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Performance Guide

# Mobius® TFF 20 System for Tangential Flow Filtration

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## How to Use This Guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® TFF 20 Solution with Single-Use 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 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.

# Introduction

**The Mobius® TFF 20 system with Flexware® Assemblies is a fully automated system designed to enable the clinical and commercial-scale operation of TFF processes for the concentration and diafiltration of Monoclonal Antibodies (MAbs), Antibody Drug Conjugates (ADC), vaccines, viral vectors, and therapeutic proteins. This system is part of the same family as Mobius® TFF 80, sharing the same design, components, and software platform.**

The Mobius® TFF 20 system is composed of a tank cart, a base cart holding the clamshell and a holder for Pellicon® Cassette, or a stand for Pellicon capsule. We also offer a bracket to hold hollow fiber devices. In addition to feed pump, and transfer pump control loops, the system also includes two automated pressure control valves on the retentate and on the filtrate lines to enable open UF and MF operations. Two flow sensors allow for feed and filtrate flow measurement as well as flux, concentration factor, diavolumes, and membrane permeability calculation. Furthermore, an optional transfer line flowmeter is available. The maximum reachable flow rate during processing may change, mainly due to the membrane area and product viscosity but the system has been designed to be capable of feeding at 20 L/min with a 1 cP solution. Inlet manifold on both the feed pump or the transfer line allow to manage WFI, caustic, buffers and product according to the process needs. Three vessel volumes can be chosen, 50 L, 100 L or 200 L with the same tank geometry as for Mobius® TFF 80 system and Mobius® mixers. In addition, all sizes are available with double jacketed carrier and temperature control. Recycle vessels come with integrated load cells, bottom-mounted mixer, automatic inflation of bag and temperature sensor. SU pH measurement and conductivity in the recycle vessel is available, as well as conductivity, UV, and pH (MU or SU) instrumentation on the TFF filtrate line.

The specific system size 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

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# 1. System hold up volume

## Background and Objectives

The hold up volume corresponds to the volume contained in the recirculation loop, it has to be added to the recirculation tank volume to determine the exact volume of solution contained in the system during a process. It is therefore useful to determine this volume to correctly monitor the progress of a process, if based on the volume of solution.

## Materials and Methods

The following tests have been performed on all Mobius® TFF 20 system tank volume. The recycle bag and the entire Flexware® are installed following the instructions provided in the user guide. The mixer motor is engaged with the impeller cup on the bottom of the bag. One CIP plate (PEH0PTCIP1) is installed between the cassette liners. The load cells are tared prior to adding 1 cP solution (WFI) to fill the bag up to 10 L. The amount of solution used to fill the bag is determined thanks to a weight scale set under the tank from which it is pumped into the bag. The system is set in recirculation mode and the feed pump is started and ramped up to 50% of speed to remove any air from flow path. The feed line drain is connected to an empty tank placed on a weight scale and tared. The feed pump is stopped, and the solution is removed from the flow path using the feed line drain, down to 5 L in the tank. Removed WFI is collected in a tank set on a weight scale to allow for the remaining water quantity measurement. For a better precision, water is furtherly removed using the retentate line sample port until reaching the hold up volume (no water in the tank but full flow path). By subtracting the recovered volume and the CIP plate volume from the initially introduced volume, we obtain the hold up volume.

**Note:** cassette configuration may vary from one process to another, therefore, measured values in this study do not take cassettes volume into account. Total retentate side volumes of cassettes must be added for exact hold up volume determination in customer process conditions.

## Results

Hold up volumes differ for each system's size as the tank cart dimensions vary in order to provide the most compact system at each size.

**Table 1: Hold up volume system**

| Tank size (L) | Measured hold up volume in average (L) |
|---|---|
| 50 L | 0.725 |
| 100 L | 0.742 |
| 200 L | 0.770 |

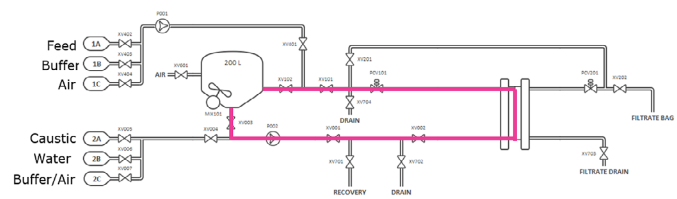


**Figure 1.** Flow path corresponding to the hold up volume

# 2. Minimum working volume against feed flow rate

## Background and Objectives

Understanding the Minimum Working Volume (MWV) within a system is critical to ensure that air is not entrained into the outlet of the recycle bag during a process. Testing performed at a range of flow rates will elucidate how low a solution can be concentrated volumetrically at a given flowrate. This is particularly important for high concentration processes. Since MWV is dependent on flow rate and viscosity, conditions from 2 to 20 L/min and viscosities of 1 cP, 25 cP and 40 cP were evaluated for the Mobius® TFF 20 system with 50, 100 and 200 L vessels.

## Materials and Methods

All Flexware® assemblies are installed into the system following the user guide instructions. The recycle vessels used in these studies are 50, 100 and 200 L. One CIP plate is installed on the holder instead of flatsheet devices. The mixer is disabled. The Membrane Recycle flowpath is opened. The retentate control valve (PCV101) is set to be 100% open. At this stage, two different methods are usually used to determine the MWV: an **additive** method and a **subtractive** method. The substractive method has been chosen to perform the tests.

## Subtractive method

The recycle bag is filled up to 15/20 L with either water (1cP solution), a 74% glycerin/water solution (25 cP solution) or 79% glycerin/water solution (40cp solution). The exact amount of liquid introduced is only known at the end when all the volume is recovered. The dead volume which is left in the system is also considered. Using a CIP plate and clamps which block the filtrate line and drain, the feed pump is slowly ramped up to the maximal flowrate (20 L/min) to remove any air from the recirculation flow path. Once the lines are filled and no air is remaining, solution is slowly removed from the flow path using the retentate sample port and poured into a container on a tared weight scale allowing for exact volume determination. Solution is removed until the minimum working volume is reached at 20 L/min. This volume is determined by the volume at which the first bubbles enter the feed flow. The volume of solution removed is used to calculate the MWV. The feed pump flowrate is decreased in 2 L/min increments down to 2 L/min (minimum flow rate of the system). By adding all volume recovered, and dead volume (unrecoverable) and subtracting the CIP plate volume, the MWV is determined for each flowrate.

At each increment, the minimum working volume is assessed in the same manner.

## Results and conclusion

The CIP plate was used in this study in place of actual TFF cassettes so that the full range of achievable flow rates could be evaluated without membrane pressure drop limitation and its possible impact on measurement.

The minimum working volumes were found similar for the different tank sizes as the slight variation in tubing lengths did not significantly affect these volumes: less than 1 L at 2 L/min and less than 4 L at 20 L/min. However, it is important to note that minimum working volumes can vary depending on the membranes used.

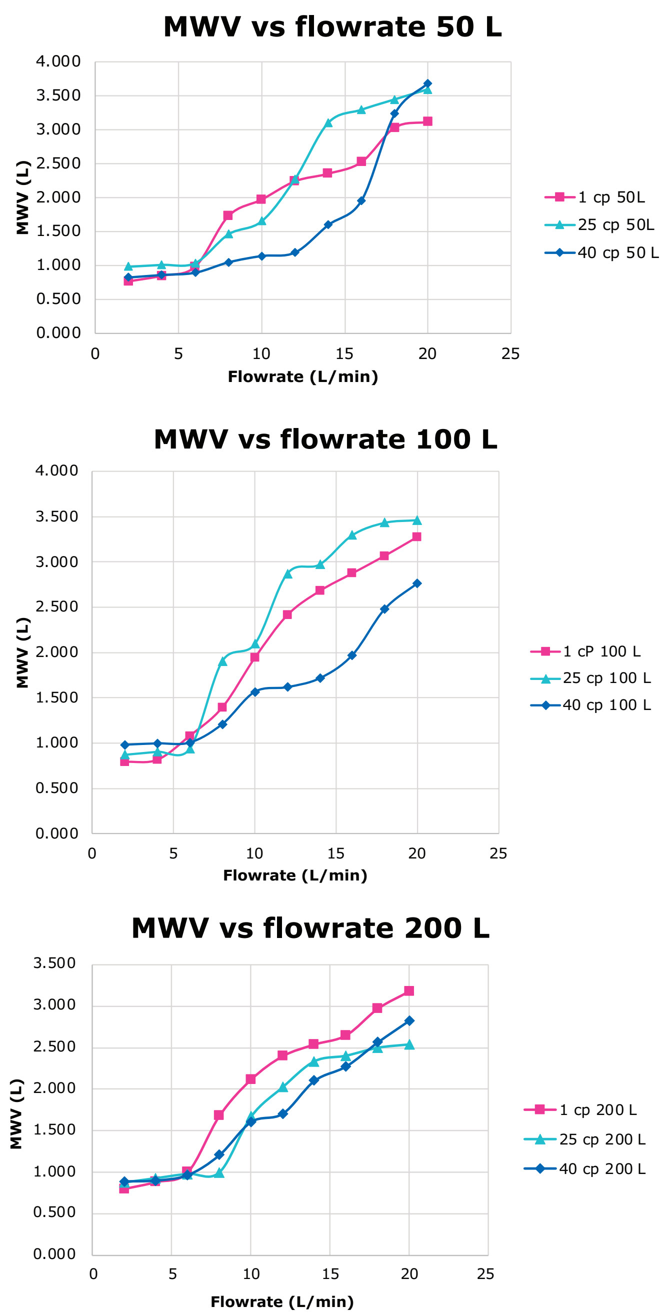


**Figure 2.** Minimum Working Volume versus Flow Rate and Viscosity for Mobius® TFF 20 system

# 3. System drainability and dead volume

## Background and Objectives

Drainability is a key factor for TFF processes that involve numbers of flushing and draining steps. Dead volumes must be prevented as much as possible to reduce the amount of solution needed to flush and to ensure optimal yield during the recovery phase. Therefore, the objective of this test is to measure the total dead volume of the system and provide an idea on its drainability.

## Materials and Methods

The recycle bag and the entire Flexware® are installed following the instructions provided in the user guide. The mixer motor is engaged with the impeller cup on the bottom of the bag. A CIP plate is installed on the cassette holder and clamps block the filtrate line and drain. The load cells are tared prior to adding 1 cP solution (water) to fill the bag up to 10 L. The exact amount of water used to fill the bag is determined thanks to a weight scale set under the DI water tank from which it is pumped. The system is set in recirculation mode (retentate line diverted to recirculation tank) and the feed pump is started and ramped up to 50% of speed to remove any air from flow path. Once no air remains, the feed pump is stopped, and the solution is removed from the flow path using the feed line drain, down to 5 L in the tank. Using the sample port from the retentate line, the volume is brought to the hold-up volume which corresponds to a known amount (see section 1).

First, the system is drained by opening XV702 (drain valve) and running the feed pump at 10% (helps to drain the feed line correctly). Then, transfer pump is also used to push liquid through the membranes line down to the XV702. The drained solution is weighted. The dead volume is calculated by subtracting the drained volume to the initial volume. Secondly, the same steps are followed but tubing's where water is retained are manually leaned (retentate sample port line leaned toward the clamshell, retentate cassette exit leaned toward the cassette and feed line cassette entry leaned towards the clamshell).

## Results

The results for each of the two draining methods are given in the table below. When draining the system, results have shown that the remaining volume in the system is lower when tubing's are manually leaned to prevent any unwanted retention area. In these conditions, undrainable volume contained in the system is around 100 mL for the Mobius® TFF 20 System. Further recovery methods can be applied to increase product yield (i.e., buffer rinse, air blowdown, etc.).

**Table 2: Dead volumes according to the draining performed for the Mobius® TFF 20 system**

| | | | |
|---|---|---|---|
| **Initial volume** | Hold up volume (mL) | 734 | |
| | CIP plate volume (mL) | 250 | |
| | **Total (mL)** | **984** | |
| **Drained volume** | | Drained without external action | Drained with tubing's lean |
| | Recovered volume (mL) | 811 | 837 |
| | Remaining volume in the CIP plates (mL) | 45 | |
| | **Total (mL)** | **856** | **882** |
| **Resulting dead volume (mL)** | | **128** | **102** |

# 4. System pressure drop versus flow rate and viscosity

## Background and Objectives

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 (PT001) 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 (PT101).

Since the Mobius® TFF 20 system 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 2 to 20 L/min and viscosities of 1 cP, 25 cP and 40 cP were evaluated.

## Results

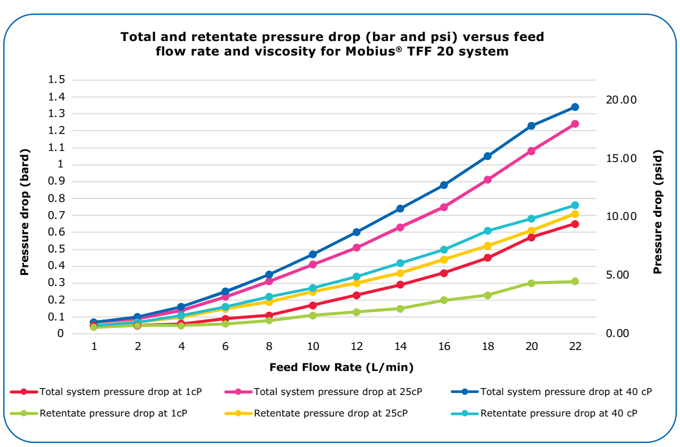


**Figure 3.** shows the total pressure drop through the TFF 20 system across the full range of achievable flow rates for viscosities up to 40 cP.

## Materials and Methods

All Flexware assemblies are installed into the system per the user guide instructions. A CIP plate (PEH0PTCIP1) is installed in the holder instead of flatsheet devices. The recycle bag is filled with approximately 15/20 L of water using the transfer pump and inlet manifold. All equipment is set to AUTO mode. The "Membrane Recycle" flow path is opened. The retentate control valve (PCV101) is set to 0% Closed. The filtrate control valve (PCV201) is set to 0% Open. The feed pump (P001) is set to a speed set point of 50% for priming. Once the system is primed, the feed pump is set to feed flow mode with a set point at 2 L/min. Once the flow and pressures stabilized, the feed flow rate (FI001), feed pressure (PT001), and retentate pressure (PT101) are recorded from the HMI display. The feed pump flow setpoint is sequentially increased in small steps and the stabilization and recordings are repeated at each new flow setpoint. After the pressure drop at flow rates from 2–20 L/min are recorded, water is drained from the system using the drain line. The entire sequence is repeated using an approximately 74% and 79% w/w glycerin/water solution to generate data at 25 and 40 cP. All tests are conducted at ambient temperature (19–24 °C) and viscosity is verified using a Brookfield viscometer.

**Note:** The CIP 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 CIP plate is a stainless steel rectangular annular ring with a thickness of 10 mm. 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 can flow through the holder without any significant pressure drop.

# 5. Lowest volume immerging the mixer/Mixing types according to mixer speed and recycle vessel volume at 1 cp

## Background and Objectives

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. It is also important to define the minimum volume in the tank allowing to keep the mixer on.

## Materials and Methods

The recycle bag and the entire Flexware® are installed 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 (water) via the transfer pump and inlet manifold to fill the bag entirely. Mixing is started at 5% and after having reached a steady state (around 1 to 2 min) the mixing type is visually determined. Mixing speed is adjusted by increments of 5% up to 100% using the HMI. Liquid surface is observed at each mixing speed increment, up to the maximum tested (100%) and mixing type is determined. Tank volume is lowered, and the mixing speed excursion is repeated, starting again at 5% mixing speed.

To determine the volume immerging the mixer, the tank was slowly drained using the retentate sample port until mixer's blade was touching the liquid surface. Resulting tank volume was then considered as minimum volume to immerge the impeller. **Table 3** shows the different mixing types considered.

**Table 3: Mixing criteria**

| Type of mixing | Description/Observation |
|---|---|
| Calm surface | No surface motion |
| Gentle mixing | Movement at the surface, no vortex |
| Mixing | Vortex starting to form but unstable or rapid movement/ dimples at the surface |
| Mixing with turbulences | Stable vortex or very strong mix without bubbles |
| Turbulences and bubbles | Full vortex and churning, bubble formation |

## Results and Conclusions

**Figure 4**, **Figure 5**, and **Figure 6** show the onset surface turbulence at 1 cP and potential areas of concern for shear sensitive operations in the 50 L, 100 L and 200 L vessels, respectively. The impeller becomes submerged around a volume of 1.4 L in the 50 L vessel, 5.7 L in the 100 L vessel and 9.1 in the 200 L vessel. As the volume increases above this level, the mixer speed at which turbulences sets in also increases. At 25 L, 50 L and 170 L in the 50 L, 100 L and 200 L vessels, respectively, the full mixer speed no longer generates troubling surface turbulences. To minimize the subjectivity of this test, more than one operator agreed on the mixing type description and all measurement were performed by the same operator to keep the same appreciation throughout both vessel measurements.

Lowest mixable volumes are summarized in **table 4**.

**Note:** Mixing types are given for information and may vary depending on the viscosity of the product and the speed of the feed pump (not considered in these measurements).

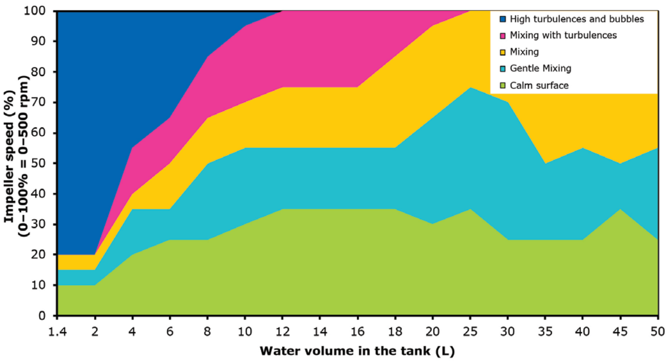


**Figure 4.** Mixer speed (%) vs. Water Volume (L) in the 50 L recycle vessel and resulting mixing type

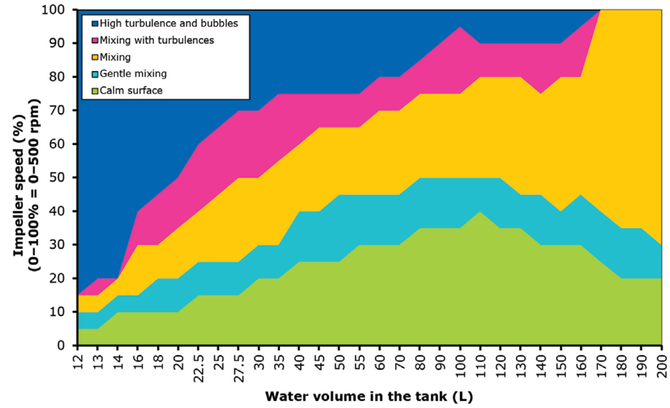


**Figure 6.** Mixer speed (%) vs. Water Volume (L) in the 200 L recycle vessel and resulting mixing type

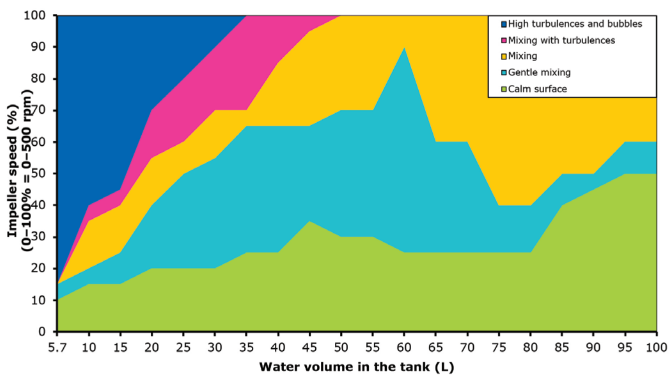


**Figure 5.** Mixer speed (%) vs. Water Volume (L) in the 100 L recycle vessel and resulting mixing type

## Table 4. Minimum tank volume required to fully immerge the impeller according to tank size

| Tank Size (L) | 50 | 100 | 200 |
|---|---|---|---|
| Minimum volume required to immerge the impeller (L) | 1.8 | 5.7 | 9.1 |

**Note:** Since the design of the 200 L tank is identical for both the Mobius® TFF 20 and the Mobius® TFF 80, the mixing characterization tests were referenced from the performance guide of the Mobius® TFF 80.

# 6. Temperature control performance of jacketed recycle vessel

## Background and Objectives

Jacketed recycle vessels are available in the standard offering of the Mobius® TFF 20 systems for temperature maintenance during process operations.

For all tank sizes, the double jacket covers the side wall and part of the bottom of the vessel.

Connections include jacket inlet, outlet and a vent. Active temperature control must be provided by an offskid Temperature Control Unit (TCU), provided by the end-user. Some cooling and heating trends illustrate basic performance of the jacketed mixers.

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

## Materials and Methods

The TCU is connected to the jacketed vessel, filled with heat transfer fluid (30% glycol) and the TCU/jacket system is vented of air. The mixer bag is installed, the mixer is engaged, and the feed/retentate lines are connected. The mixer bag is inflated using the on-board pressure regulator to ensure correct seating of bag. The mixer bag is filled with respective amount of water to simulate product volume. Two volumes are tested for each vessel, approximately ½ and full volume. Lines are then clamped to prevent any recirculation. For each volume, two heating steps (from 20 °C to 45 °C and from 4 °C to 20 °C) and one cooling step (from 45 °C to 4 °C) are performed. All three steps are performed in the same test run for each volume.

**Table 5: Volume tested for each tank size**

| Vessel size (L) | Volume tested (L) |
|---|---|
| 50 | 25 |
| | 50 |
| 100 | 50 |
| | 100 |
| 200 | 100 |
| | 200 |

**Table 6: Summary of the different heating and cooling steps performed for each volume tested**

| Step | Heat/Cool | Tini (°C) | Tfin (°C) | TCU setpoint (°C) |
|---|---|---|---|---|
| 1 | Heat | 20 | 45 | 50 |
| 2 | Cool | 45 | 4 | 0 |
| 3 | Heat | 4 | 20 | 50 |

**Table 7: General information on the performed test**

| General information | 50 L | 100 L | 200 L |
|---|---|---|---|
| TCU used | Lauda T 4600 | | Lauda VC 10000 |
| Ambient temperature (°C) | 20-27 °C | | 20-23 °C |
| Vessel mixer speed (in rpm) | 500 | 250 | 340 |
| Vessel mixer speed (in %) | 50% | | 68% |
| Product used | DI water | | |

The system is capable of controlling an external TCU with two modes: bath temperature and product temperature, therefore, heating and cooling trials are conducted using a single CCP recipe. This recipe is written to stabilize the vessel temperature at 20 °C by applying a bath temperature of 19 °C. The vessel mixer is turned on. Once the vessel temperature is stable at 20 °C during 10 min the bath temperature setpoint is switched to 50 °C to start the heating phase from 20 °C to 45 °C. Once the vessel reached a temperature of 45 °C for over 2 minutes, the bath temperature setpoint is switched again to 0 °C to bring the vessel temperature at 4 °C. Once the vessel reached a temperature of 4 °C for over 2 minutes, the bath temperature setpoint is switched back to 50 °C to reach a vessel temperature of 20 °C. Once reached the bath temperature control is stopped. During the entire recipe, the mixing speed is stable and set as shown in the previous tables.

In addition to the inbuilt temperature recorder, thermocouples are used to monitor temperatures in different places inside the vessel. The time to achieve the target end point was recorded.

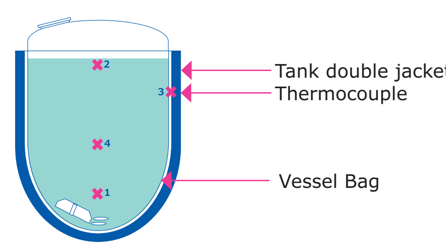


**Figure 7:** Transversal view of the system vessel with corresponding thermocouples number and placement for the 50 L and 100 L tank

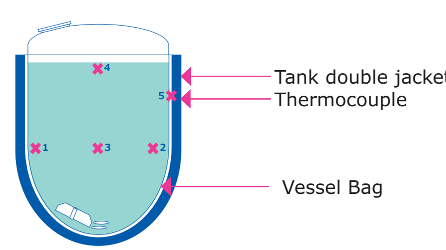


**Figure 8.** Transversal view of the system vessel with corresponding thermocouples number and placement for the 200 L tank

**Table 8: Temperature sensors number and placement description**

| Temperature sensor number | Position (50 and 100 L) |
|---|---|
| 1 | In the bag, at the bottom of liquid |
| 2 | In the bag, at the top of the liquid surface |
| 3 | Between the double jacket and the bag (**not in the solution**) |
| 4 | In the bag, in the middle of the liquid (only for full volume) |
| **Temperature sensor number** | **Position (200 L)** |
| 1 | On the interior surface of the bag above the impeller |
| 2 | On the interior surface of the bag at the opposite side of the impeller |
| 3 | In the middle of the vessel |
| 4 | In the middle of the vessel at the liquid surface |
| 5 | Between the double jacket and the bag (**not in the solution**) |

## 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.

**Table 9: Temperature control performance test results for the 50 L vessel filled at 50%**

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| 50 L | 25 L | 1 | 20 | 45 | 02:19:00 |
| | | 2 | 45 | 4 | 03:12:30 |
| | | 3 | 4 | 20 | 00:32:00 |

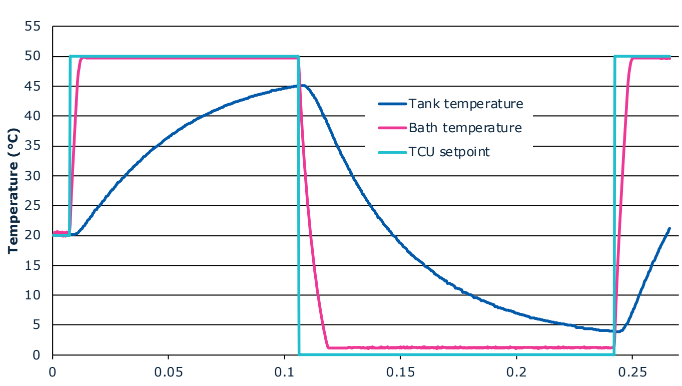


**Figure 9.** 50 L jacketed vessel heating and cooling test; volume in feed bag was 25 L

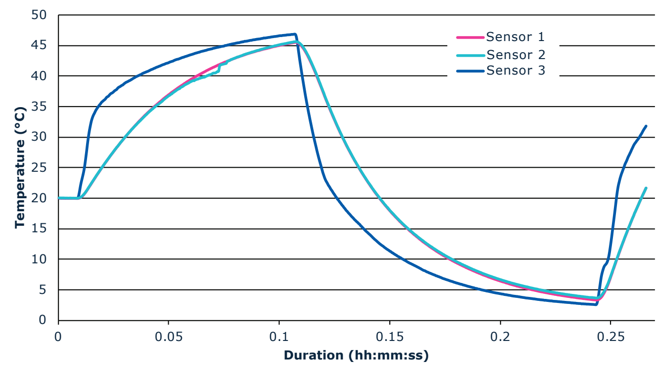


**Figure 10.** Sensors measurements in the 50 L jacketed vessel during heating and cooling test; volume in the bag was 25 L

**Table 10: Temperature control performance test results for the 50 L vessel filled at 100%**

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| 50 L | 50 L | 1 | 20 | 45 | 01:53:30 |
| | | 2 | 45 | 4 | 03:09:00 |
| | | 3 | 4 | 20 | 00:31:00 |

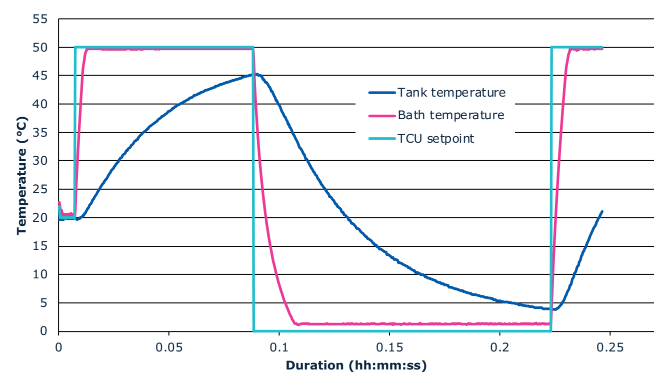


**Figure 11:** 50 L jacketed vessel heating and cooling test; volume in feed bag was 50 L

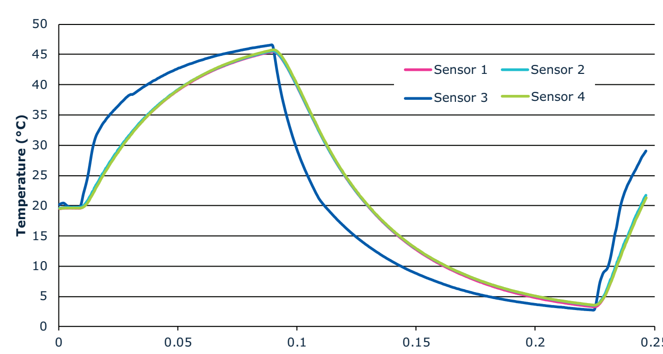


**Figure 12.** Sensors measurements in the 50 L jacketed vessel during heating and cooling test; volume in the bag was 50 L

### Table 11: Temperature control performance test results for the 100 L vessel filled at 50%

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| | | 1 | 20 | 45 | 02:11:30 |
| 100 L | 50 L | 2 | 45 | 4 | 03:26:00 |
| | | 3 | 4 | 20 | 00:37:00 |

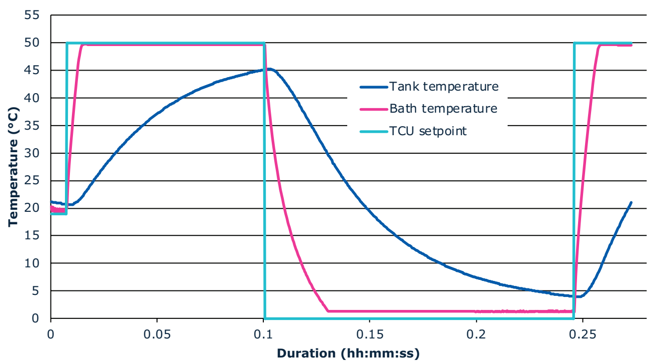


**Figure 13.** 100 L jacketed vessel heating and cooling test; volume in feed bag was 50 L

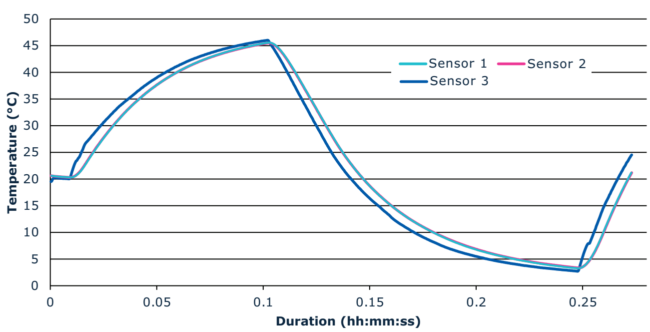


**Figure 14.** Sensors measurements in the 100 L jacketed vessel during heating and cooling test; volume in the bag was 50 L

### Table 12: Temperature control performance test results for the 100 L vessel filled at 100%

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| | | 1 | 20 | 45 | 02:27:00 |
| 100 L | 100 L | 2 | 45 | 4 | 04:38:00 |
| | | 3 | 4 | 20 | 00:42:30 |

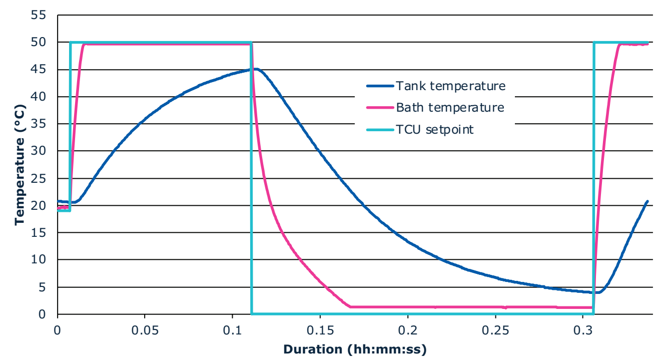


**Figure 15.** 100 L jacketed vessel heating and cooling test; volume in feed bag was 100 L

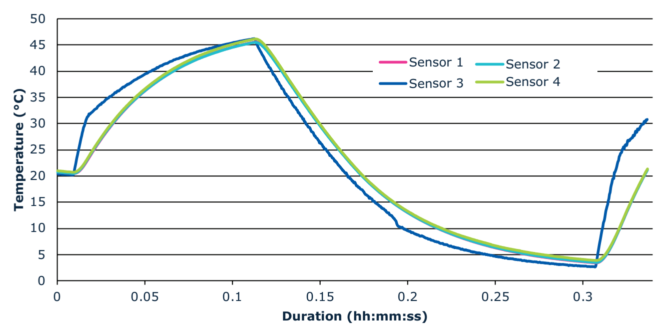


**Figure 16.** Sensors measurements in the 100 L jacketed vessel during heating and cooling test; volume in the bag was 100 L

## Table 13: Temperature control performance tests results for the 200 L vessel filled at 50%

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| 200 L | 100 L | 1 | 20 | 45 | 2:35:16 |
| | | 2 | 45 | 4 | 4:17:36 |
| | | 3 | 4 | 20 | 0:49:06 |

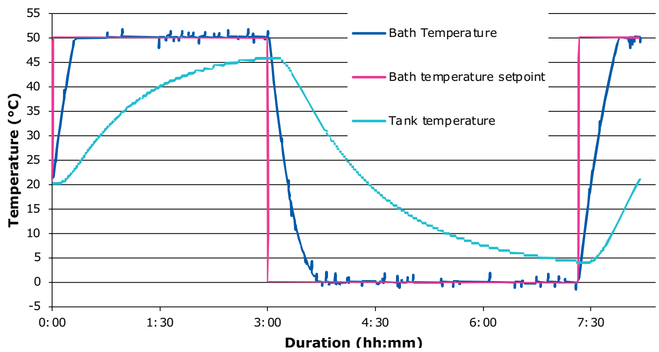


**Figure 17.** 200 L jacketed vessel heating and cooling test; volume in feed bag was 100 L

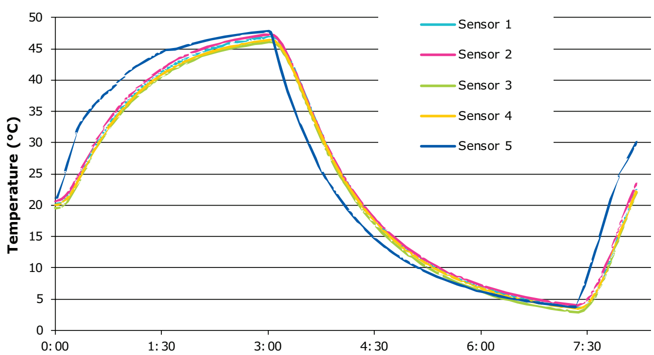


**Figure 18.** Sensors measurements in the 200 L jacketed vessel during heating and cooling test; volume in the bag was 100 L

## Table 14: Temperature control performance tests results for the 200 L vessel filled at 100%

| Vessel size | Volume (L) | Test | Tini °C | Tfin °C | Time needed (h:mm:ss) |
|---|---|---|---|---|---|
| 200 L | 200 L | 1 | 20 | 45 | 2:55:20 |
| | | 2 | 45 | 4 | 4:51:10 |
| | | 3 | 4 | 20 | 0:57:10 |

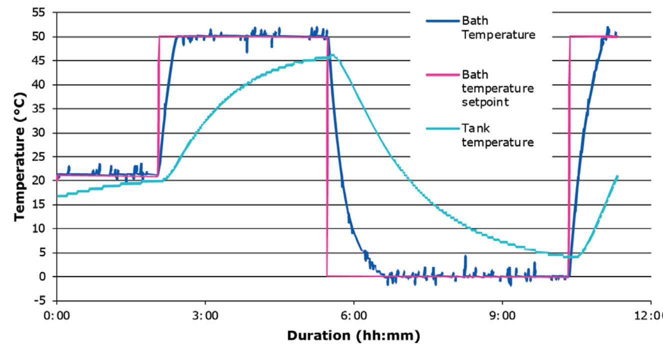


**Figure 19.** 200 L jacketed vessel heating and cooling test; volume in feed bag was 200 L

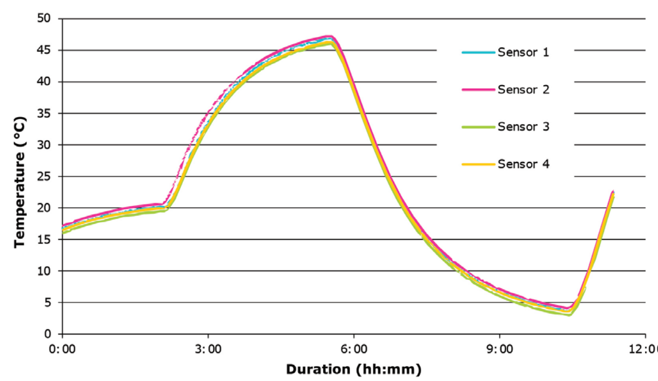


**Figure 20.** Sensors measurements in the 200 L jacketed vessel during heating and cooling test; volume in the bag was 200 L

**Note:** Since the design of the 200 L tank is identical for both the Mobius® TFF 20 and the Mobius® TFF 80, the temperature tests are referenced from the performance guide of the Mobius® TFF 80.

# 7. Protein UF/DF and recovery step

## Background and Objectives

Ultrafiltration is used to purify and concentrate a retained solute. Diafiltration 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 has been run on the Mobius® TFF 20 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 were used for the UF/DF.

## Materials and Methods

All Flexware® assemblies are installed into the system following the User Guide instructions. The recycle bag outlet is connected to the feed pump inlet. The TFF 20 recycle vessels used in this study is the 50 L tank. The Flexware® bag retentate port is connected to the clamshell return via the retentate sampling assembly. One Pellicon® 3 membrane (1.14 m² Cat# **P3B010A10**) is installed on the cassette holder. The cassette is flushed with water according to the Pellicon® 3 Cassettes Installation & user guide (**AN1065EN00**) and using the following system procedures.

**Membrane flush in WFI:** The transfer inlet manifold is connected to the transfer pump, themselves connected to the clamshell through the transfer flowmeter. The flush assembly on the retentate outlet and the filtrate outlet are directed to drain. Caustic solution and water are connected to the TFF 20 via XV005 and XV006 transfer manifold valves, respectively. Using the HMI controls, the “Water Source” and “Single-pass Flush from Inlet Manifold” flow paths are engaged. The retentate control valve (PCV101) and filtrate control valve (PCV201) are fully opened.

Flow control mode is engaged on the feed pump and set to 6.8 L/min. Retentate pressure control is engaged on the PCV101 and set to 1.2 barg (25 psig), to target a conversion ratio of around 30%. Once stable, the retentate PCV was fixed in position control (keeps the position corresponding to the previous TMP control). The filtrate draining valve (XV703) is pulsed several times to rinse the line. After around 30 L of water is flushed through the membrane to drain, conductivity reading confirmed that the target of 2.5 µS/cm is reached (conductivity of water used). Removing the feed line from the source of water allowed the lines to be pumped empty before switching off the pump.

**Sanitization:** Sanitization is done using a 0.5 M NaOH solution prepared in a Mobius® Power MIX 100 L and connected to the feed pump inlet XV005. The retentate line drain (XV704) is connected to the Mobius® Power MIX 100 L to create a recirculation loop. Flow control mode was engaged on the feed pump and set to 6.8 L/min. Retentate pressure control is engaged on the PCV101 and set to 1.2 barg (25 psig), to target a conversion ratio of 30%. The filtrate draining valve (XV703) is pulsed several times to rinse the line with the caustic solution and ensure caustic presence in this part of the membrane during the entire sanitization. Recirculation duration is 1 h. The PCV101 is completely opened and the feed pump is shut down prior to draining the system using the feed line drain (XV702). Post sanitization flushing is made following the same procedure as for the cassette preservation solution removal described previously.

**NWP measurement:** A NWP measurement is conducted to determine the value following the initial contact with caustic. The water source is connected to valve XV006, and the “single pass flush” flowpath is used. The flow control mode on the feed pump is activated and set to 7 L/min. Additionally, retentate pressure control is enabled on PCV101, set to 1 barg. After achieving stability, the NWP is measured for 5 minutes, resulting in an average logged NWP of 172.8 L/m²/h/bar.

**Membrane integrity test:** Retentate flow path is drained, and integrity test is performed using the Integritest® 5 connected to post pump transfer line dedicated connection.

**Equilibration:** Equilibration buffer (0.1 M Sodium Bicarbonate) is connected to transfer pump and the tank is filled up to 10 L. The retentate and permeate PCV valves are fully opened and both retentate and permeate lines diverted back to the tank. Recirculation loop is opened, and the feed pump is started at 6.8 L/min. Retentate PCV valve is partially closed to reach a TMP of 1.2 bar.

**Product fill and UF/DF:** 300 g Bovine Serum Albumin (BSA) is diluted to 6 g/L with PBS Phosphate Buffer Salin to a volume of 50 L in a Mobius® MIX 50 which is connected to the XV402 inlet. An additional 0.1 M 70 L of bicarbonate sodium solution is prepared as diafiltration buffer in a Mobius® MIX 100 which is connected to the XV403 inlet. The "Feed Source Open" and the "Recycle Bag Fill" flow paths are engaged to transfer 50 L of protein feedstock into the recycle bag using the transfer pump at a speed setpoint of 30%. The mixer is enabled in Auto mode.

Using a pre-programmed recipe, the following processes are executed: a concentration based on volume from 50 L to 9.14 L total volume setpoint, a 5-diavolume diafiltration and a final concentration to 4.13 L. The feed pump flow control is set at 6.8 L/min, and the retentate pressure control is set at 1.2 bar. Level control during diafiltration is set at 9.14 L in the tank, which corresponds to the wanted 10 L without hold up volume and cassette volume. After the final concentration step is complete, a recommended depolarization step is performed by engaging the "Membrane Recirculation" flow path, setting the permeate return to retentate, opening the retentate control valve, and setting a feed pump flow of 5 L/min to recirculate for 10 minutes, with active mixing.

**Product recovery:** Product recovery is performed by opening the recovery line (XV701) and pushing with the feed pump. The transfer pump is used to push through the retentate line and through the membranes.

An additional buffer recirculation is used to recover any remaining protein from the system and complete the protein mass balance (load versus recovery). Yields are calculated for both recovery steps (before and after buffer recirculation) using a spectrophotometer.

Cleaning commenced using 0.5 M NaOH connected to XV005 inlet and standard procedures. Post cleaning NWP measurement is conducted using dedicated solution.

## Results and conclusion

All the steps of a protein UF/DF process are executed in this trial, from system setup and membrane flushing through to product recovery, including a 5x VCF, 5x DF and final concentration of 2 VCF. Automation features such as flow paths, feed flows and pressure control loops, and recipe control including step transition criteria, are employed. The mass balance of the process is the primary metric for this UF/DF trial and is shown in **Figure 22**. The bulk recovery from the vessel and using feed pump internal air blow down for the tubing and cassette yielded 92.7% of the initial protein quantity. Another 3.7% came from the buffer recirculation. The total mass balance recovered represented 96.3%.

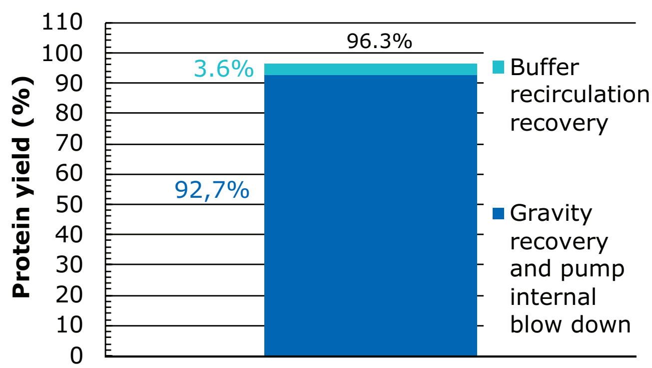


**Figure 21.** Yield of protein UF/DF Process on Mobius Smart Flexware® TFF 20 depending on the recovery method

# 8. Feed bag sampling and pH measurement volume limit

## Background and Objectives

The Mobius TFF 20 system Flexware® is available with optional sampler, conductivity patch and pH sensor port on the recycle vessel bag. This feature may be ideal for solution concentration and diafiltration monitoring via pH / conductivity or to collect a sample without any flow. Due to the tank geometry, the ports are usable down to a certain volume. The objective of this chapter is to determine this lower volume limit at which both ports are usable.

## Materials and Methods

The Mobius® Flexware® bag is installed into the tank following the User Guide instructions. Retentate and feed ports are clamped as close as possible to the bag. The bag is inflated using the inbuilt inflation system. The tank is tared and filled from the top with 50 L DI water (1cP solution). The bag sample port is opened allowing water to flow out in a bucket until no more water can flow through the sampler meaning that the minimal volume is reached. The weight of the tank is recorded.

For pH measurement, the pH probe is placed in the Flexware® bag, and by using the retentate sample port, the tank is slowly drained until the pH probe is not able to read a pH value anymore.

**Note:** Neither impeller nor feed pump is running during measurements, induced turbulences/waves could potentially higher or lower the volume.

## Results and conclusion

All available Flexware® bags are tested (50 L, 100 L and 200 L). Results given in the table below are corresponding to the volume at which the sampler and the pH sensor port are not usable anymore. When referring to these number for process applications, it is important to:

- Add at least the amount of solution to be sampled (and an eventual safety margin) to the given volume to ensure that sampling is possible.
- Remember that the measured volumes only represent the amount of solution needed in the tank (rest of the flowpath not considered)
- Stay above given values when pH measurement is needed. Note: limit may change according to the pH sensor used.

**Table 15. Summarized volume limits regarding sampler and pH sensor port according to the tank size**

| Tank Size (L) | 50 | 100 | 200 |
|---|---|---|---|
| Sampler volume limit (L) | 10.8 | 22.7 | 32 |
| pH / conductivity sensor volume limit (L) | | | |

# 9. Minimum volume for temperature control

## Background and Objectives

Jacketed recycle vessels are available in the standard offering of the Mobius® TFF 20 systems for temperature maintenance during process operations. 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 lowest temperature possible with the pump operated at its maximal capacity: 20 L/min.

## Materials and Methods

All Flexware® assemblies are installed into the system following the User Guide instructions. The recycle bag outlet is connected to the feed pump inlet. The TFF 20 recycle vessel used in this study is the 100 L tank. The Flexware® bag retentate port is connected to the recycle bag return via the retentate sampling assembly. One CIP plate is installed on the holder instead of membrane cassettes. The Membrane Recycle flow path is opened. The retentate control valve (PCV-101) is set to be 100% opened and the permeate control valve (PCV-201) is set be 100% closed. The vessel mixer was activated, adjusting its speed according to the volume of the tank.

The system is capable of controlling an external TCU, therefore, trials were conducted using a CCP recipe. This recipe was written to decrease the vessel temperature from 20 °C to 4 °C by applying a bath temperature of 0 °C. Once the vessel temperature reached 4 °C, the feed pump was started to recirculate the fluid at a rate of 20 L/min for approximately one and a half hour. Then, the tank volume was reduced by slightly opening the permeate valve, allowing 2 L/min to flow through the permeate side to simulate a concentration until the minimum volume required to immerge the impeller, 5.7 L, was reached. The temperature was monitored for several hours.

In addition to the inbuilt temperature recorder, a thermocouple was used to monitor the liquid temperature, results are displayed in the graph below.

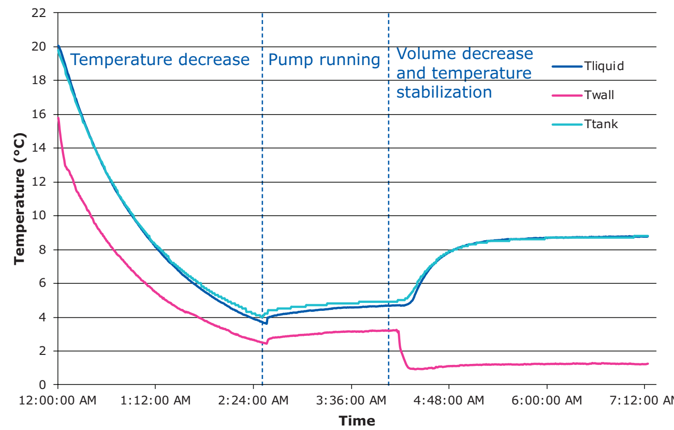


**Figure 22.** Temperature evolution during the decrease of volume

## Results and conclusion

The Mobius® TFF 20 system can maintain a temperature below 5 °C at a volume of 50 Liters in the 100 L tank when the pump is running at 20 L/min. However, as the volume approaches the minimum working level, it becomes more challenging to sustain such a low temperature due to the reduced volume and the high pump speed. Nevertheless, the temperature remained stable, not exceeding 9 °C for several hours.

**Note:** The results of this test are given for information and may vary depending on the volume, ambient temperature, target temperature, TCU model, viscosity of the product, type and area of membranes and the speed of the feed pump.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany



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