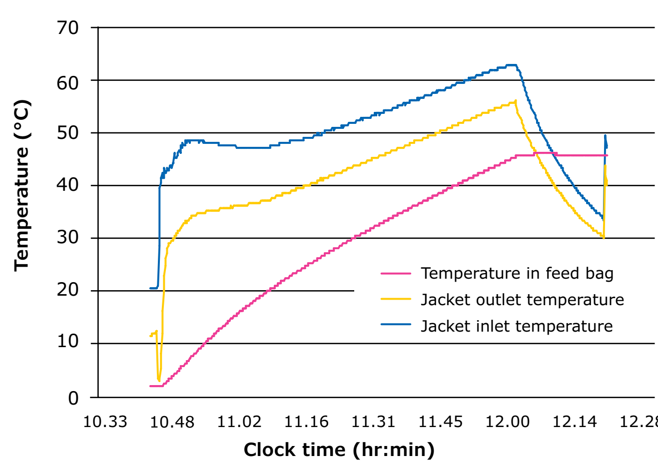

Figure 9H. 100 L (Mobius® MIX 100 L mixer) jacketed vessel heating time from 4°C to 45°C; volume in feed bag was 95 L

Vessel	Volume (L)	Test No.	Time
200 L (Mobius® MIX 200 L mixer)	100	1	1:55
		2	0:17
		3	0:49
	195	1	3:16
		2	N/A
		3	1:19

Table 6. Temperature control performance test results for 200 L vessel (Mobius® MIX 200 L mixer)

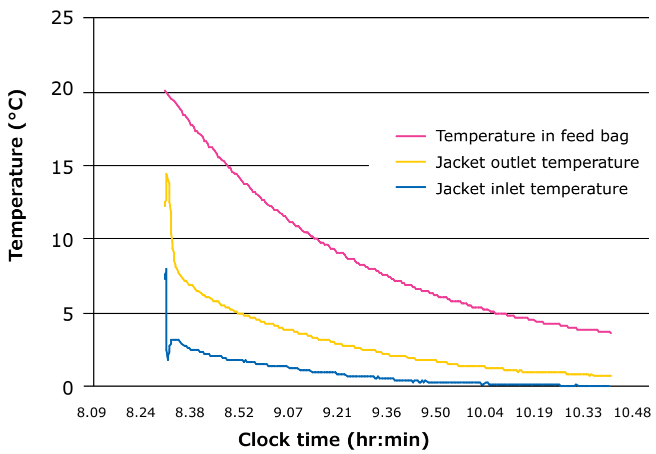

Figure 9I. 200 L (Mobius® MIX 200 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 100 L

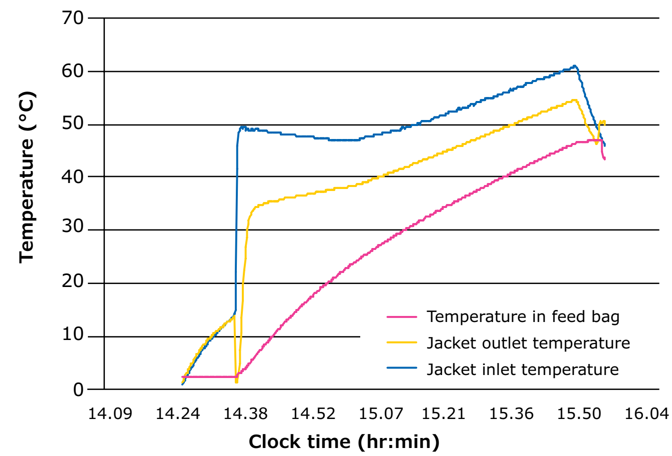

Figure 9J. 200 L (Mobius® MIX 200 L mixer) jacketed vessel heating time from 4°C to 45°C; volume in feed bag was 100 L

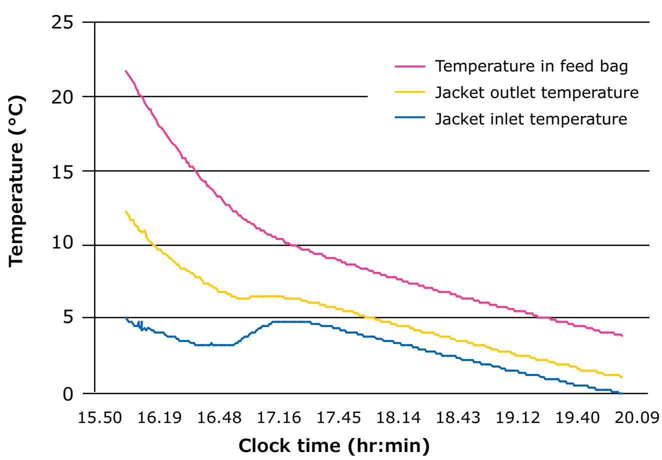

Figure 9K. 200 L (Mobius® MIX 200 L mixer) jacketed vessel cooling time from 20°C to 4°C; volume in feed bag was 195 L

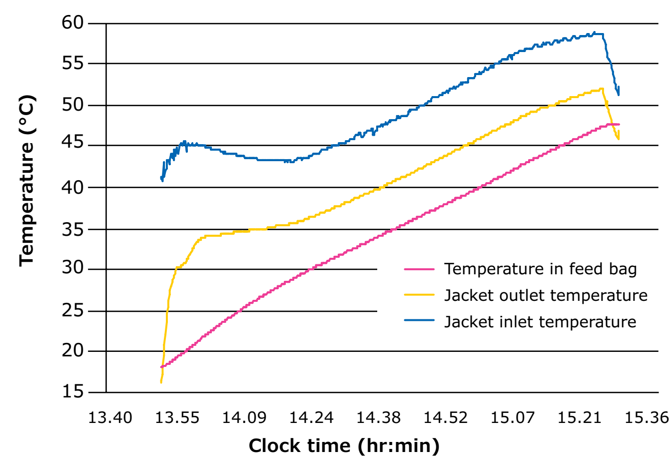

Figure 9L. 200 L (Mobius® MIX 200 L mixer) jacketed vessel heating time from 20°C to 45°C; volume in feed bag was 195 L

12. Temperature maintenance at low tank volume during TFF

Objective

Maximum jacket area is available to provide heat transfer when the jacketed vessel interior is full. The effective heat transfer area decreases as volume in the vessel drops (i.e. during concentration) and less sidewall is contacted. There is also a partial jacket covering the cone bottom of the vessel. This additional test explored a low vessel volume where the effective heat transfer area could maintain the vessel contents at 4°C.

Materials and Methods

Refer to section 9 for details of the setup and the operation parameters for the TCU. A certain low volume was filled in each vessel according to the table. The volume in the 50 L was below the cone, but in the 100 L and 200 L vessels the volume was up to the cone. The vessel mixer was turned on. Thermocouples were used to monitor temperatures.

Results and Conclusions

The temperature was maintained below 4°C for >3 hours in the 50 L and 4 hours in the 100 L and 200 L vessels. The 50 L test was 3 hours by design.

Vessel	Volume (L)	RPM	TCU recirc (L/min)	4°C Maintained (hrs)
50 L	3	120	10	3
100 L	cone	150	10	4
200 L	cone	250	5	4

Table 7. Low tank volume temperature maintenance test parameters

Below is an example of the 50 L temperature maintenance data. Thermocouples were placed at three locations within the vessel: the liquid surface at the center, the wall, and at the bottom of the cone.

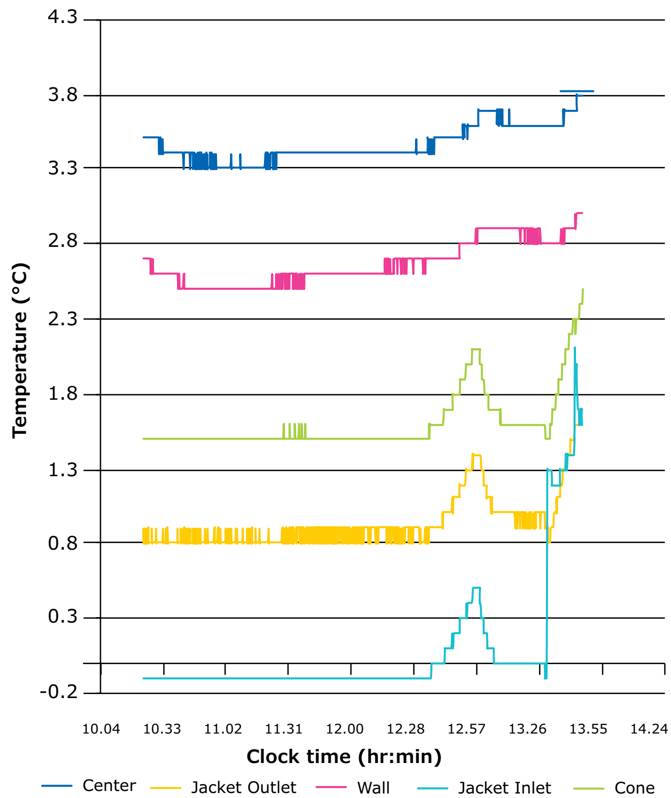

Figure 10A. Maintenance of 4°C at low volume of 3 L in the 50 L (Mobius® MIX 50 L mixer) jacketed vessel

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