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Application Note

Running a TFF optimization study with Cogent® Lab Systems and Bio4C® Control Software

Introduction

This application note describes the recommended methods and techniques for optimizing a tangential flow filtration (TFF) process using a Cogent® Lab system.

While the steps outlined are the same as a typical TFF, adaptations for streamlining the run with Bio4C® Control Software for Cogent® Lab systems are presented.

The following steps are highlighted:

- Optimization
- Concentration (and concentration in fed batch)
- Diafiltration
- Final concentration and recovery

Note that single-pass TFF (SPTFF) mode is not included here but can easily be run on the Cogent® Lab systems by selecting the SPTFF mode at the start of the experiment.

The full guide for general TFF operation using Pellicon® Cassettes can be found in the following document: *AN2700EN00 – A hands-on guide to ultrafiltration/ diafiltration optimization using Pellicon® Cassettes.*

Optimization

The optimization step is performed after the flushing/ equilibration and normalized water permeability (NWP) preparation steps which are typically completed by manual control of the system.

This optimization step identifies and helps set the transmembrane pressure (TMP) and flux that will be used for the remainder of the run. Typically, three different fluxes (measured in liters per minute per meter squared or LMM) are explored, with several TMP increments tested at each flux.

The optimization step can be controlled manually or automated via a recipe where the system regulates its flux and TMP and switches setpoints based on a timestamp (it is recommended to allow a minimum of 5 minutes once setpoints are reached to allow for stabilization).

Figure 1 provides an example of a recipe for an optimization run. Macrosteps are pre-defined automated sequences with pre-programmed actuators and end criteria adapted to main operations of a TFF process. For an optimization run, drag and drop each macrostep, starting with “Total Recirculation”. For a fully automated run, select “Auto Start ON” to allow each step to start without confirmation, otherwise, a pop-up prompt will appear after each step and require user confirmation. Configure the macrostep as follows:

- Set a flow control loop for the feed pump (P001)
- Indicate the chosen flux as setpoint
- Set a TMP control loop for the retentate PCV (PCV101)
- Put the lower TMP value as setpoint and set 5 minutes as end criteria
- Drag a second “Total Recirculation” macrostep with the same settings, except for PCV101, where the TMP setpoint is increased
- Add as many macrosteps as there are number of increments in the TMP setpoint
- End the recipe with the macrostep “Stop Equipment” (note that a recipe can contain a maximum of 9 macrosteps)
- Save the recipe

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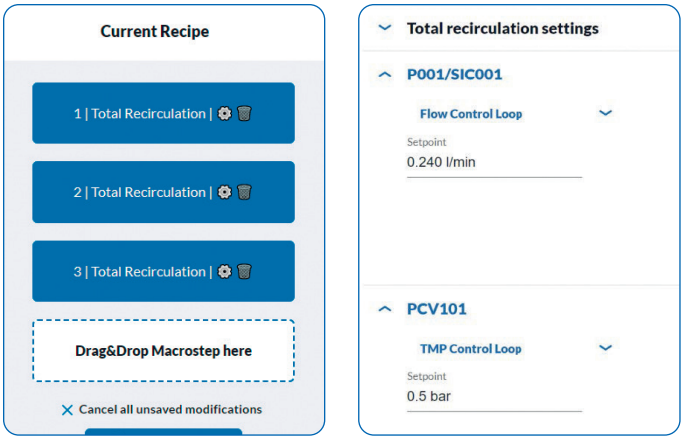


Figure 1.

Total Recirculation macrosteps in series (left) and bloc example details (right).

Upon command, the system can record all data points during a set recipe, however, it is recommended to record the values manually as each of the flux/TMP pairings need to be plotted on top of each other, and not chronologically (which is how they will appear in the record file of the Cogent® Lab system). Figure 2 provides an example of flux versus TMP optimization curves.

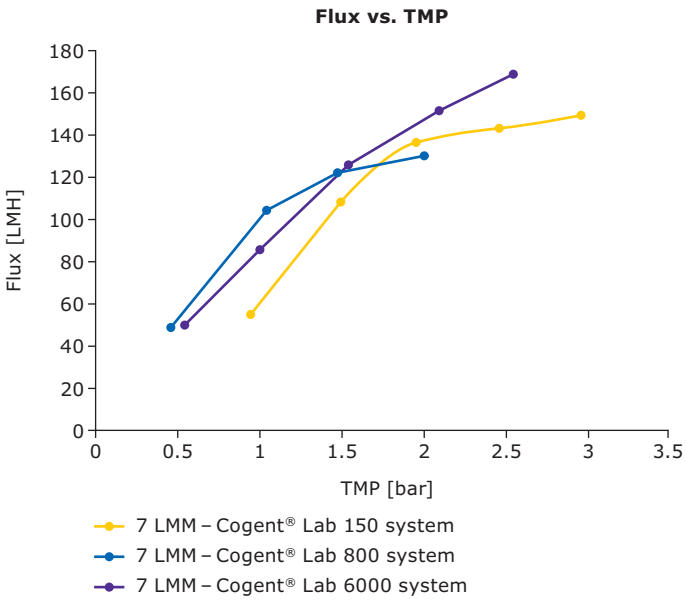


Figure 2.

Example of optimization curves.

In order to perform the same run at a different flux, multiply the recipes created (one per flux explored). To avoid setting up the parameters again, the previous recipe can be duplicated and only the feed pump setpoint changed in each macrostep.

Concentration

The remaining steps of the TFF run (concentration, diafiltration, final concentration, and recovery) can be controlled via a single recipe, using the pre-defined macrosteps.

Figure 3 shows an example of the macrosteps to be used for a typical TFF run.

Figure 3.

TFF run recipe example.

If working in fed-batch mode, where the feed volume is more important than the tank maximum capacity, a diafiltration macrostep can be used to automate concentration by fed-batch until the volume of feed is reduced to the maximum volume capacity of the tank. In this case, the first macrostep will be Diafiltration, with end criteria being either the number of diavolumes (in this case, the fed batch ratio, i.e., total volume of feed divided by maximum tank volume) or, if this option is present on the equipment, the total volume recorded by the filtrate flowmeter FI201 (FT201 threshold end criteria). This macrostep would be followed by a concentration step as required.

To enable automation and setup of the recipe as needed for the entire run, within Bio4C® Control software you must configure the Human Machine Interface (HMI) to include all the elements (actuators and sensors) that will be needed throughout the entire run. Figure 4 is a view of the menu used to select the elements to include from the P&ID screen.

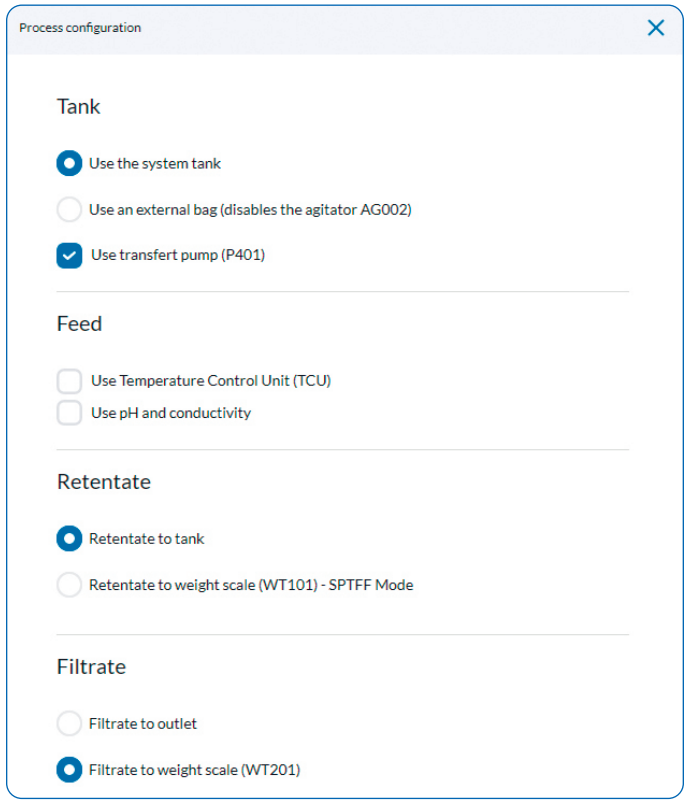


Figure 4.

Process configuration menu.

The concentration run can be programmed in the Bio4C® Control Software settings, fixing parameters such as TMP and flux, or by setting a target volumetric concentration factor (VCF), after which the system will progress onto the next macrostep (typically diafiltration). When running in VCF control mode, make sure that the feed tank weighing scale (WI001) has been tared before the product has been added, as this is the base volume upon which the system will calculate the VCF.

The concentration run can also be controlled by time stamp instead of VCF.

Figure 5 is an example of a typical concentration recipe block.

In this case, one single recording file can be started for the whole TFF run (i.e., it will record everything from initial concentration – or fed-batch, through diafiltration, to final concentration).

Live graphs are also available for consultation with up to a 10-minute time range (Figures 6 and 7).

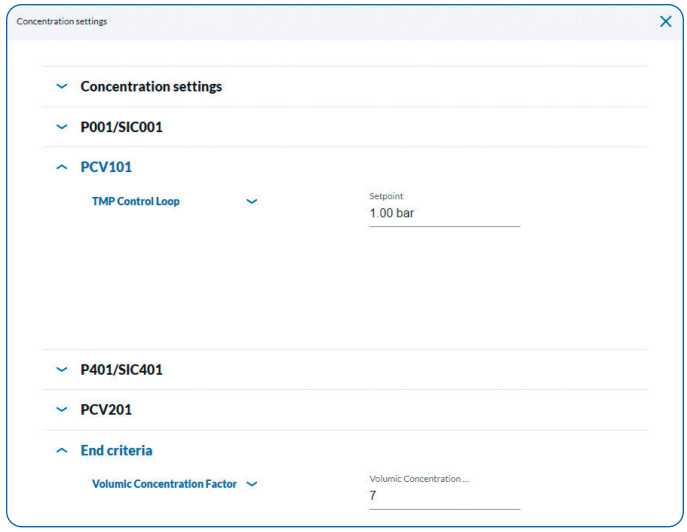


Figure 5.
Concentration bloc parameter examples.

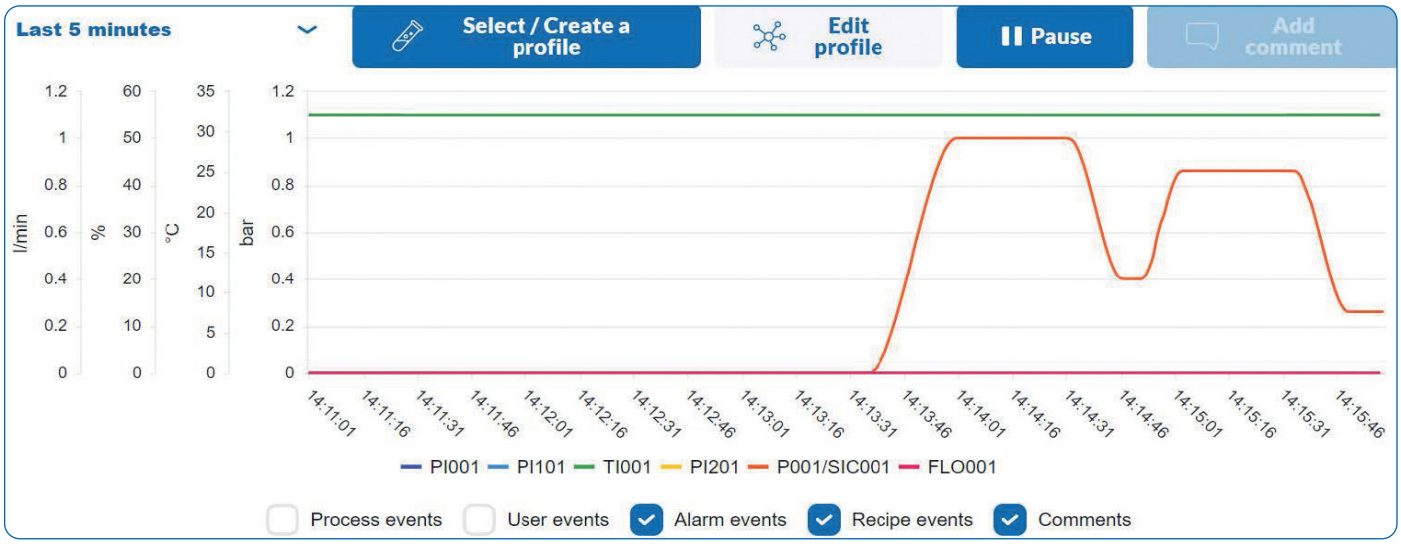


Figure 6.
Live graph example.

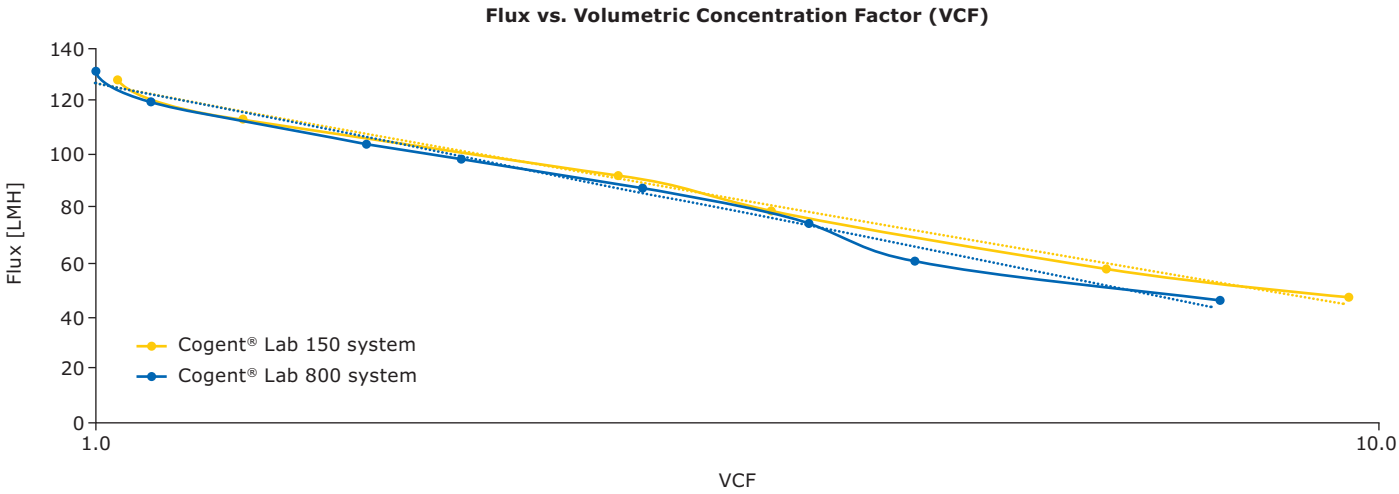


Figure 7.
Example of concentration run curves (Flux vs. VCF).

Diafiltration

It is important to note that if a diafiltration is planned, the Bio4C® Control Software for Cogent® Lab systems must be set up by selecting the diafiltration accessories (typically, the transfer pump; Figure 8).

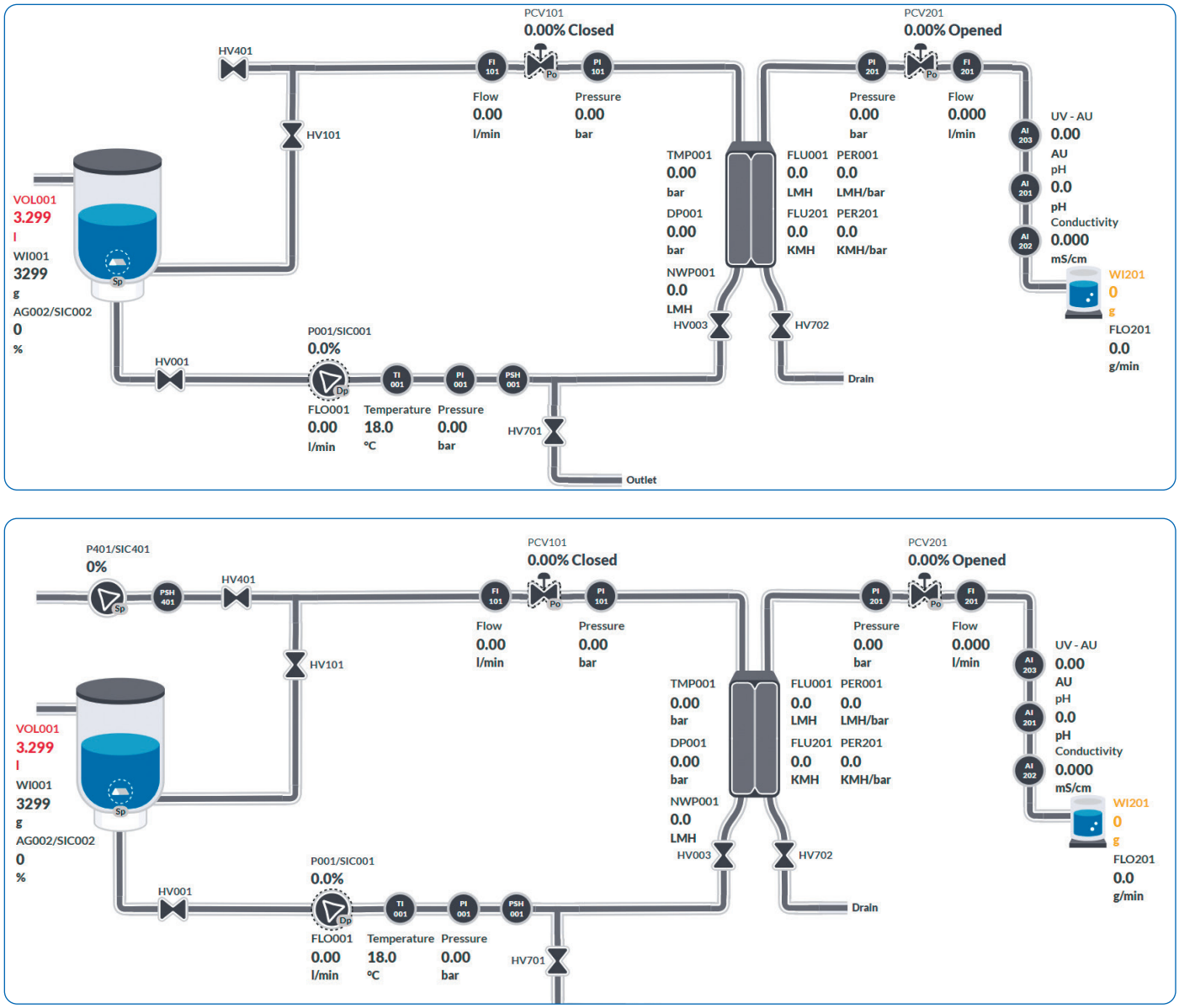


Figure 8.

P&ID without (top) and with (bottom) transfer pump.

The diafiltration macrostep, when positioned right after the concentration macrostep, will automatically start once the end criteria for the concentration run has been met (final VCF factor, time stamp, etc.).

The diafiltration macrostep is set in a similar manner to the concentration step, with the exception of the transfer pump setting; note that this does not apply to the Cogent® Lab 150 system which does not have a transfer pump option. The pump can be controlled by target tank weight and speed, meaning that it will start and stop at a defined speed to maintain a target weight in the tank.

The diafiltration can be controlled by diafiltration volume target or by time stamp (not available on the Cogent® Lab 150 system).

To run an automated diafiltration on Cogent® Lab 150 system, a separate pump can be connected to one of the two top tank ports and used to feed the main tank at the same flow as filtrate flow during diafiltration. Alternatively, a void can be created in the tank by closing the vent port and connecting a buffer supply to one of the two top ports on the tank. To lessen the lag between void creation and beginning of buffer suction, a syringe connected to the vent port can be used to create a void and prime buffer flow. In the macrostep setup, the initial product volume will need to be filled manually to allow for diavolume calculation; filtrate weight will then be used to calculate an end point.

Figure 9 is an example of a diafiltration recipe macrostep and Figure 10 shows an example of diafiltation curves.

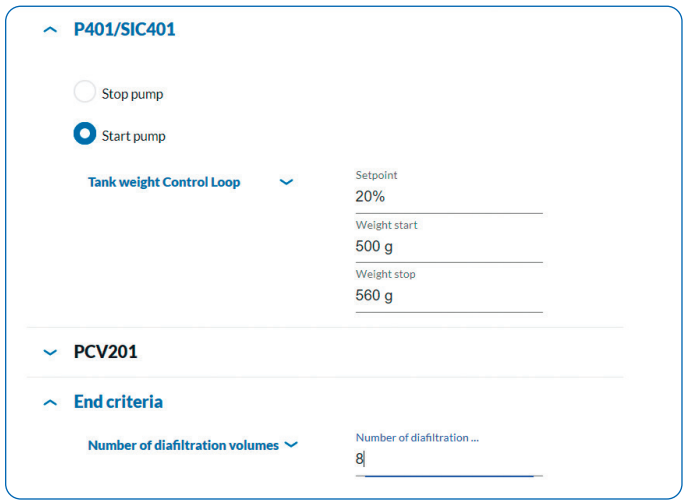


Figure 9.

Diafiltration bloc parameter examples.

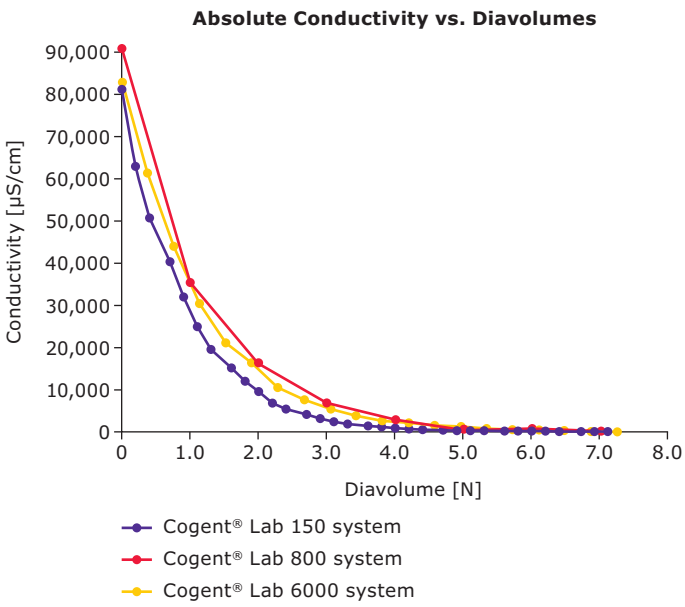


Figure 10.

Example of diafiltration curves (conductivity vs. diavolumes).

Final concentration and product recovery

The final concentration, if set in the overall general recipe with all the previous steps, will start as soon as the diafiltration run reaches its end criteria (final number of diavolumes or time stamp). The final concentration can be set up in the same way as the first concentration in the software.

If, however, the product is highly viscous and concentrated at the end of the first steps of the process or requires the addition of a substance to alter its consistency, this final concentration can be excluded from the general recipe and performed through manual control.

The product recovery step can also be included in the recipe and a pause can be added to the recipe at the end of the final concentration step by introducing a pop-up which waits for user confirmation. This allows the operator to switch the outlet line into a collection vessel. Note that for this option, the Total Recirculation macrostep will need to be used.

Figure 11 shows the option of selecting a pop-up prompt.

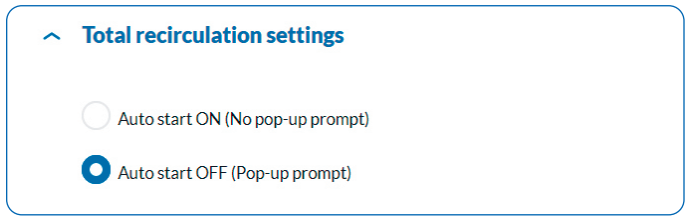


Figure 11.

Pop up selection.

Figure 12 shows how the product recovery step can also be set up via a “Tank Emptying” macrostep.

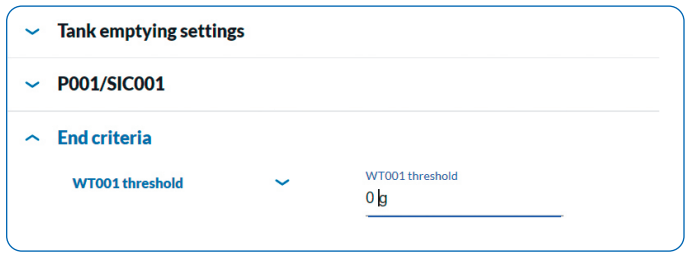


Figure 12.

Tank Emptying bloc parameter example.

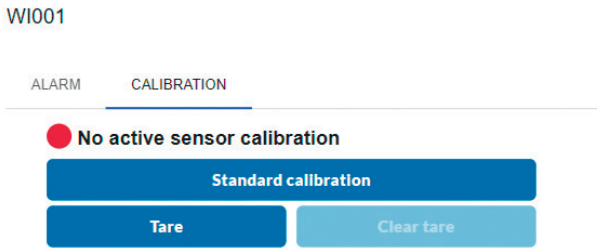
At the end of a recipe, the final macrostep, “Stop Equipment”, needs to be included. The recording is stopped manually outside of recipe control.

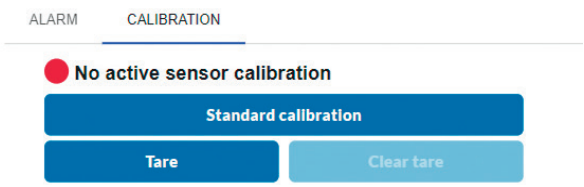
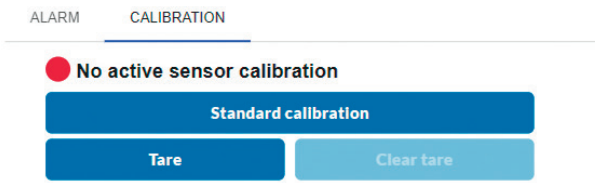
Suggested Recipe Structure

The table below summarizes the different recipe structures that can be used depending on process objectives and steps.

Table 1.

Recipe Structures.

Process objectives	Suggested macrostep succession	Notes
TMP/flux excursion	**Feed flux excursion** • Flux highest to lowest (Flux 1 > Flux 2 … > Flux n) • TMP values lowest to highest (TMP A < TMP B … TMP N) **Flux 1:** Total Recirculation • Auto start: ON • P001: Flux 1 set-point (speed, delta-P, flow) • PCV101: TMP A set-point (% closure, retentate pressure value, TMP control loop) • End criteria: 5 minutes Repeat for as many TMP set points as desired (max 9 in total), changing PCV101 to desired TMP value Save recipe **Flux 2:** Duplicate recipe created for Flux 1 Duplicate Recipe Modify P001 setting in each macrostep by Flux 2 set-point Save recipe	Set flow path in total recirculation mode. Run each recipe one after the other, from highest to lowest flux. Remember to press "Start" in the recording control box to begin the recording. The curves generated during the different recipes cannot be superposed in the Trends pane directly. For comparison purposes, export data to Excel and plot datasets on the same graph in Excel.
	Repeat recipe creation for as many fluxes as desired	
Fed-batch concentration	Concentration from V0 feed volume split between side recipient and equipment tank **Reduction of feed volume V0 to tank volume VT:** Select diafiltration macrostep Diafiltration Input initial volume in the field "Manual Initial Diafiltration Volume" or let equipment measure it by weighing tank Note: when using "Auto Initial Diafiltration Volume", remember to tare WI001 through calibration, clicking on "WI001" on the P&ID  Set up actuators appropriately and select end criteria, either: • "Number of diafiltration volumes" where N is the fed batch ratio minus one (V0/VT-1) to stop concentration when feed volume is reduced to one tank volume (if fed batch ratio is not an integer, round up or down as appropriate) • FT201 threshold (with flowmeters option), with value total feed volume minus tank volume (V0–VT) **Reduction of feed volume when volume is equal to or lower than tank volume:** Select Concentration macrostep: Concentration Proceed with macrostep setup according to pre-configured actuators and desired end criteria Save recipe as is or add further macrosteps as desired (up to 9 in total)	Set flow path in total recirculation mode, retentate to feed tank, filtrate to container. When running a concentration experiment for data plotting analysis (to determine gel concentration for example), it is desirable to work without fed batch. Reduce and split starting feed volume as required.

Process objectives	Suggested macrostep succession	Notes
Diafiltration	Buffer displacement and/or exchange with or without accessory transfer pump **Cogent® Lab 800 and Lab 6000 systems:** If accessory transfer pump is available, select and add parameters to diafiltration step as follows: Diafiltration Input initial volume in the field “Manual Initial Diafiltration volume” or let equipment measure it by weighing the tank Note: when using “Auto Initial Diafiltration Volume“, remember to tare WI001 through calibration, clicking on “WI001” on the P&ID WI001  Set up transfer pump P401: • Either based on speed (pump calibration curve will have to be used to adjust transfer pump flow to match filtrate flow) • Or based on tank weight, defining volume variation range (high and low) and speed at which the pump will refill the tank between those two values Set up other actuators appropriately and select end criteria, either: • “Number of diafiltration volumes” where N is the ratio of buffer volume on feed volume (round up or down as appropriate if N is not an integer) • FT201 threshold (with flowmeters option), with total diafiltration buffer volume Save recipe as is or add further macrosteps as desired (up to 9 in total) **Cogent® Lab 150 system:** Select and add parameters to diafiltration step as follows: Diafiltration Input initial volume in the field “Manual Initial Diafiltration volume” or let equipment measure it by weighing tank Note: when using “Auto Initial Diafiltration Volume”, remember to tare WI001 through calibration, clicking on “WI001” on the P&ID WI001  Set up actuators appropriately and select end criteria, either: • “Number of diafiltration volumes” where N is the ratio of buffer volume on feed volume (round up or down as appropriate if N isn’t an integer) • FT201 threshold (with flowmeters option), with total diafiltration buffer volume Save recipe as is or add further macrosteps as desired (up to 9 in total)	Set flow path with retentate to feed tank, filtrate to container. For the Cogent® Lab 150 system or in the absence of connected transfer pump, buffer input will either take place using an independent pump or through vacuum suction in the tank (closing top vent, connecting buffer line to one of the top ports).

For additional information, please visit **SigmaAldrich.com/TFF-systems**
To place an order or receive technical assistance, please visit **SigmaAldrich.com/contactAF**

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