Alternatives to ATF as a cell retention device

Levitronix® Bioprocessing Conference 2026 Boston

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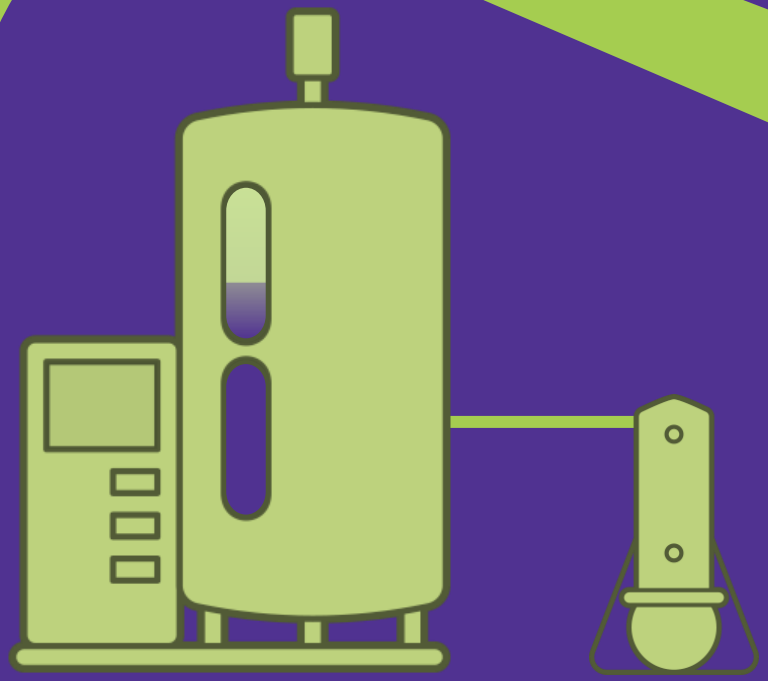
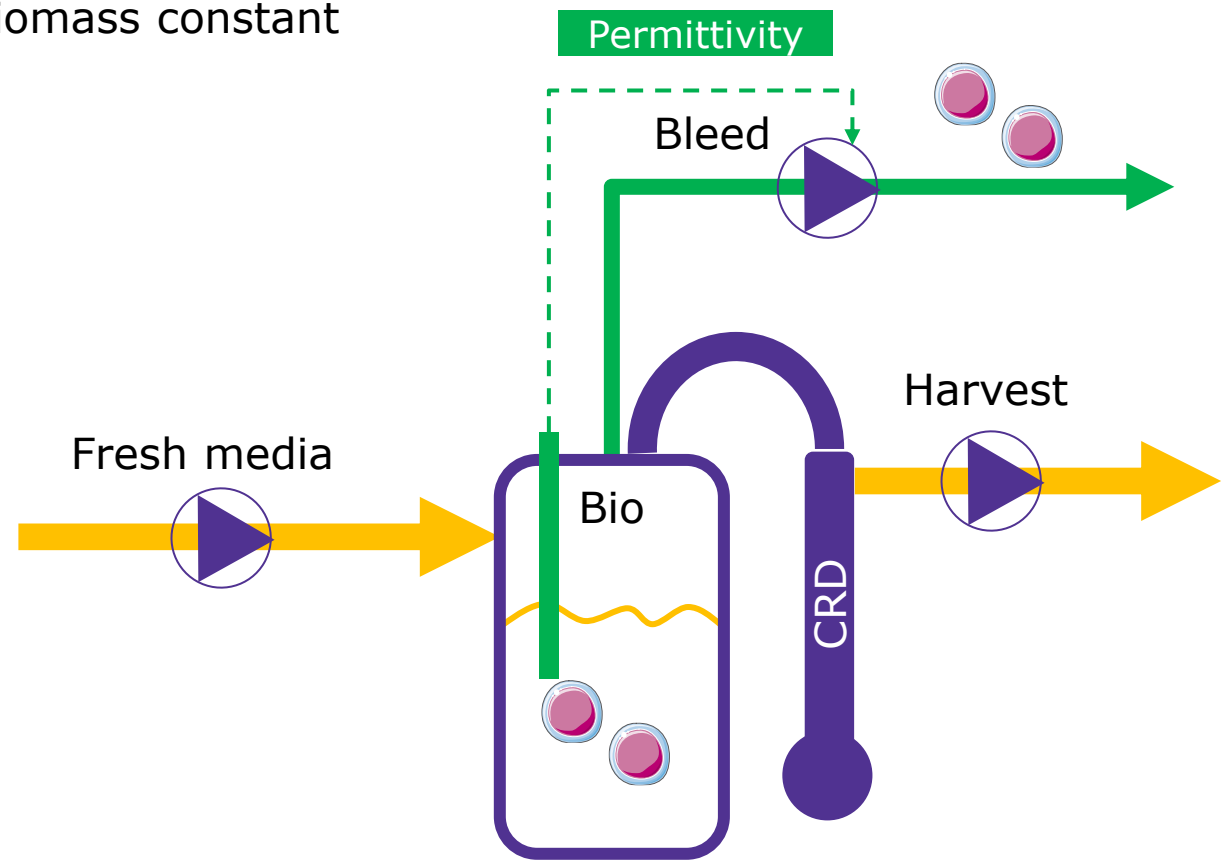
18.06.2026

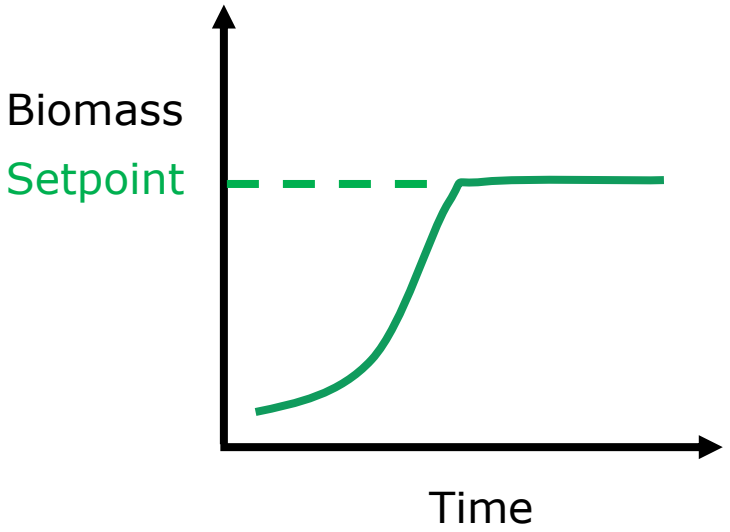
Perfusion platform



Merck perfusion platform
Perfusion process

- Maintain all process parameters constant
- Maintain cell growth and cell renewal
- Keep the biomass constant



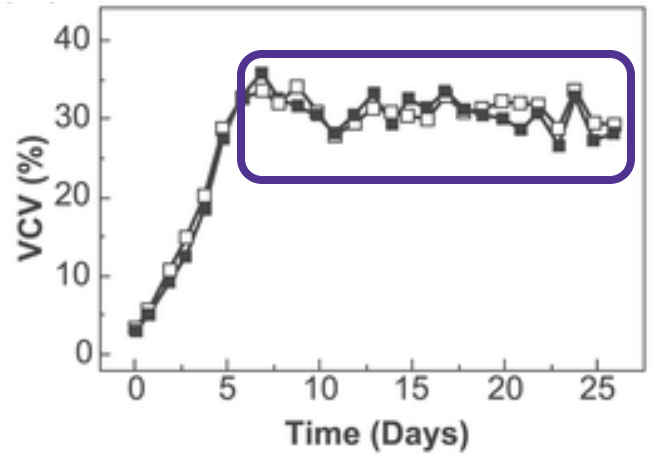


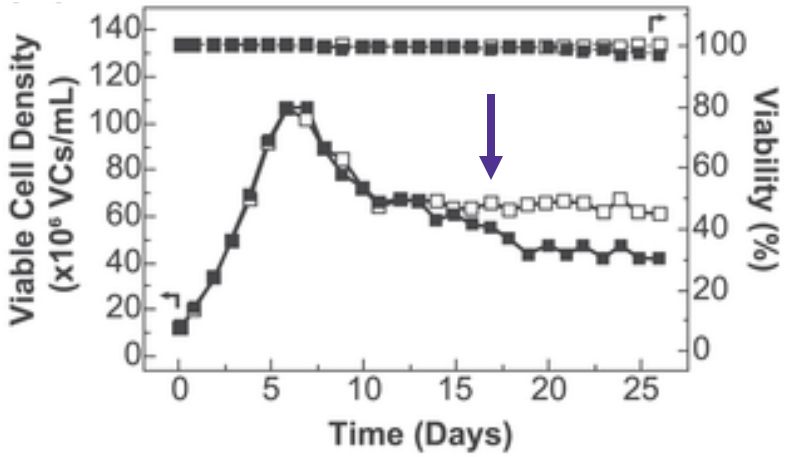
CRD = Cell Retention Device

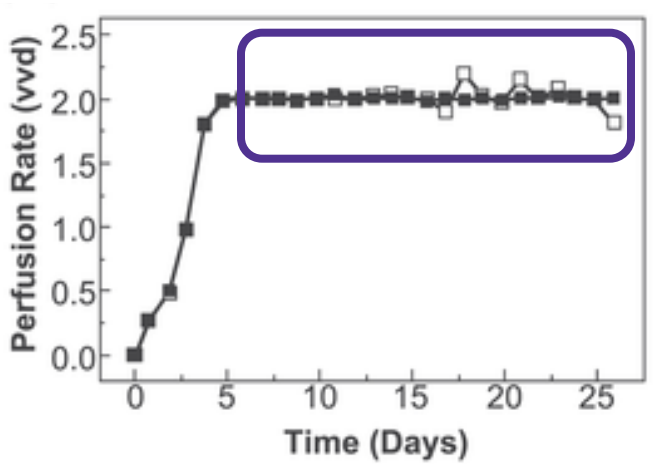
Merck perfusion platform
Biomass and Viable Cell Volume

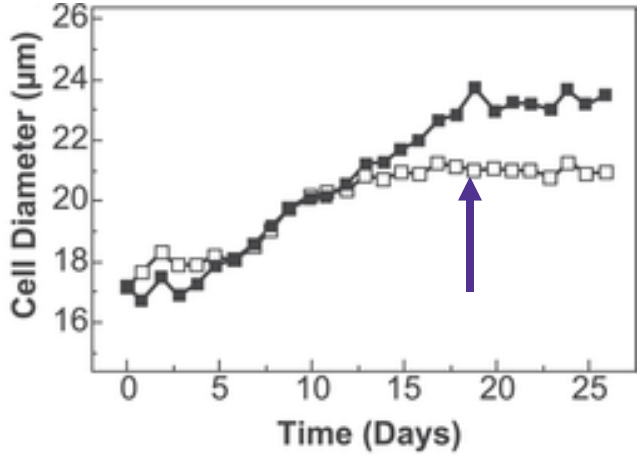
Biomass = Viable Cell Volume

Biomass gives a better indicator than Viable Cell Density (VCD), due to cell diameter shift.



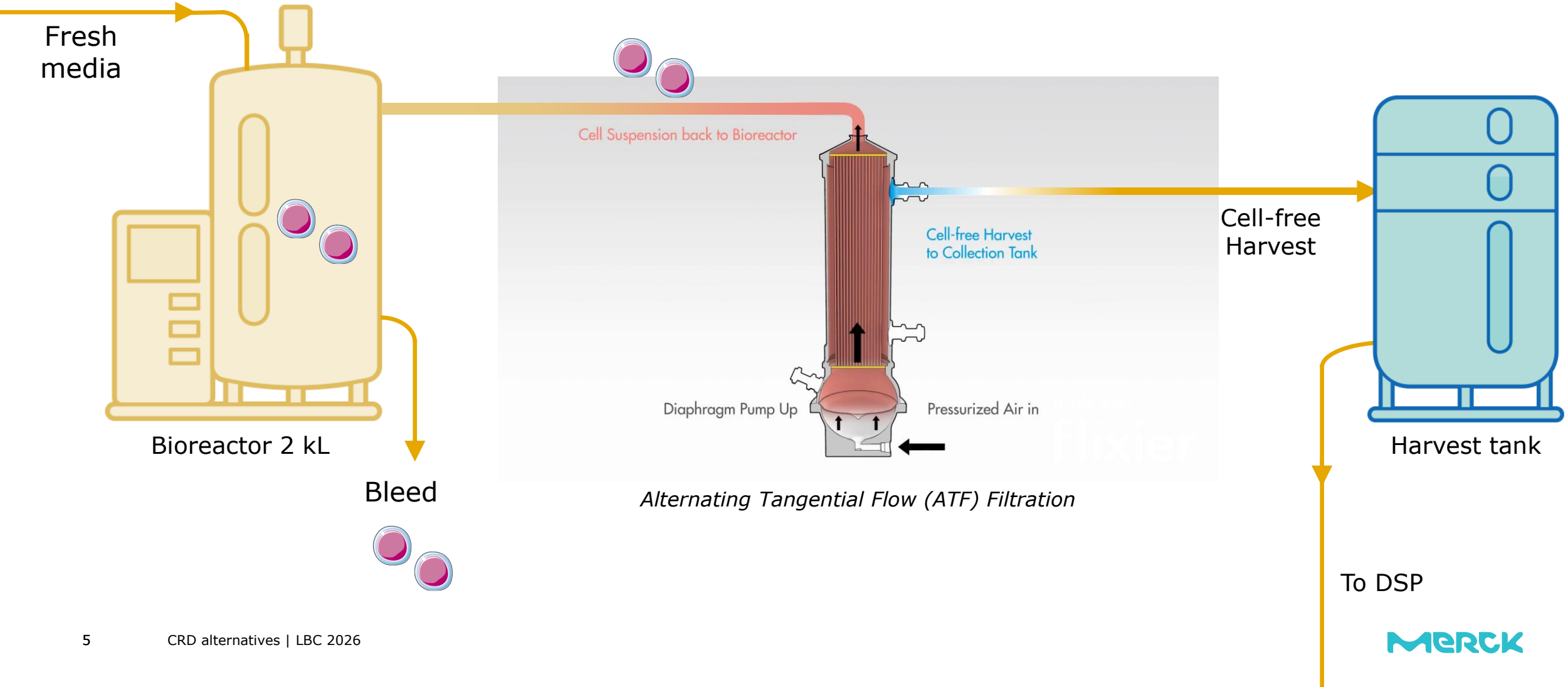






Caso, Stefania & Aeby, Mathieu & Jordan, Martin & Guillot, Raphael & Bielser, Jean-Marc. (2022). Effects of pyruvate on primary metabolism and product quality for a high-density perfusion process. Biotechnology and Bioengineering. 119. 10.1002/bit.28033.

Merck perfusion platform
Overview of Current Filtration Process



Alternating Tangential Flow (ATF) Filtration

Merck perfusion platform
ATF filters scalability

XCell ATF 1 Single-use Device

XCell ATF 2 Single-use Device

XCell ATF 6 Single-use Device

XCell ATF 10 Single-use Device

12 75 1440 6720

#Fibers

Basic principle: Increase the number of 60 cm fibers through the scales

→ All parameters will stay close through the scales: Shear rate, residence time, exchange rate, ATF to filtration ratio.

Merck perfusion platform
ATF filters scalability

Limited to 1000 L/day

XCell ATF 1 Single-use Device

XCell ATF 2 Single-use Device

XCell ATF 6 Single-use Device

XCell ATF 10 Single-use Device

12	75	1440	6720

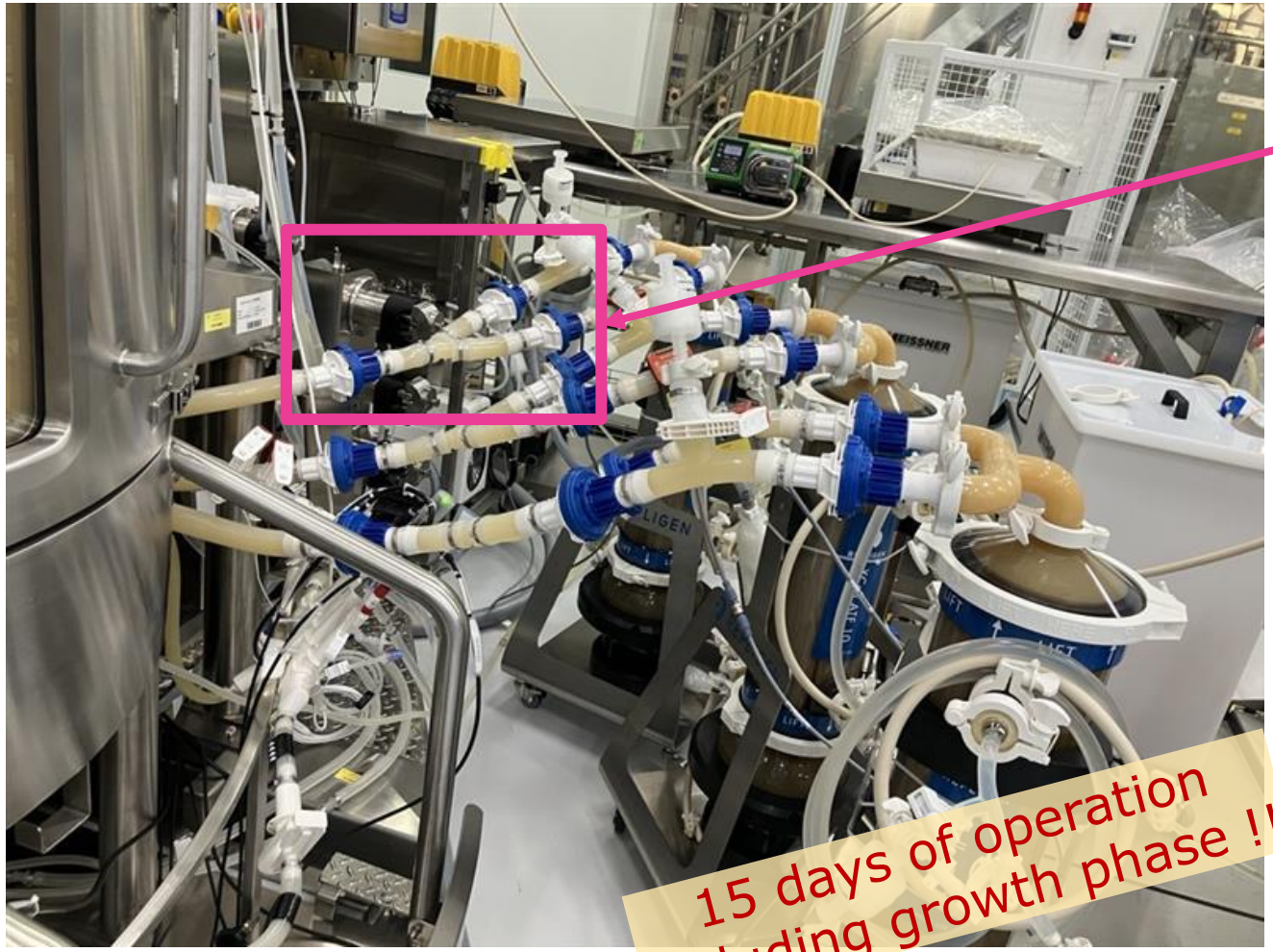
#Fibers

Basic principle: Increase the number of 60 cm fibers through the scales.

→ All parameters will stay close through the scales: Shear rate, residence time, exchange rate, ATF to filtration ratio.

Merck perfusion platform
Technical limitations

3 x ATF10 in degraded mode

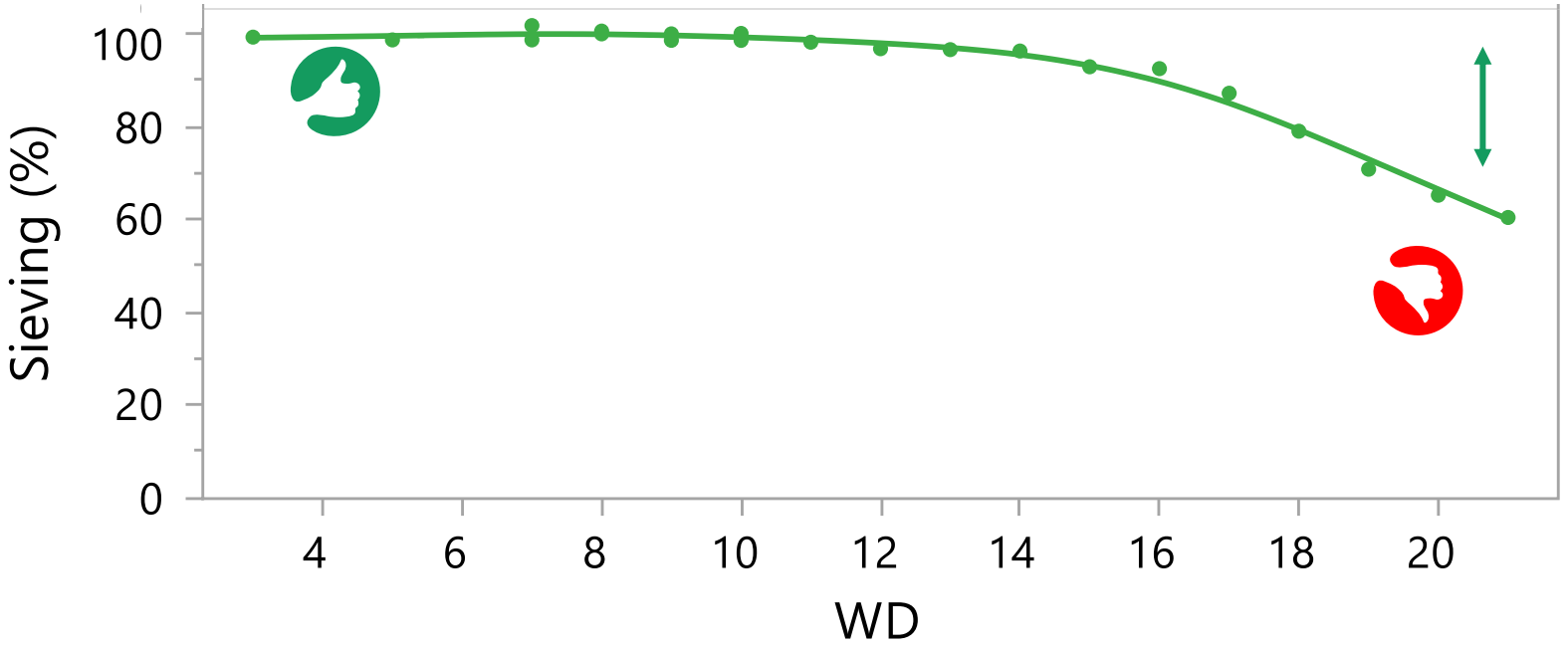


- To achieve perf rate of process, we are operating in degraded mode – with **'Y'** pieces with increased pressure head.
  - 2 ATF10 correctly connected
  - 4 ATF10 in degraded mode
- **Y** pieces are not recommended by Repligen.
- No reconnection possible to the same port.

Info: last 2 kL run had a 10 days of control state before clogging (3000 L/day of harvest).

Limitations of ATF technology at large scale – sieving issues

Sieving at 2000 L scale using ATF



- Product retention
- Quality drift due to an increase in product residence time
- Due to filter fouling

$$\text{Sieving} = 100 - \text{product retention} = \frac{\text{Harvest titer}}{\text{Bioreactor titer}} \times 100$$

WHY An alternative cell retention device

Operational failure

30% of ATF fail integrity in Pilot

1-2 deviations on ATF operations per GMP run (aborted)

Platform Limitation

16 days steady state, 2000 L bioreactor at 2 Working Volume/Day is very challenging to manage with ATF nowadays

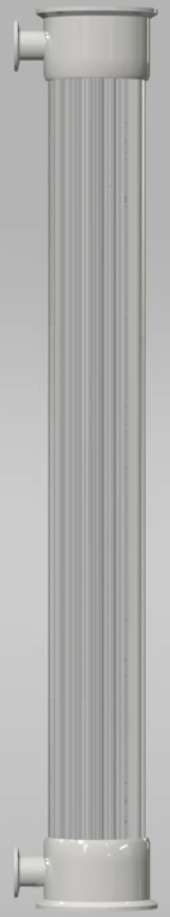
Loss of trust in the alternative tangential flow technology

ATF is a monopoly

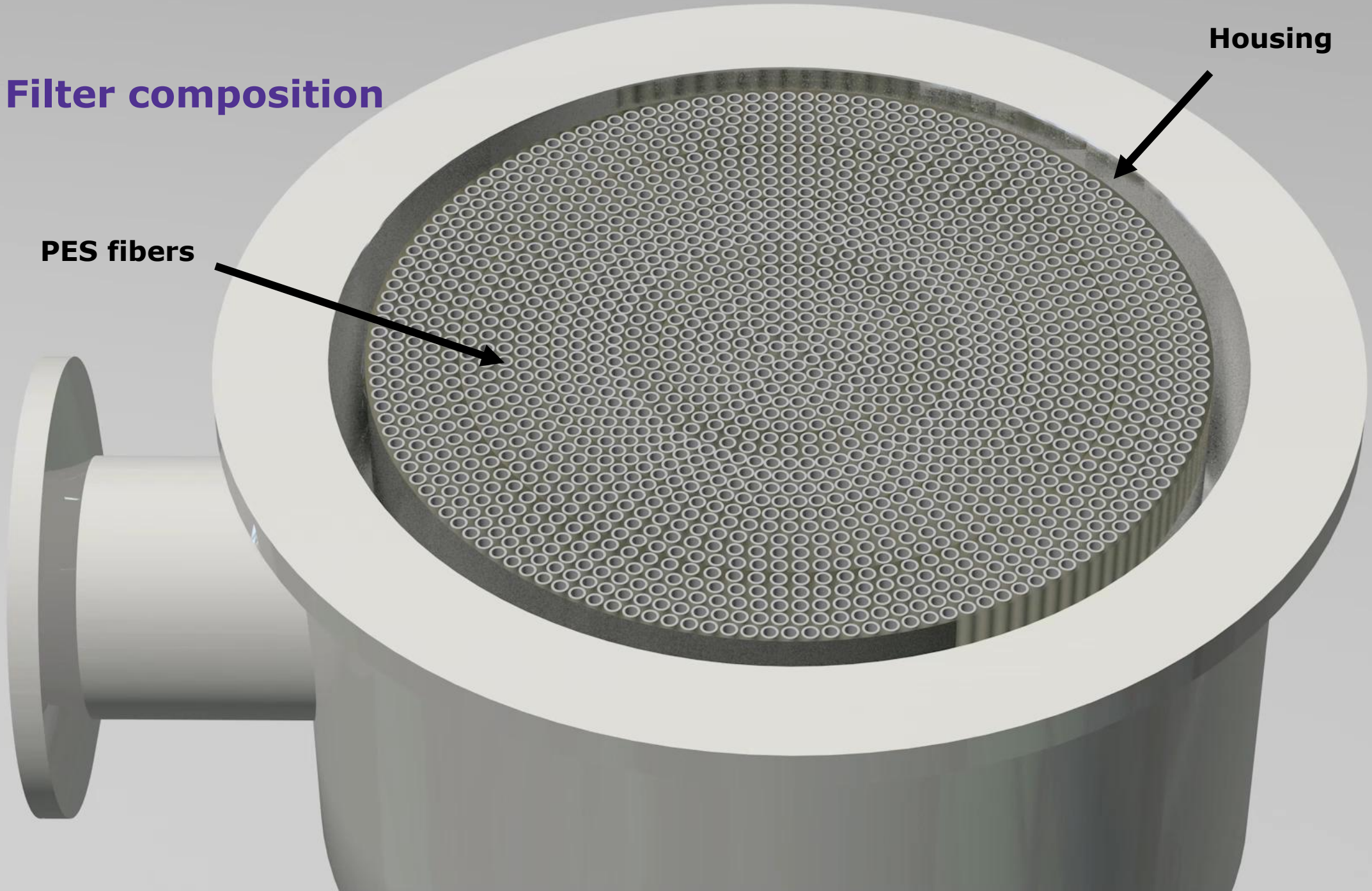
Cost of cell retention device is ~20% of a USP run of 2000 L 16 days at steady state

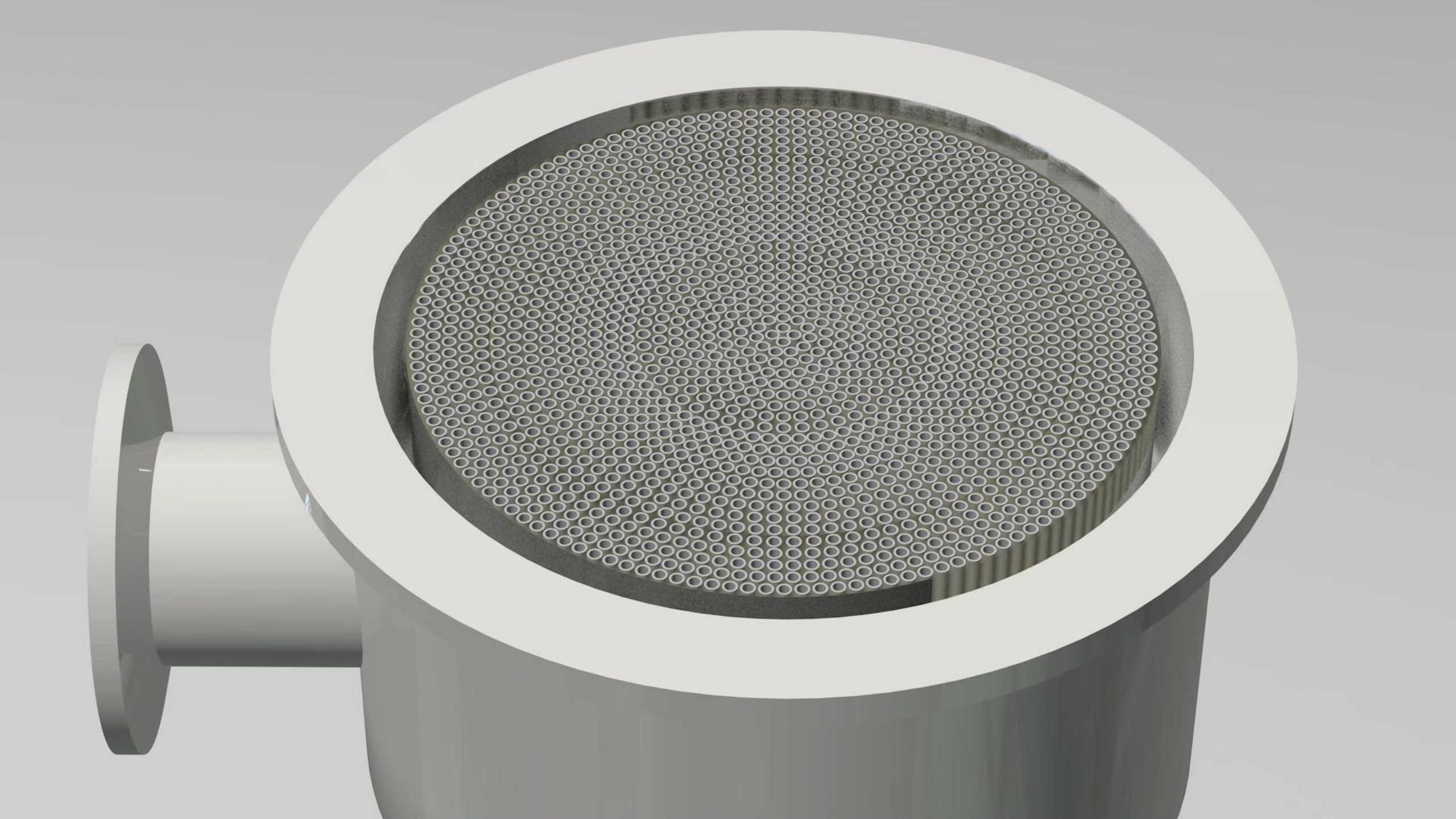
Hollowfiber filters design

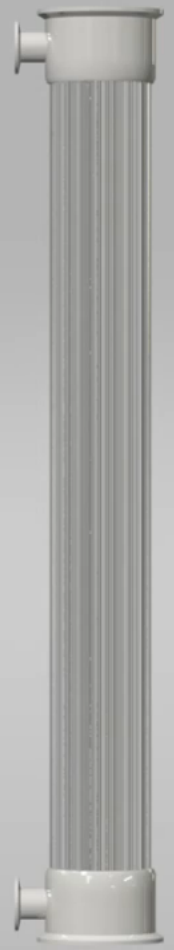
Filter overview



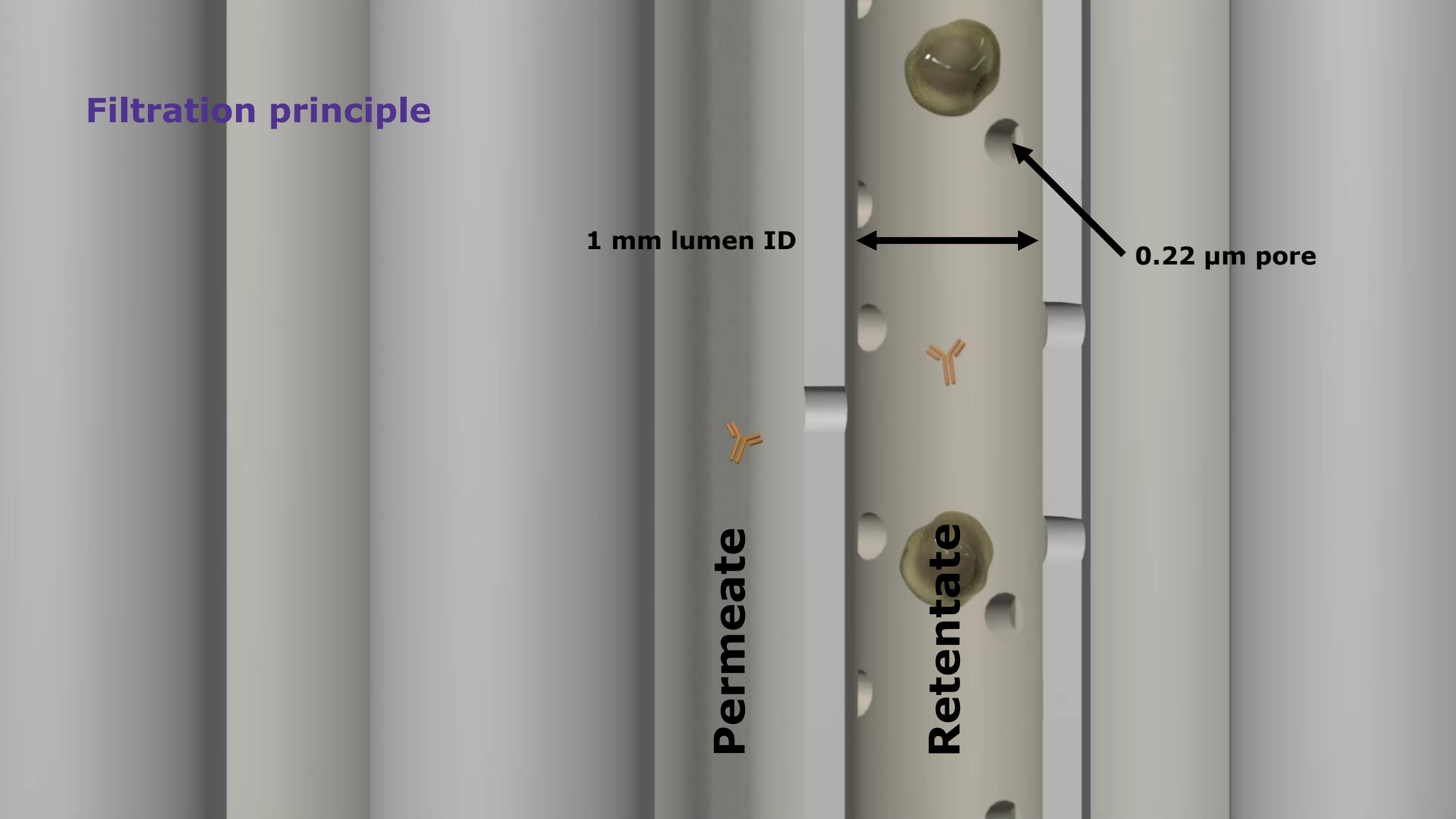
Filter composition







Filtration principle



Starling effect

Starling effect

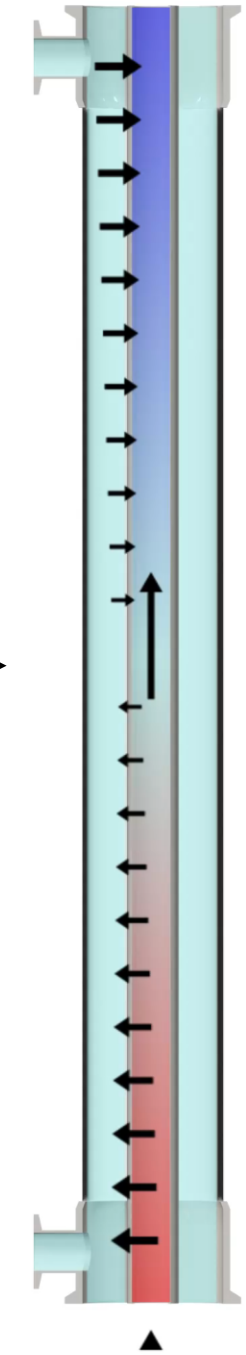
retentate pressure < permeate pressure

Cleaning of the filter

Equilibrium
permeate pressure = retentate pressure

retentate pressure > permeate pressure

Fouling of the filter

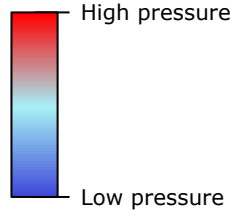


Starling effect

- Can generate a cleaning of the filter
- Generate fouling of the filter
- Only the first half of the filter is used for filtration in a TFF

Harvest flow is considered negligeable (~50x smaller)

Romann et al. (2023). Co-current filtrate flow in TFF perfusion processes: Decoupling transmembrane pressure from crossflow to improve product sieving. Biotechnology and Bioengineering, 121. doi:10.1002/bit.28589



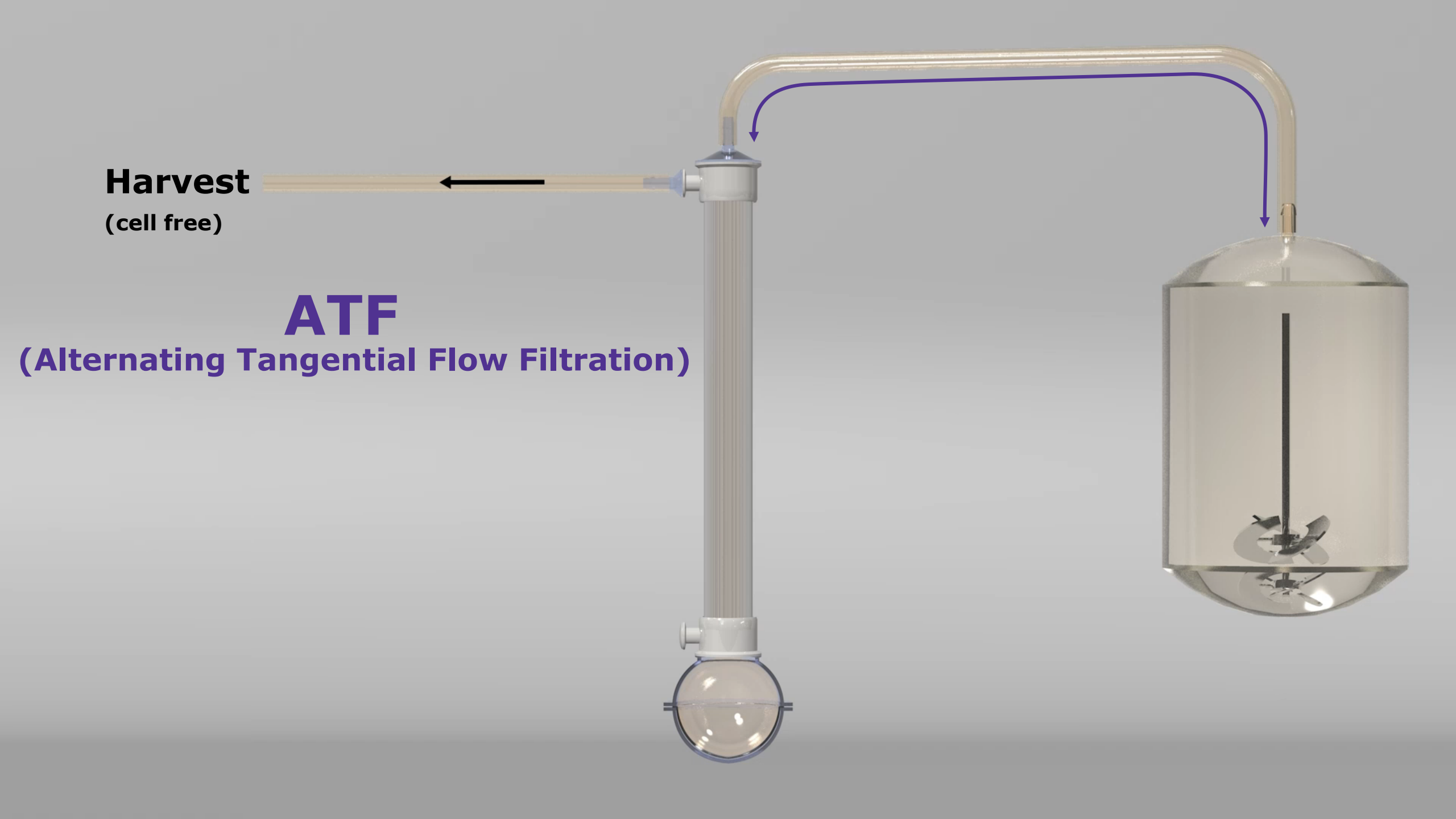
Alternating Tangential Flow Filtration (ATF)

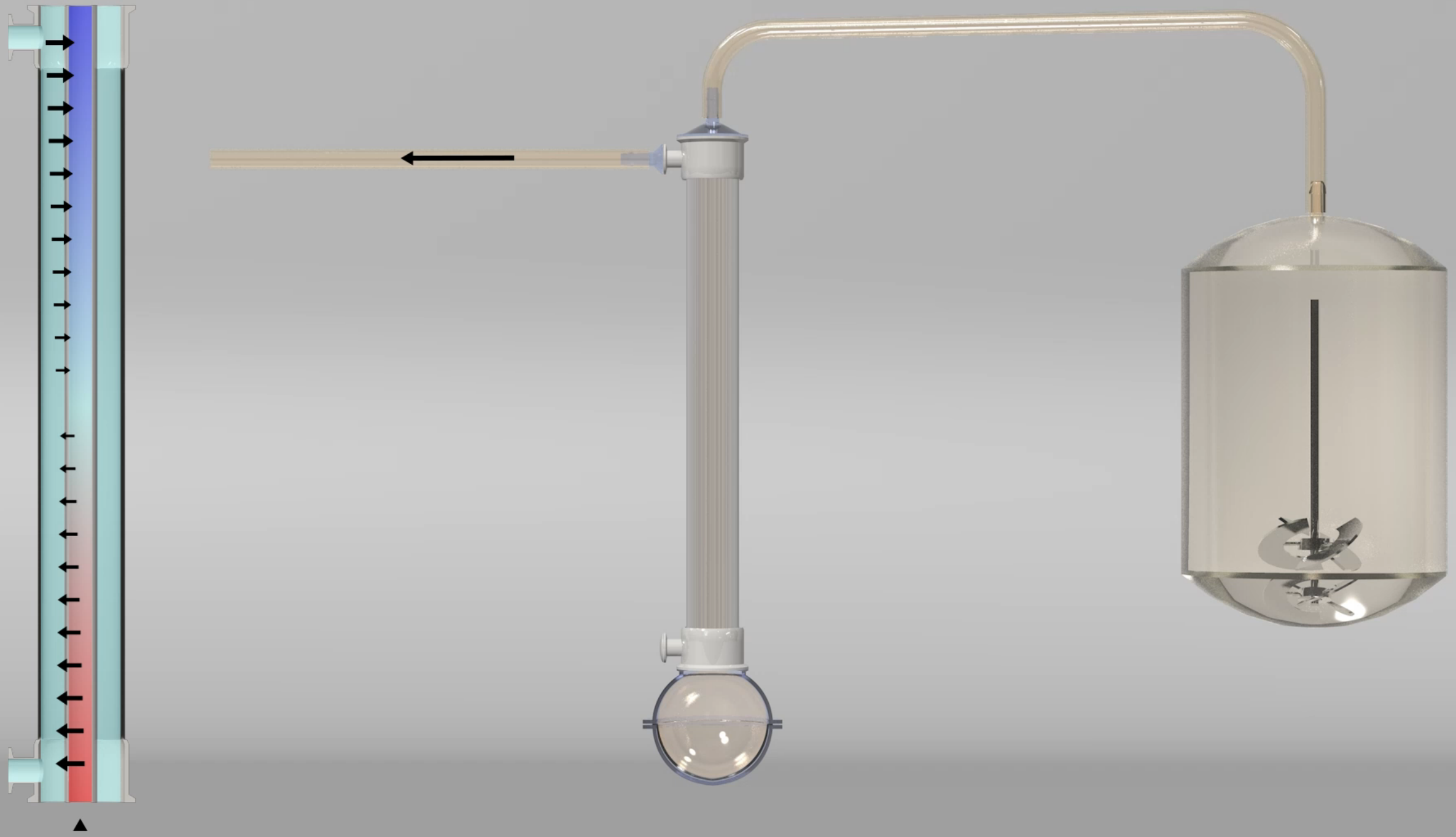
Harvest

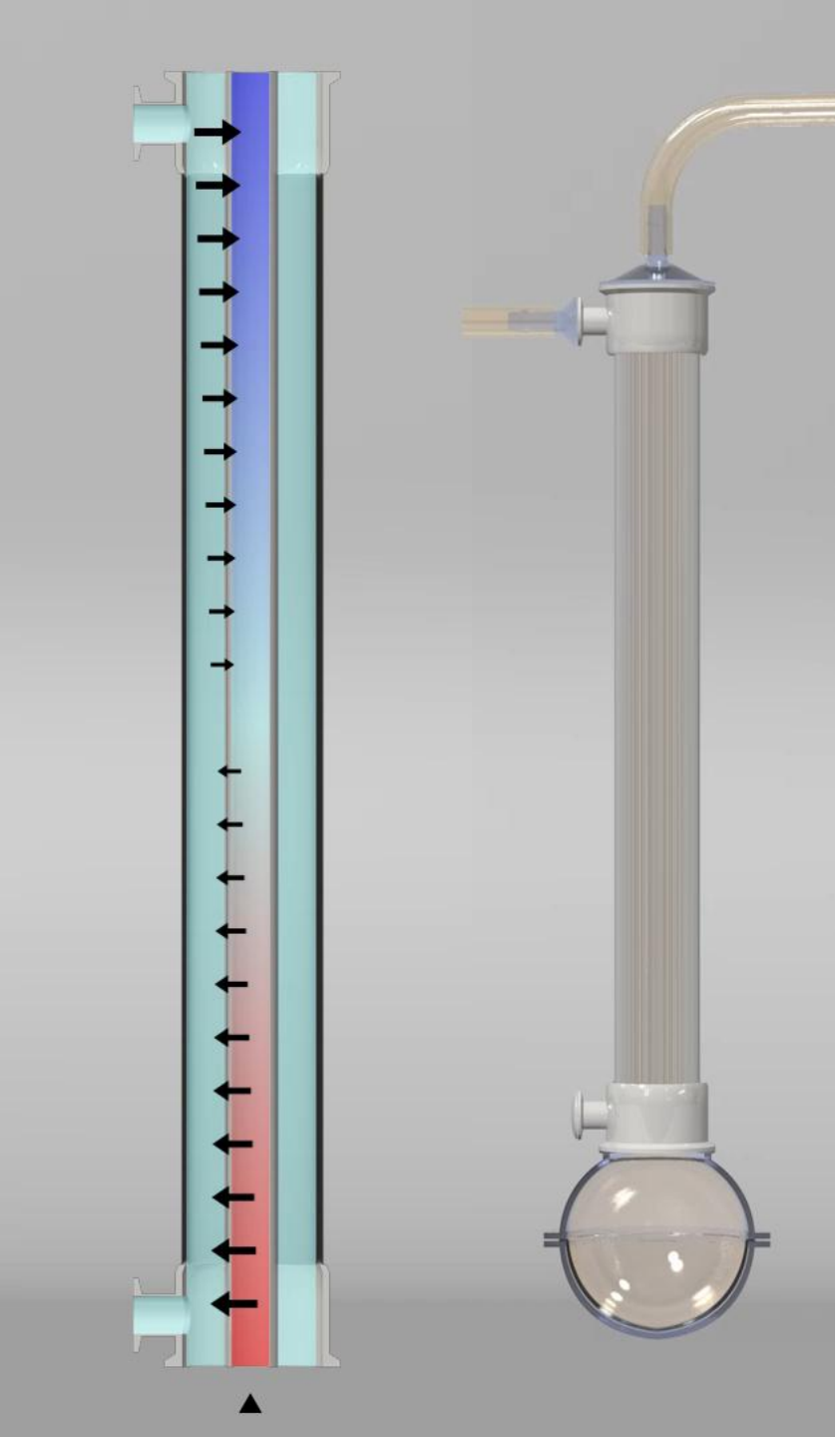
(cell free)

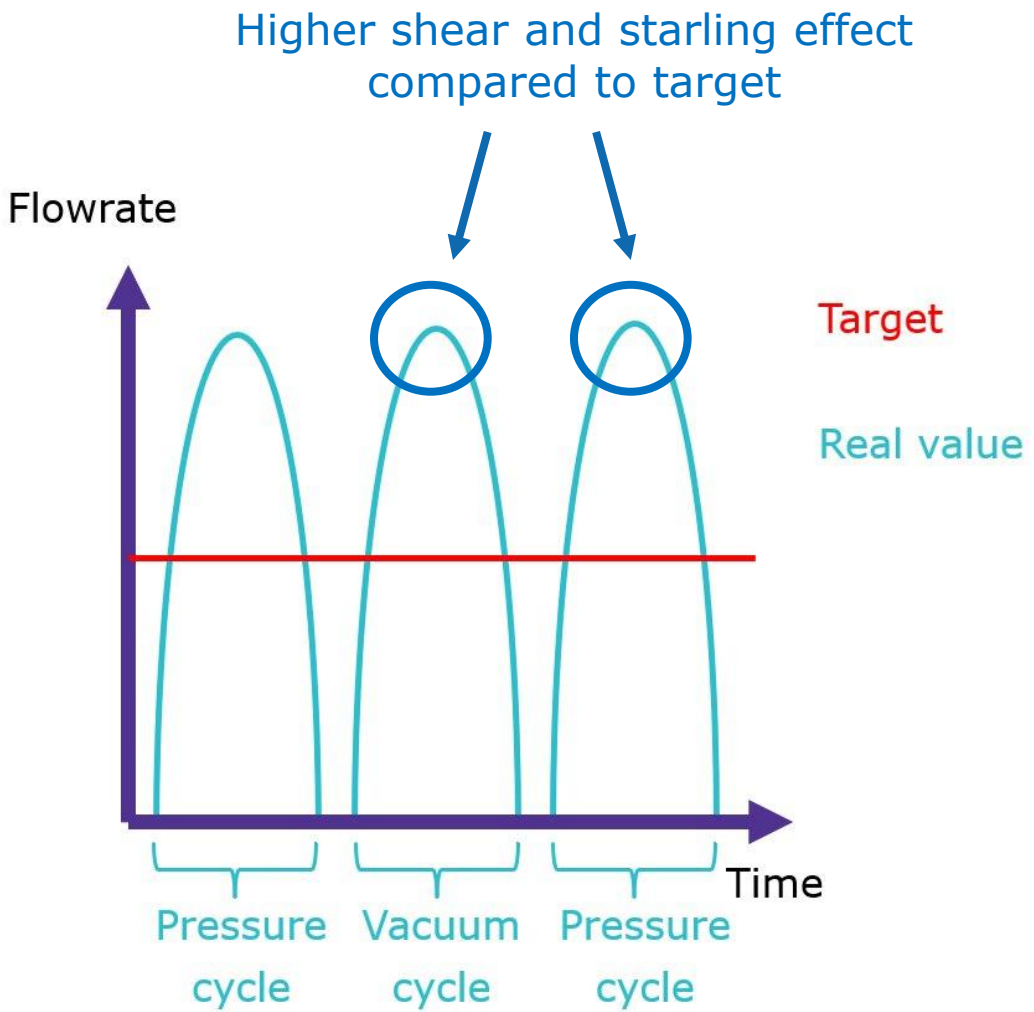
ATF

(Alternating Tangential Flow Filtration)








Higher shear and starling effect
compared to target
Flowrate
Target
Real value
Time
Pressure
cycle
Vacuum
cycle
Pressure
cycle

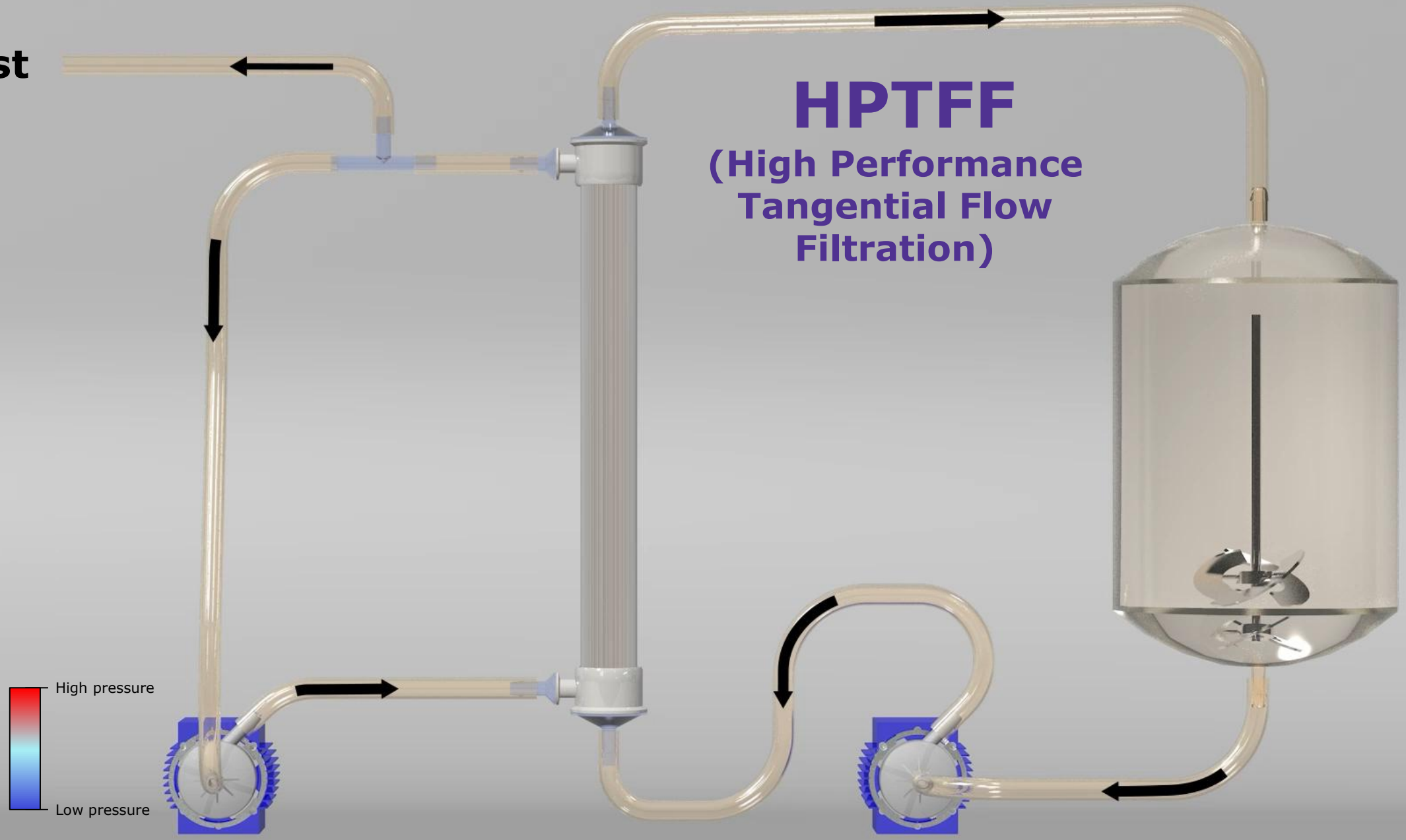
High performance TFF (HPTFF)

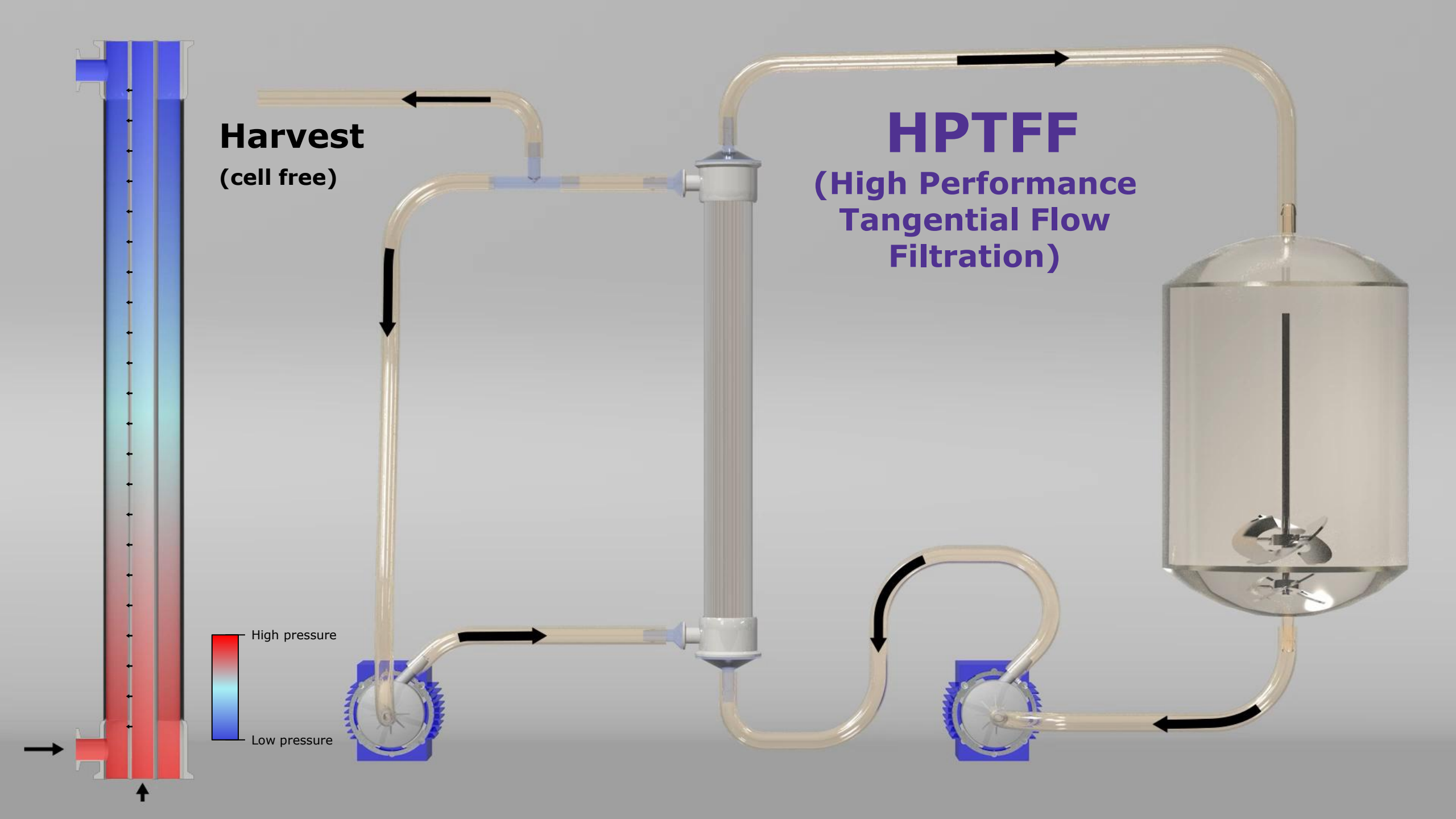
Harvest

(cell free)

HPTFF

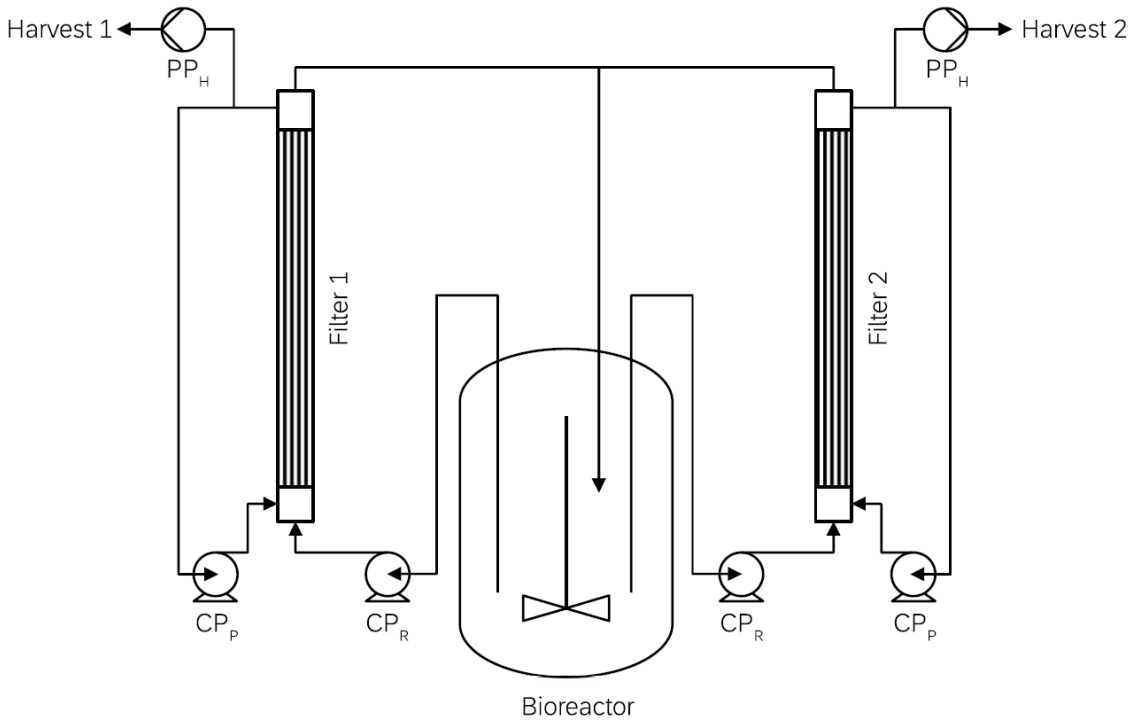
(High Performance Tangential Flow Filtration)




Harvest
(cell free)
HPTFF
(High Performance Tangential Flow Filtration)
High pressure
Low pressure

HPTFF
3.5 L crossflow comparison

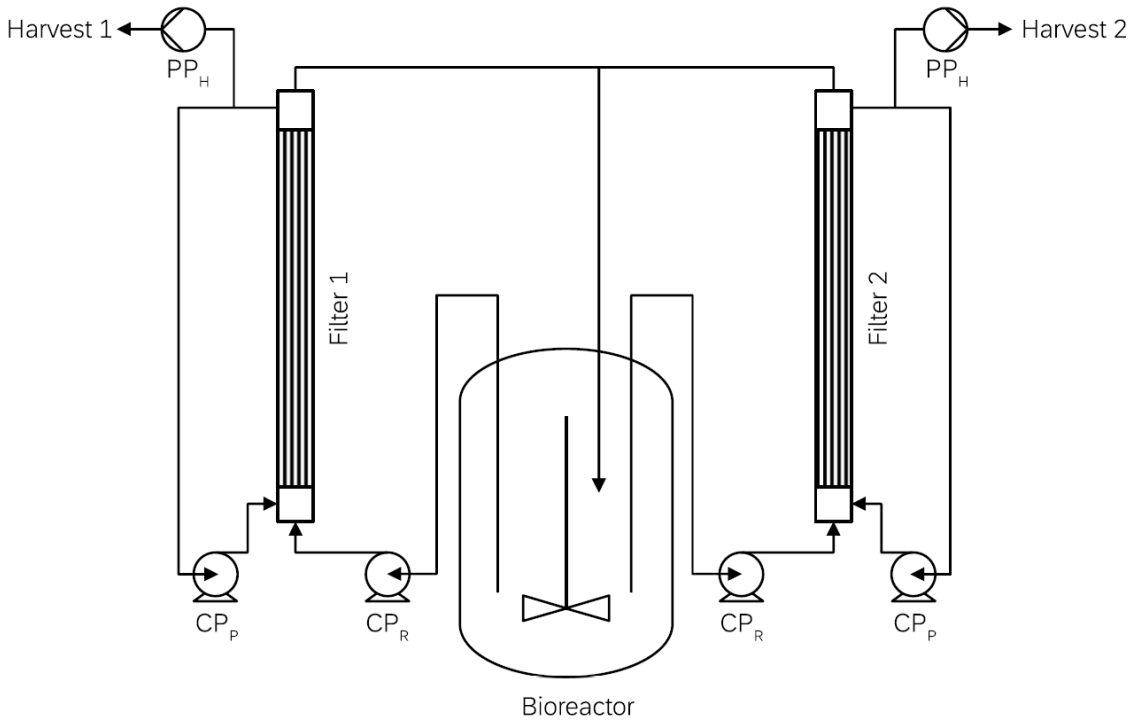
Aim: create equivalent conditions for the 2 membranes.



- 3.8 LMH applied per filter
- 10% biomass

HPTFF
3.5 L crossflow comparison

Aim: create equivalent conditions for the 2 membranes.



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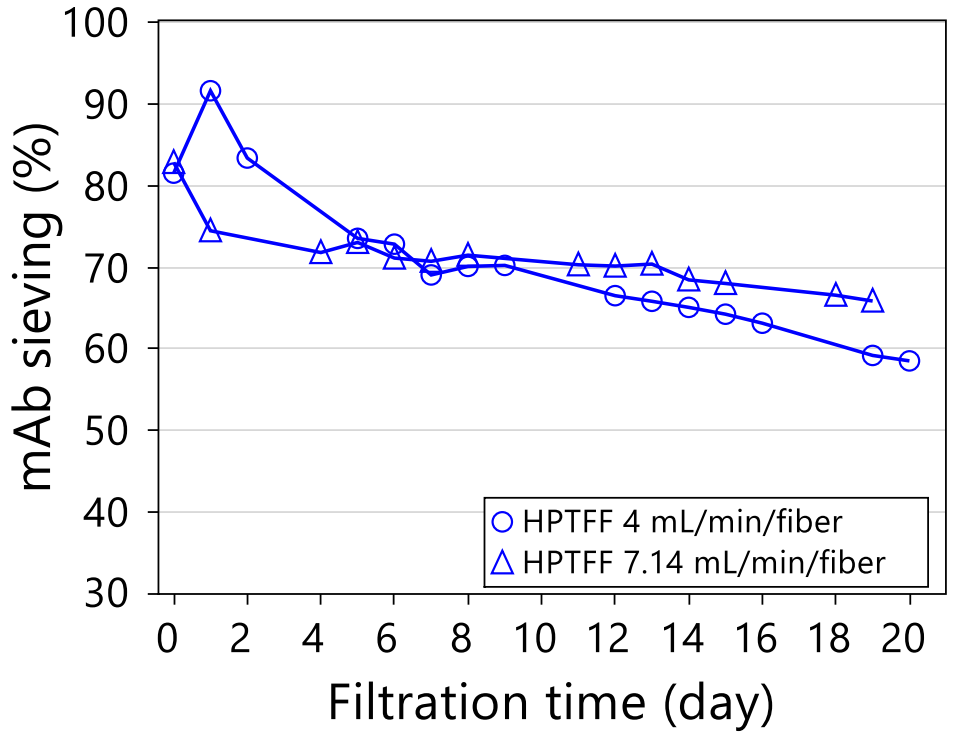


In this case: Higher flow improves performance, but too high flows might damage cells due to shear (filter or/and pump); an optimum is needed.

HPTFF
20 L perfusion wave bag

HPTFF
20 L perfusion wave bag

Filter:

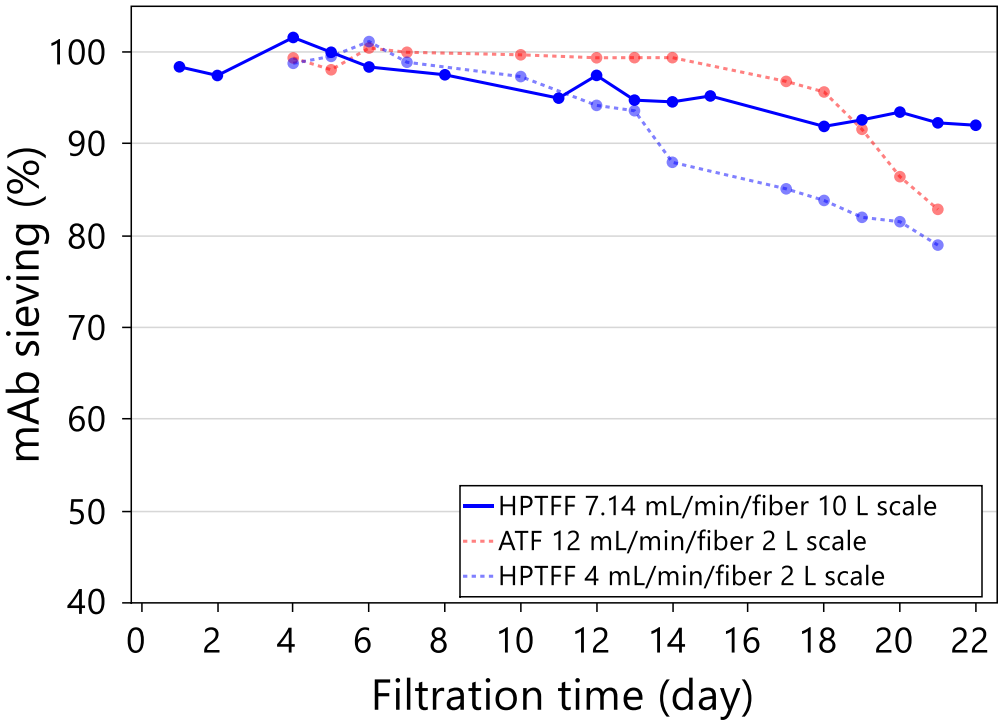
- 3.8 LMH
- 7.14 mL/min/fiber

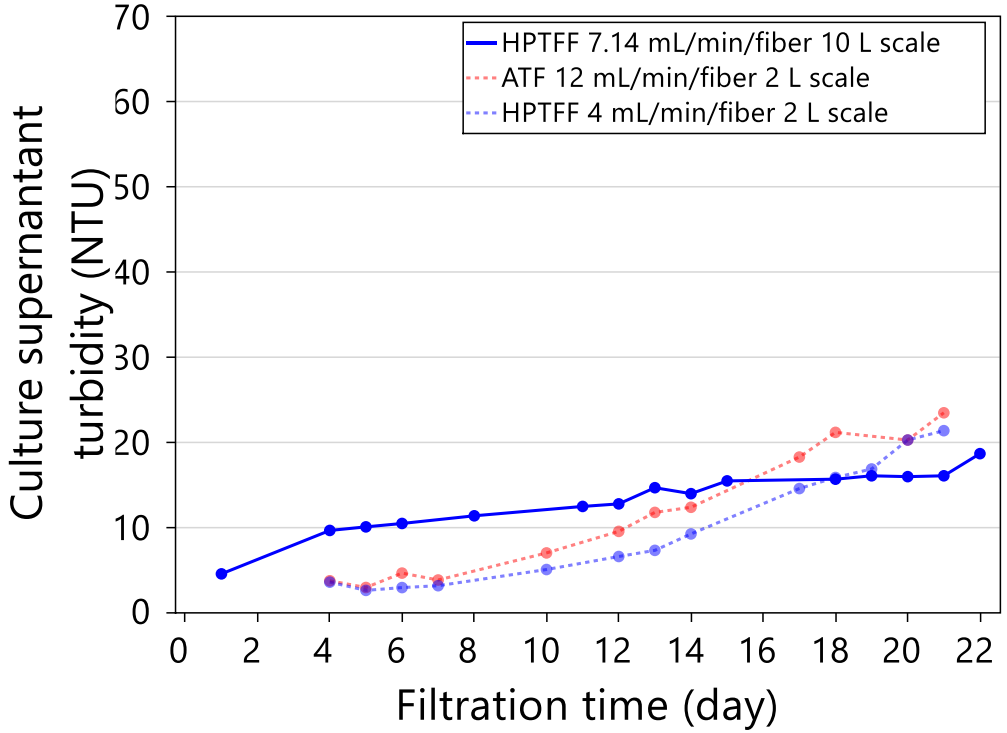
Culture:

- 10 L working volume
- 1.3 vvd
- 8.3% biomass or VCV

HPTFF
10 L perfusion wave bag

Test the best HPTFF retentate flow that was identified at a larger scale : **7.14 mL/min/fiber**





- HPTFF demonstrated good scalability in term of performances
- Pump scaling gets easier when going to higher scale

HPTFF
Conclusion

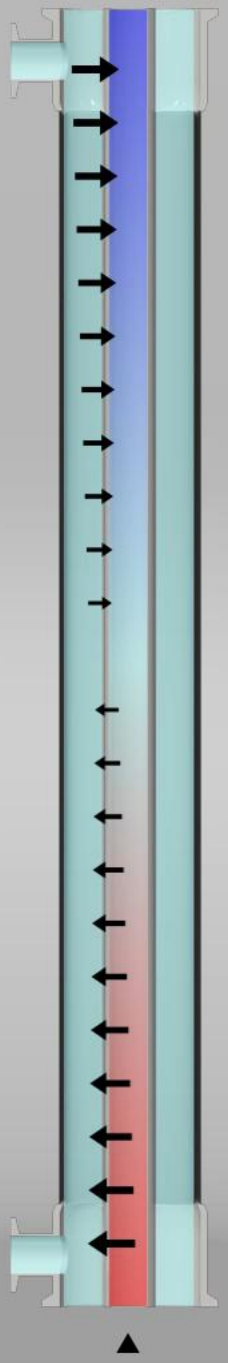
- HPTFF demonstrate a good scalability
- HPTFF is able to achieve good performances in specific conditions

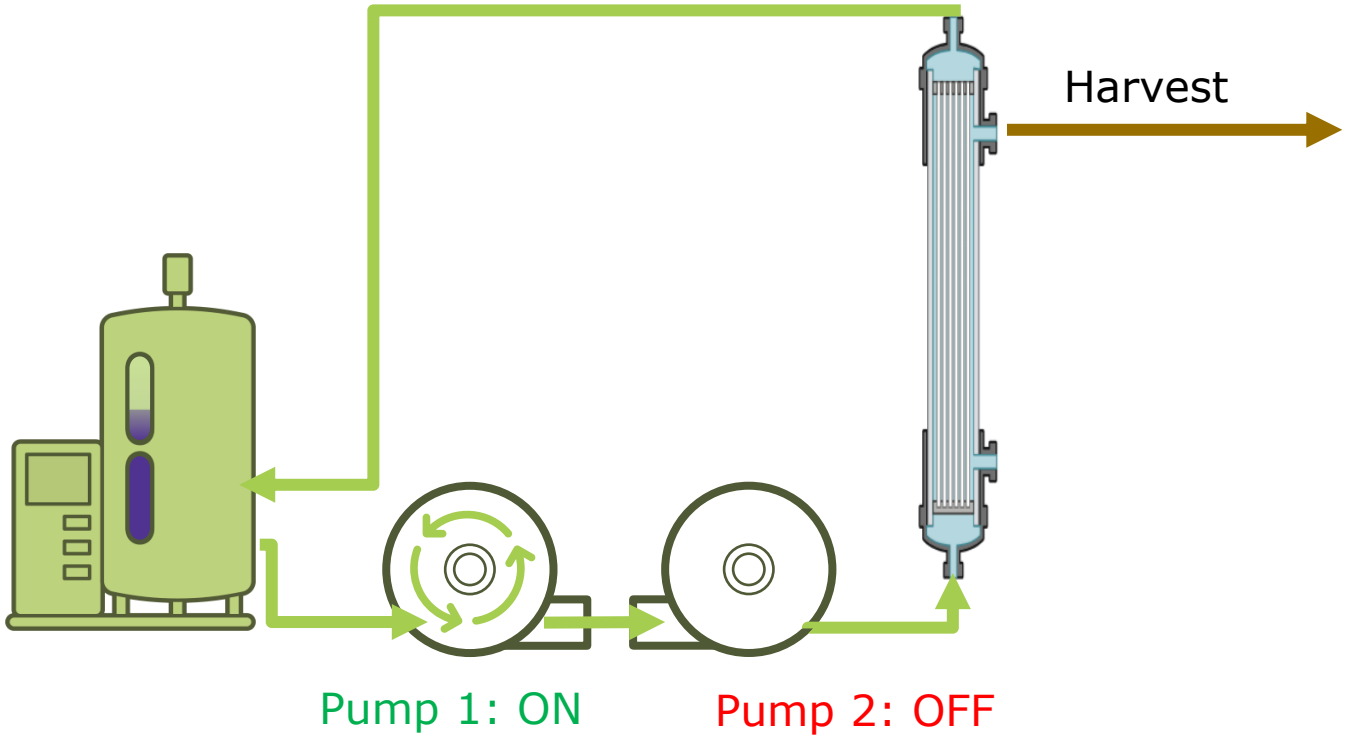
However:

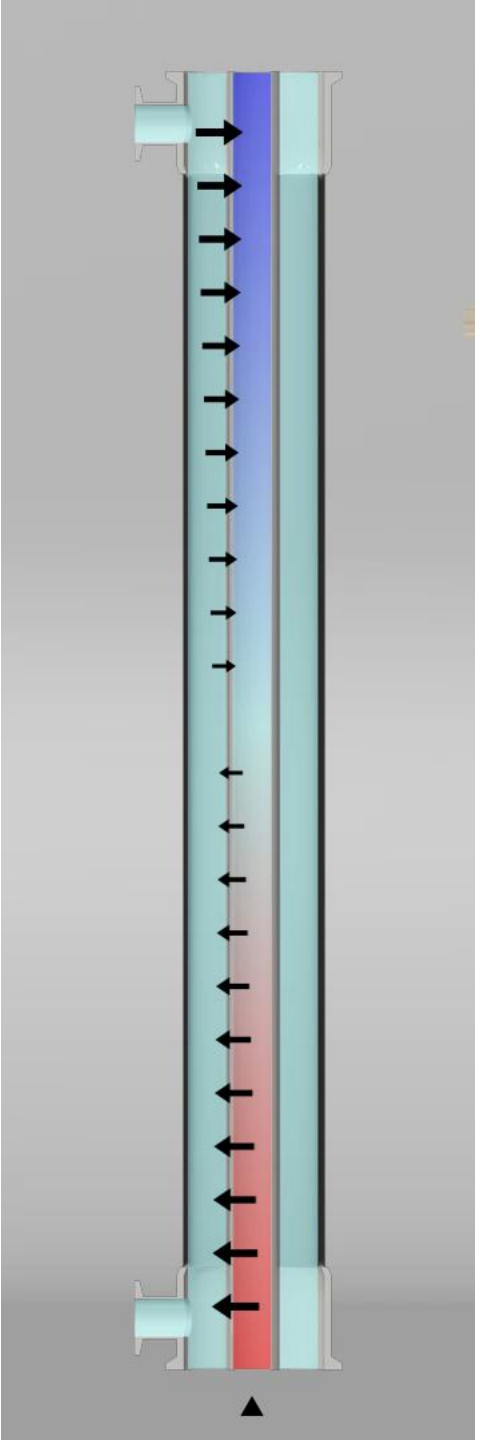
- The system is complex (needed accurate pressure sensors)
- The system is less flexible than rTFF
- One directional flow might lead to filter inlet clogging
- Scale down model (3.5 L) is complicated to achieve due to fluidics constraints

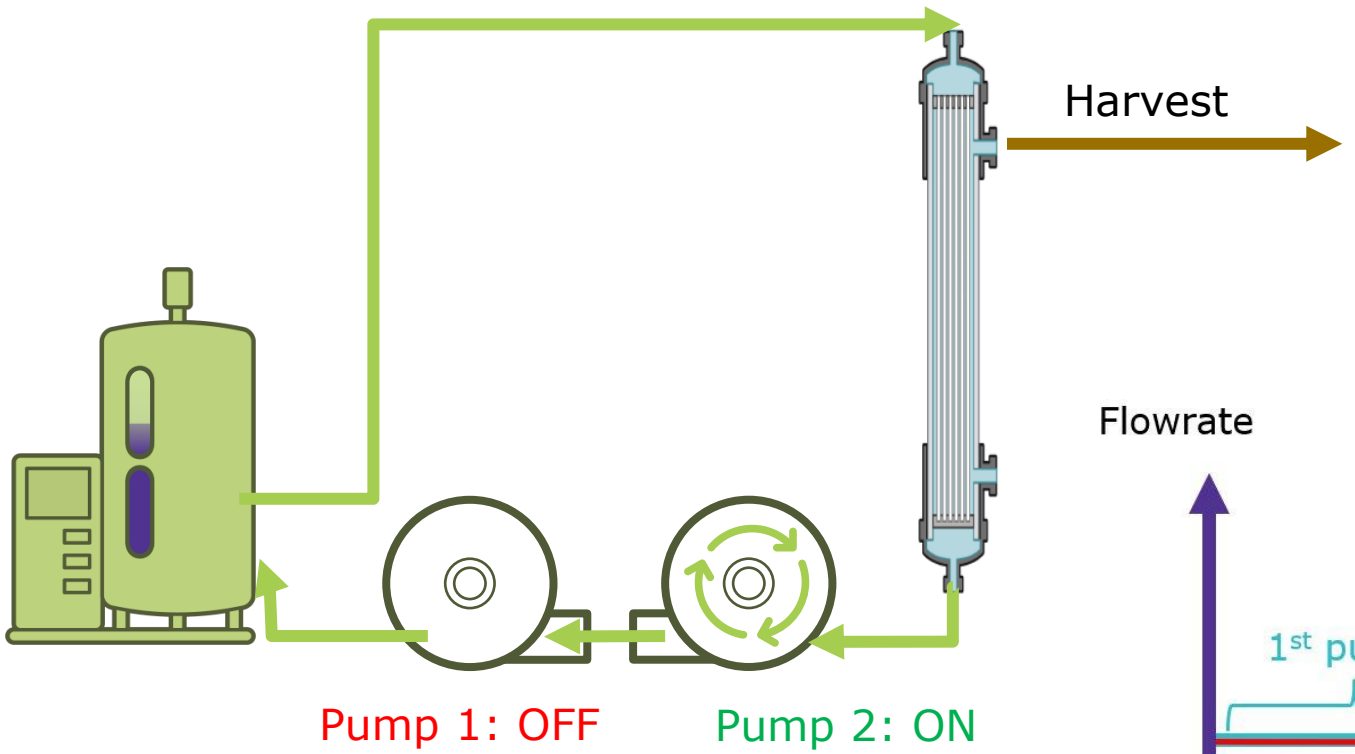
Cell aggregates at filter inlet

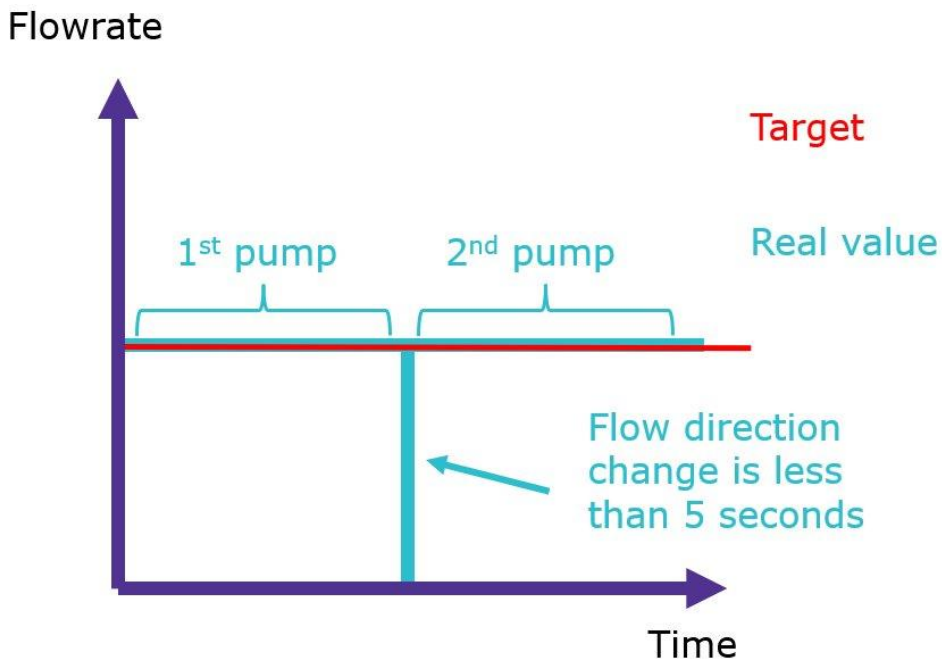
Reverse TFF (rTFF)



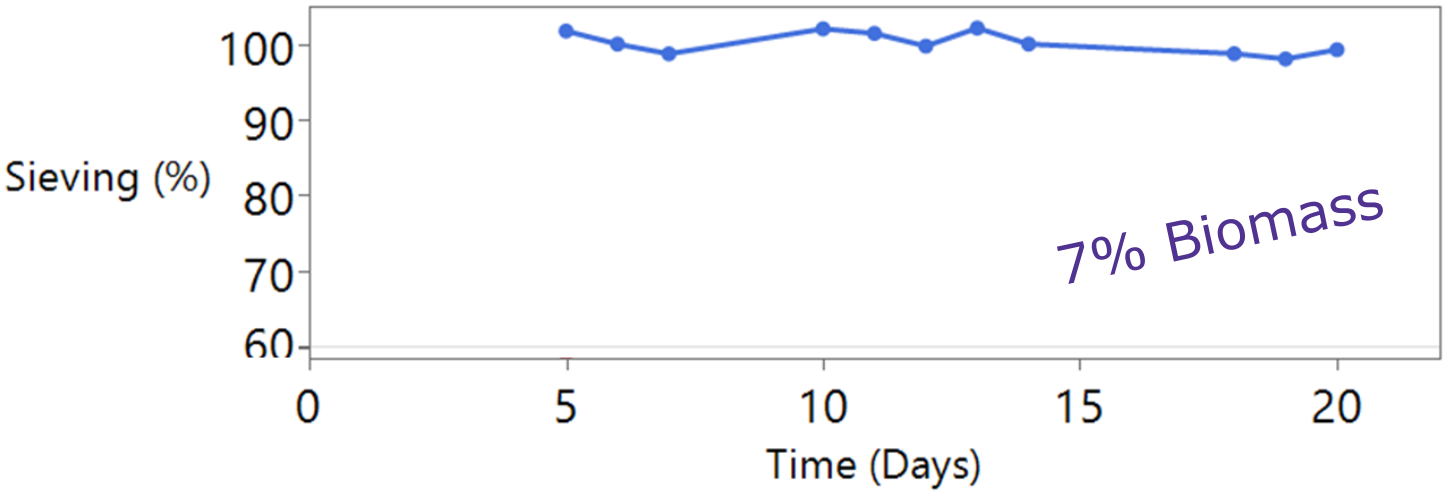

Harvest
Pump 1: ON
Pump 2: OFF




Harvest
Pump 1: OFF
Pump 2: ON


Flowrate
Target
Real value
1st pump
2nd pump
Flow direction change is less than 5 seconds
Time

rTFF
20 L test in wave-bag



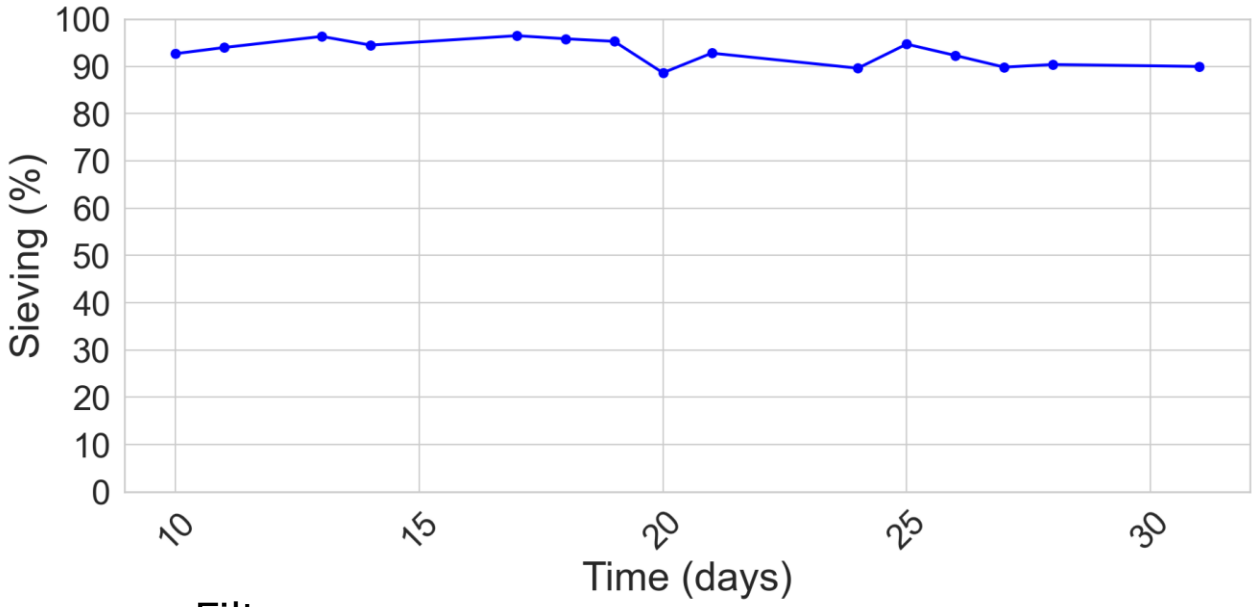
Filter:

- 3.74 LMH
- 6.70 mL/min/fiber

Culture:

- 15.1 L Working Volume
- 0.89 vvd
- 6.85% biomass or VCV

rTFF
Test 50 L in wave-bag

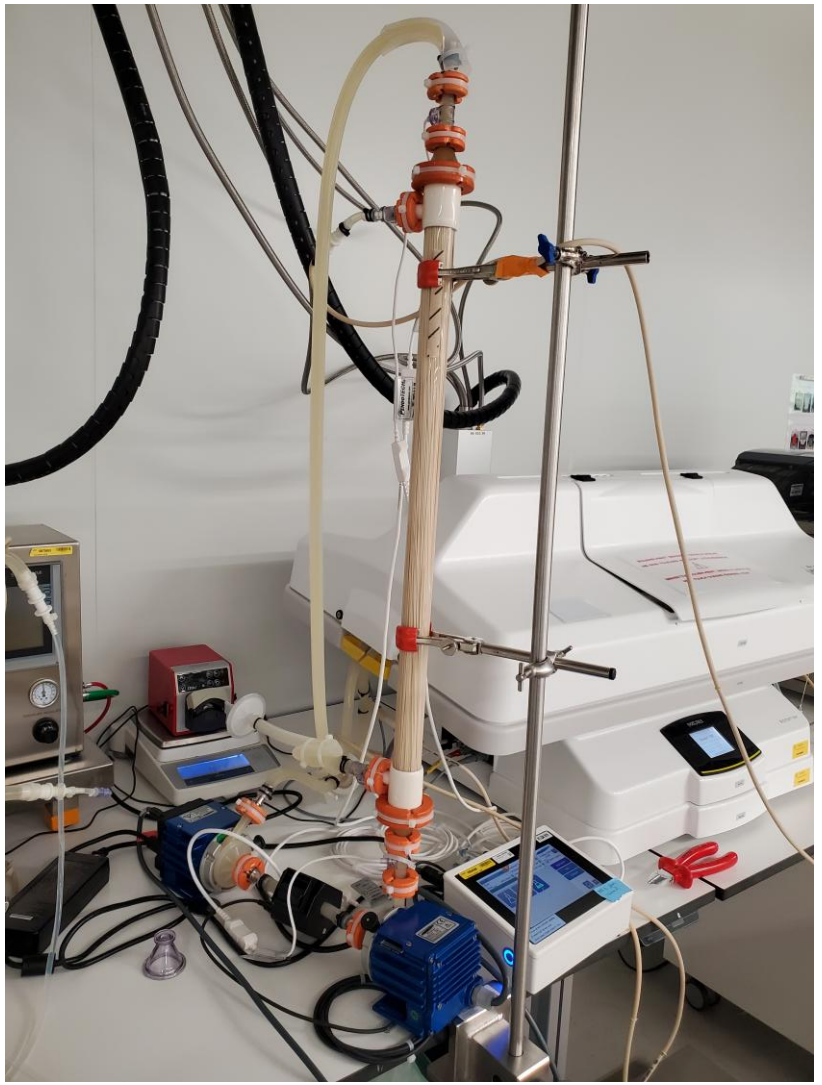


Filter:

- 3.79 LMH
- 1000 s^{-1}

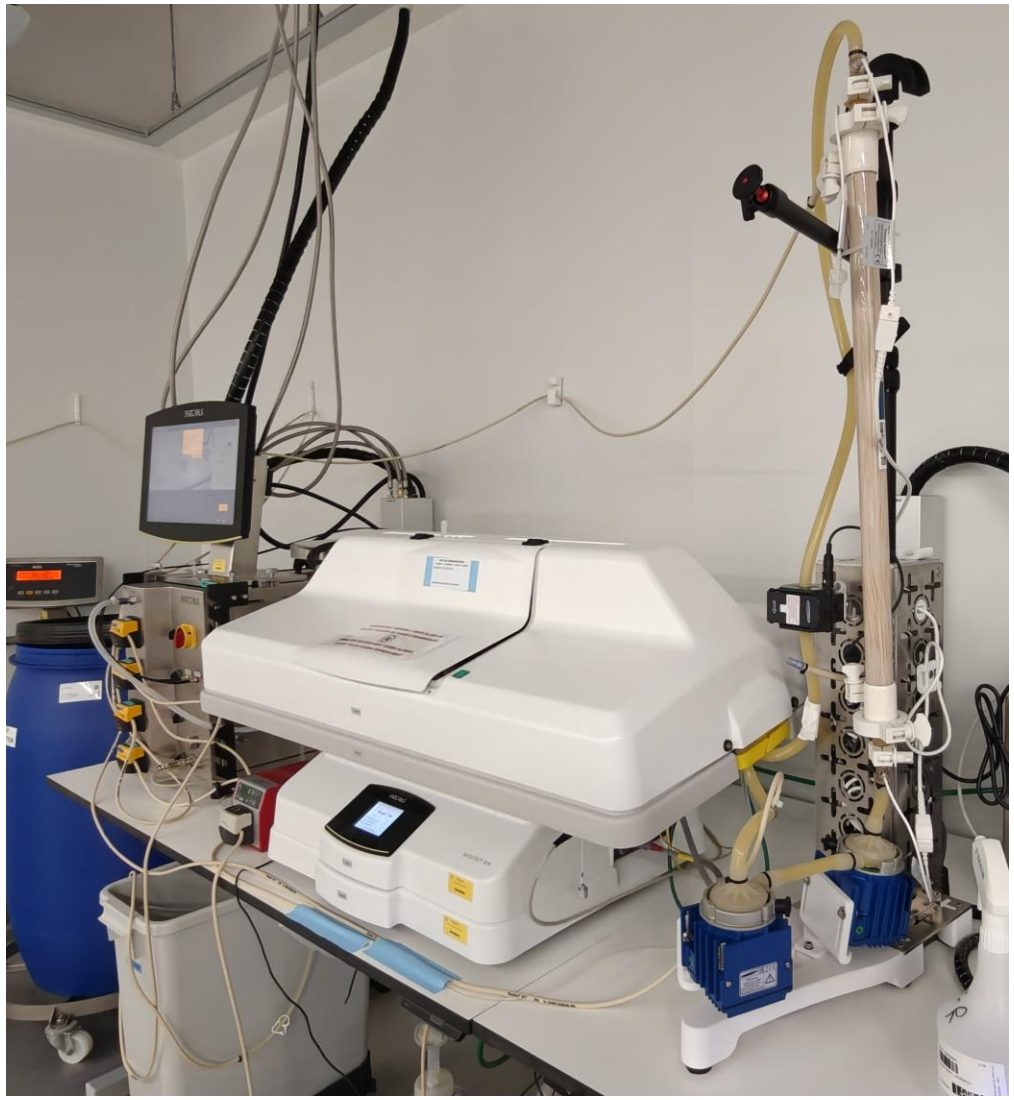
Culture:

- 25 L Working Volume
- 2 vvd
- 20% biomass or VCV



rTFF

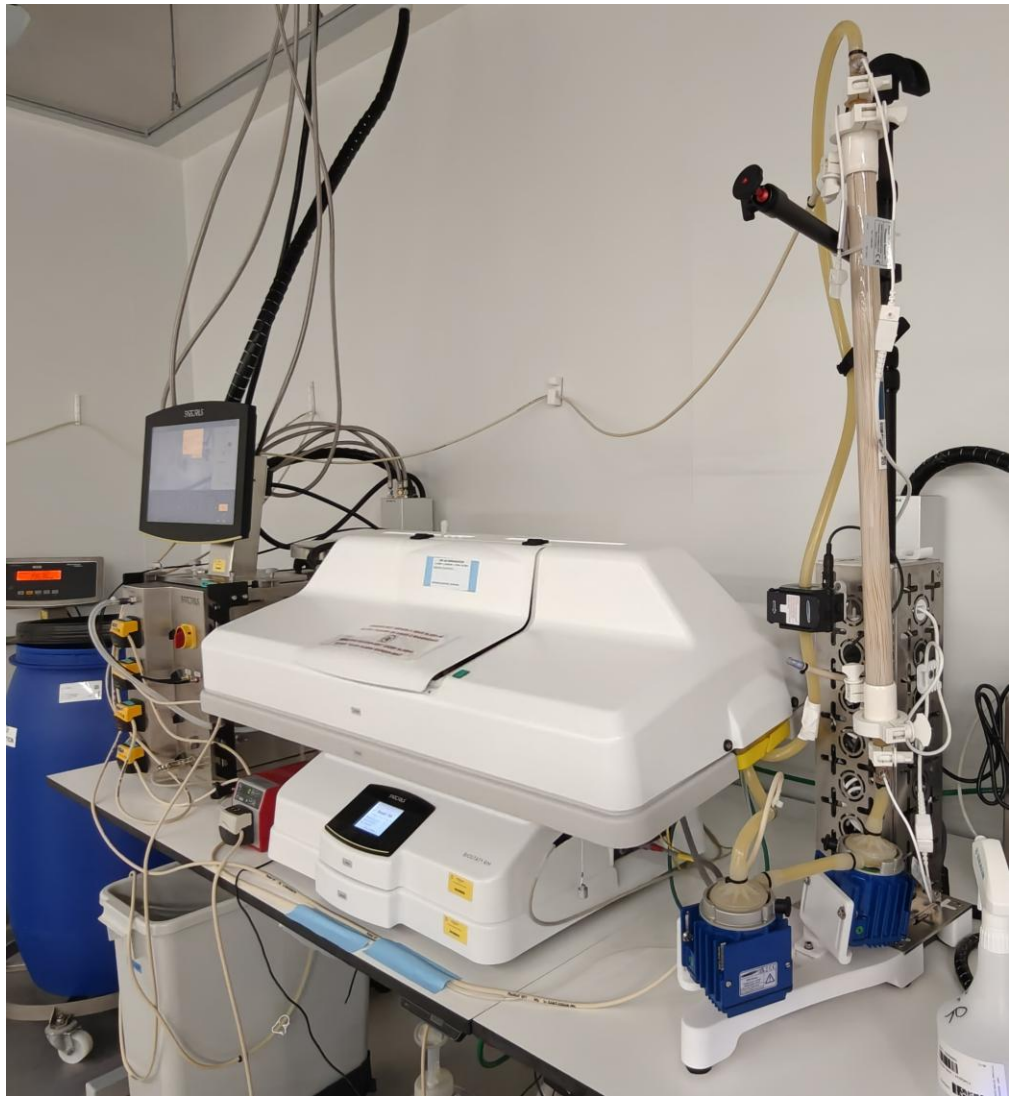
Early material generation in 50 L wave bag



Filter set-up developped with Levitronix and wave bag adaptation with larger tubing and deep tubes for better compatibility with rTFF.

rTFF

Early material generation in 50 L wave bag



Filter set-up developped with Levitronix and wave bag adaptation with larger tubing and deep tubes for better compatibility with rTFF.

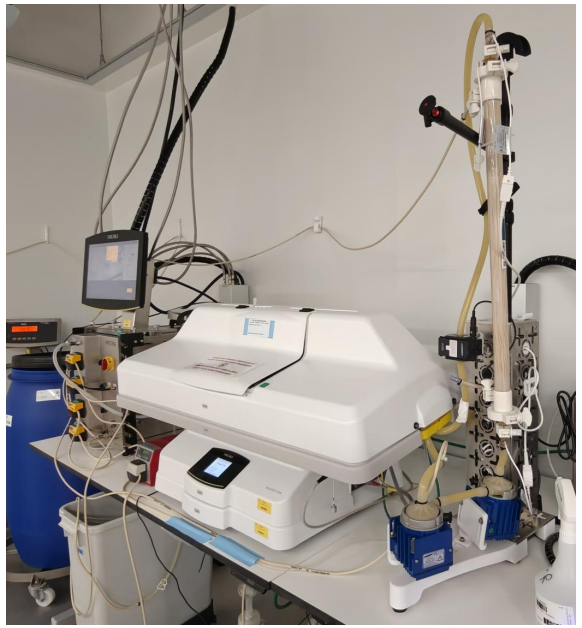
Filter:

- 4.1 LMH
- 7.14 mL/min/fiber

Culture:

- 25 L Working Volume
- 2 vvd
- 50% biomass or VCV

rTFF
Early material generation in 50 L wave bag



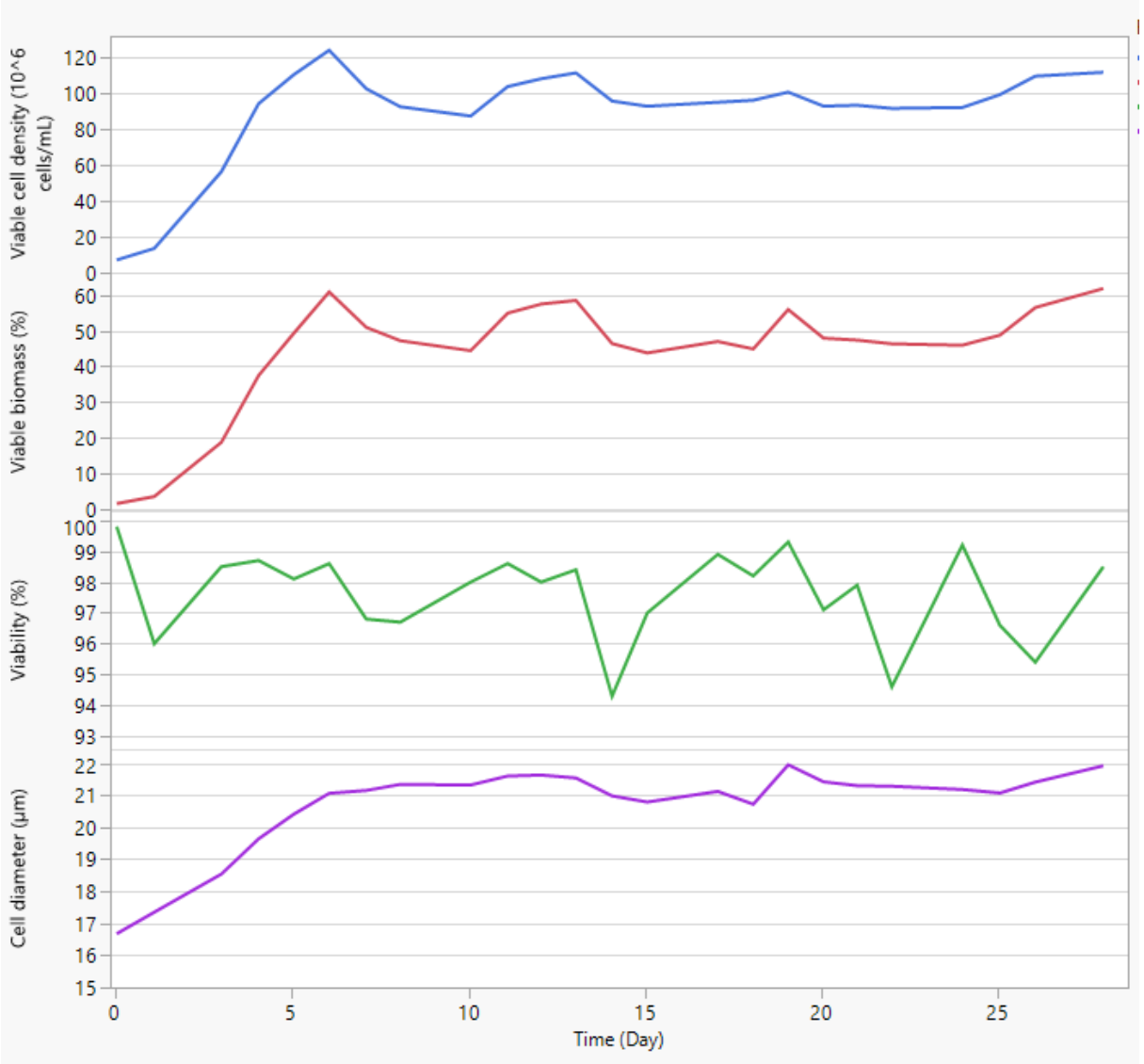
Filter set-up developped with Levitronix and wave bag adaptation with larger tubing and deep tubes for better compatibility with rTFF.

Filter:

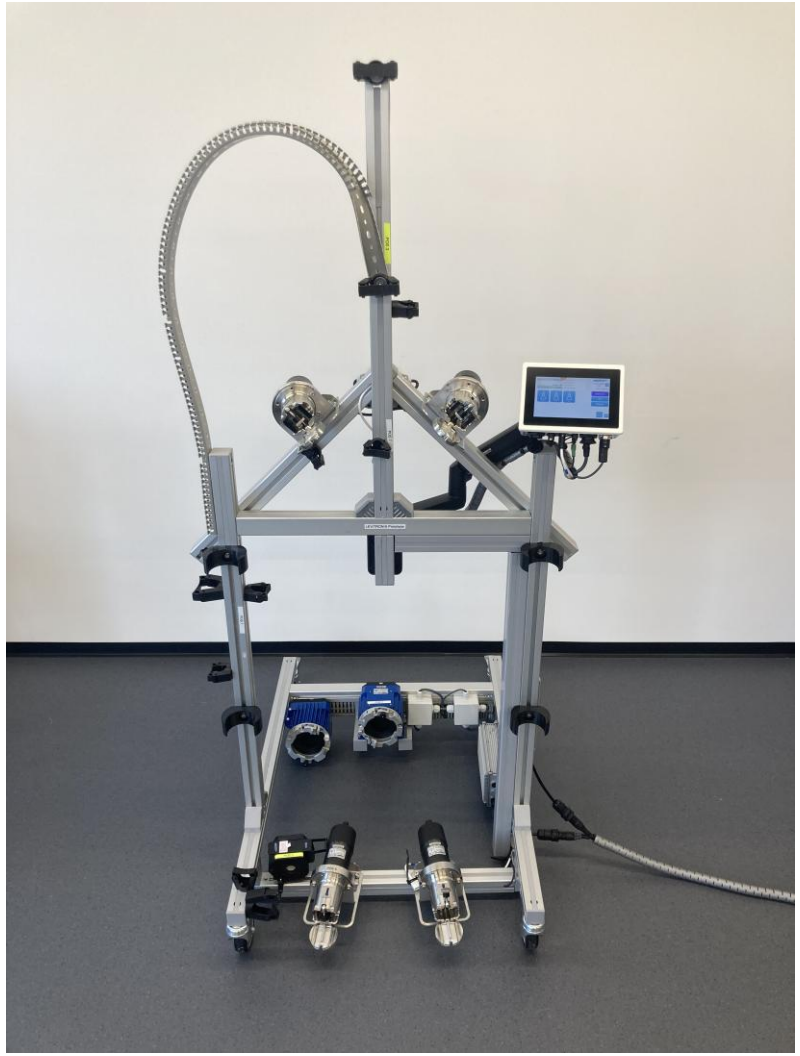
- 4.1 LMH
- 7.14 mL/min/fiber

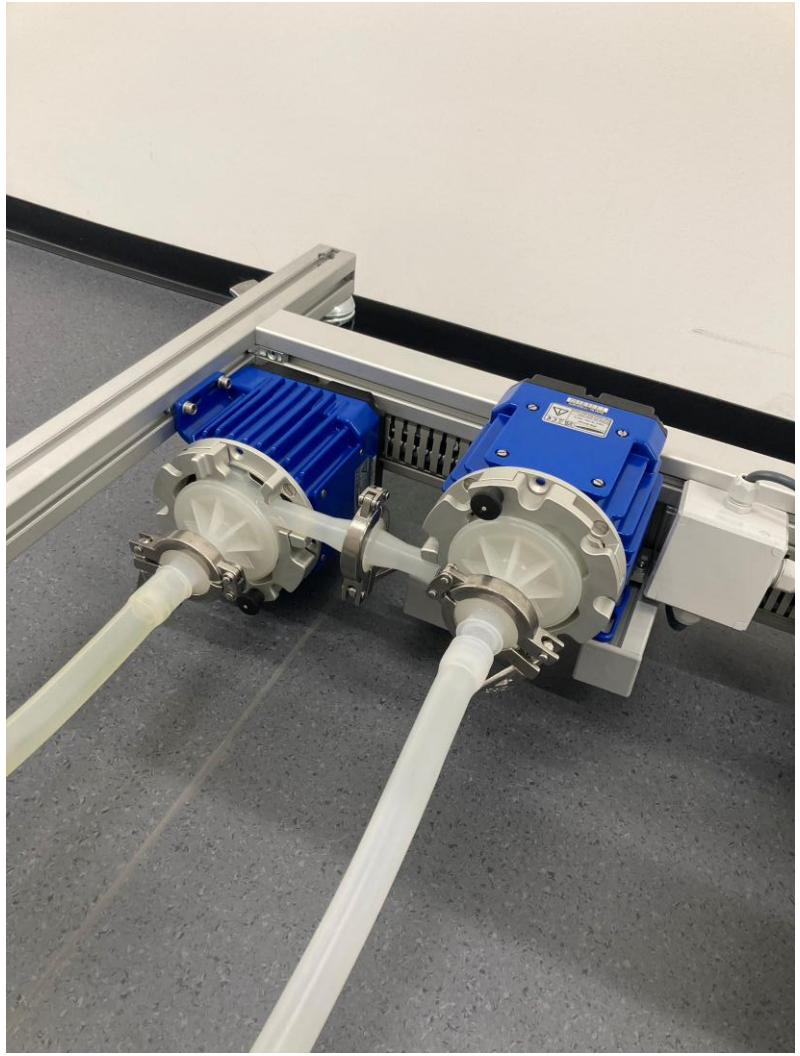
Culture:

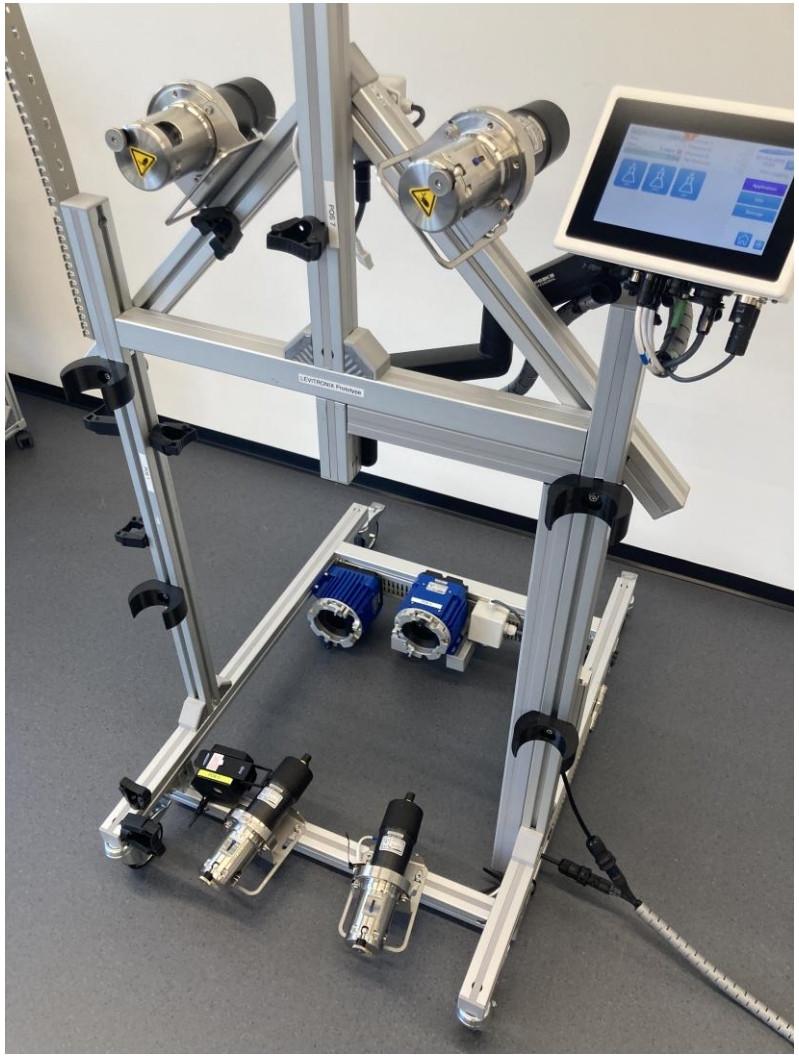
- 25 L Working Volume
- 2 vvd
- 50% biomass or VCV



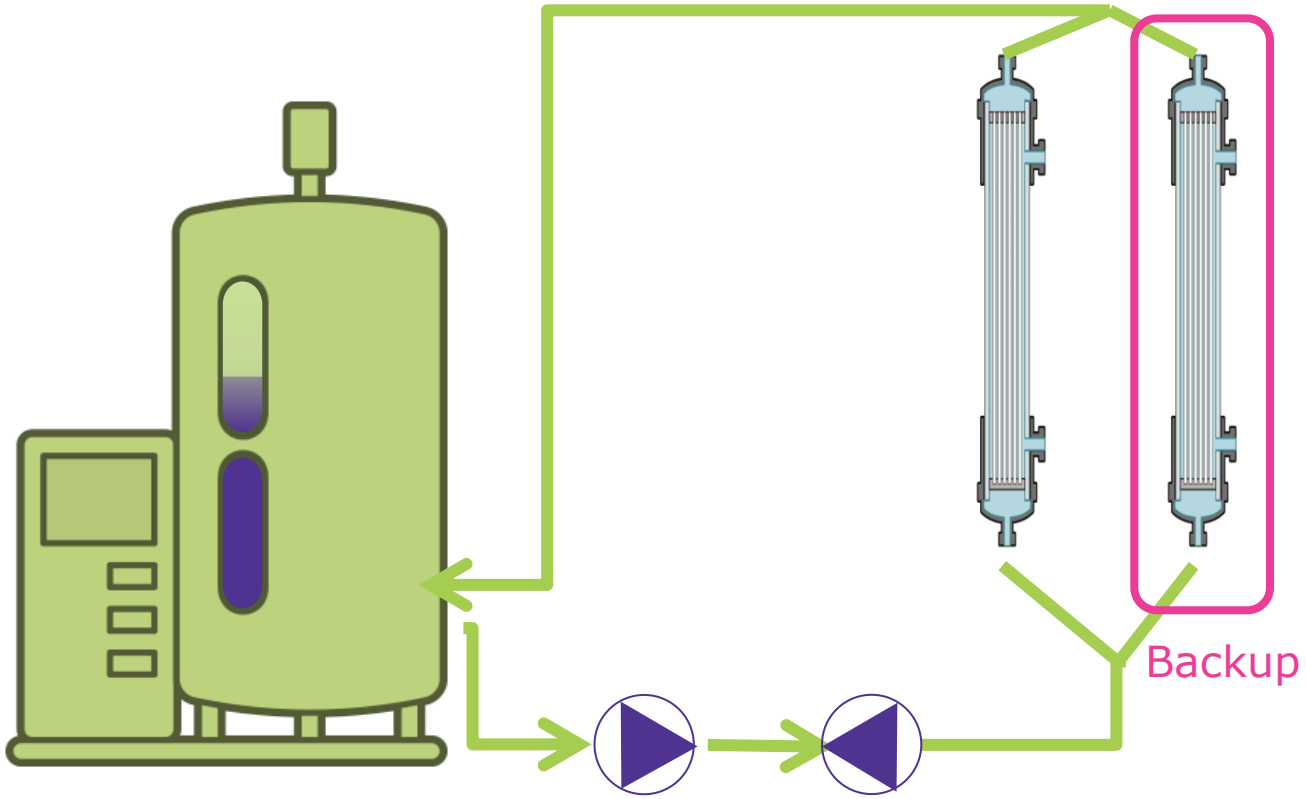
200 L rTFF
Pilot-Scale Skid Prototype

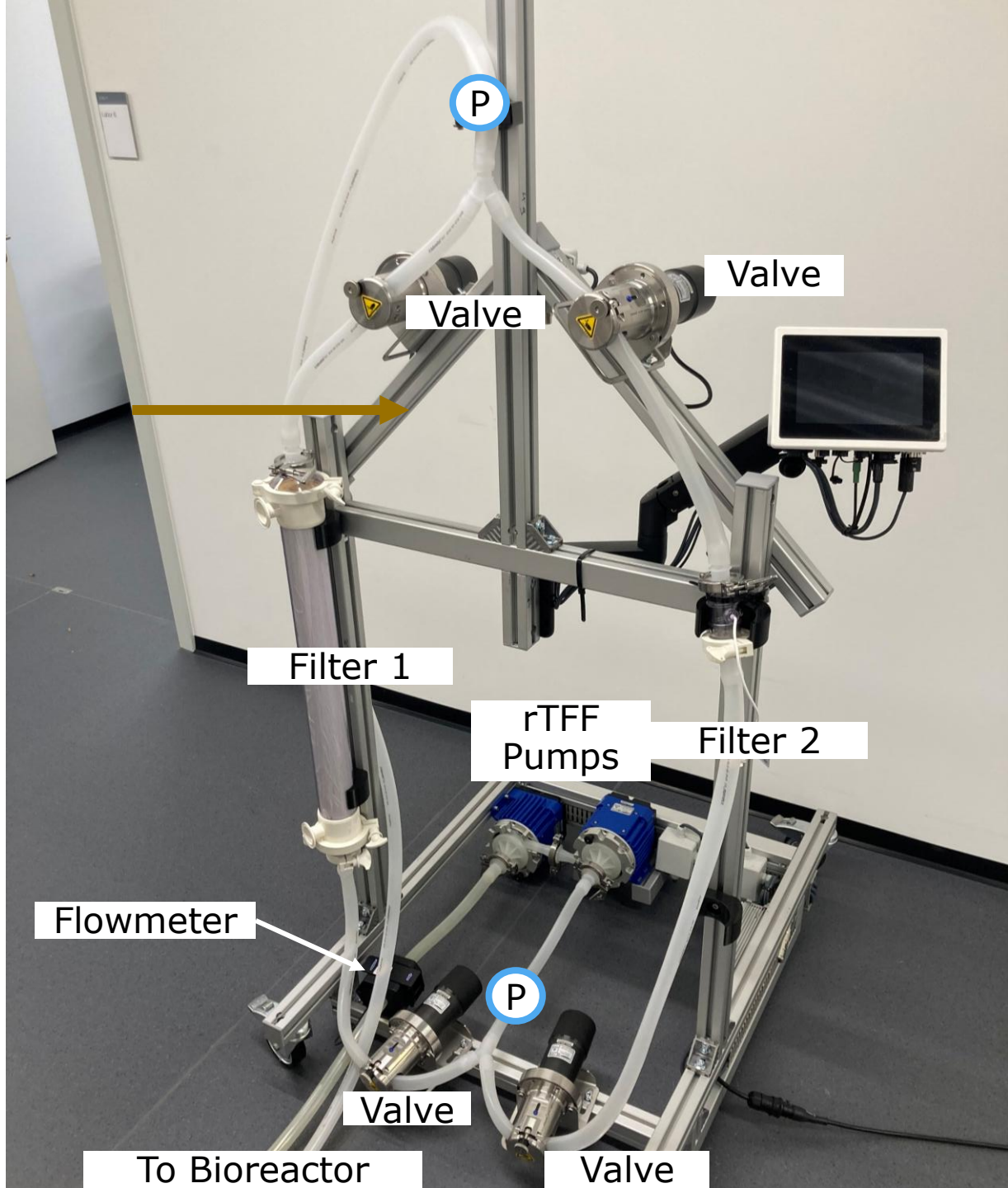






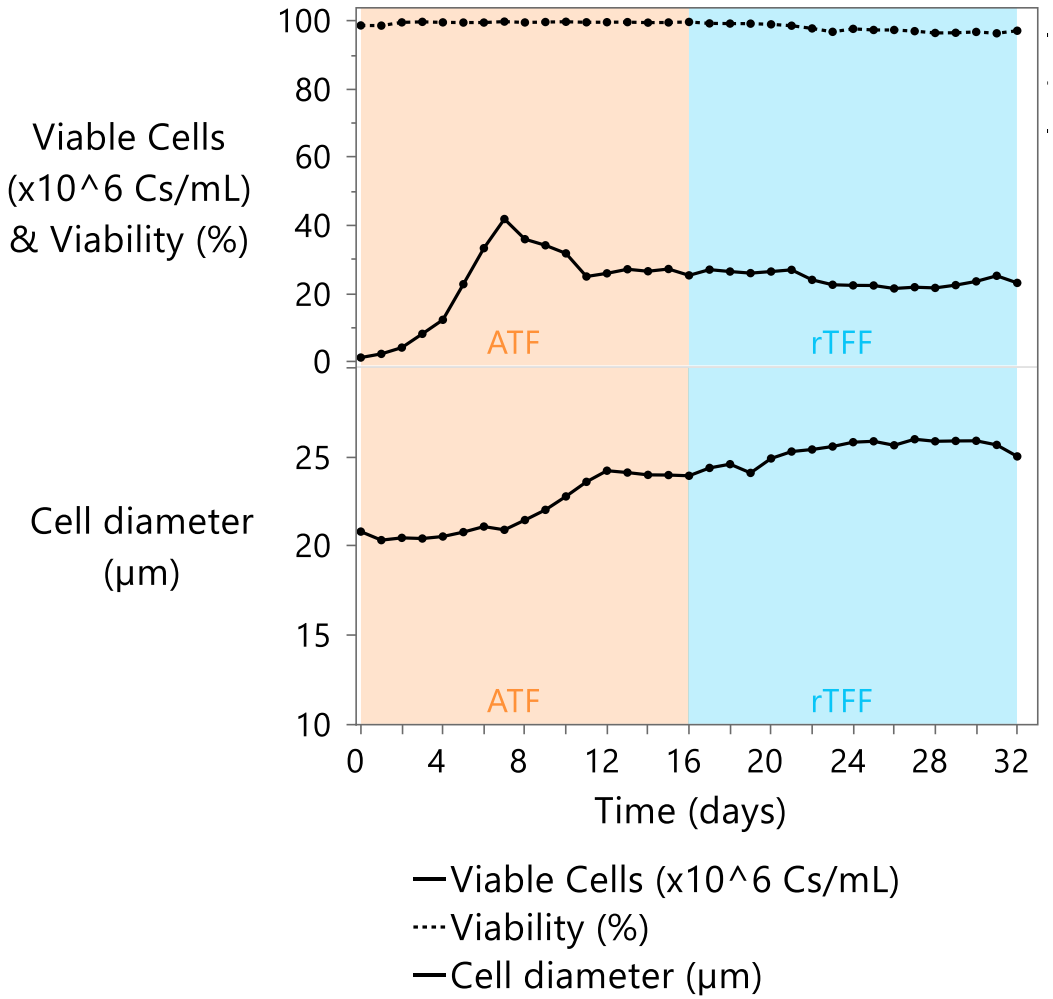
200 L rTFF
Prototype with backup option

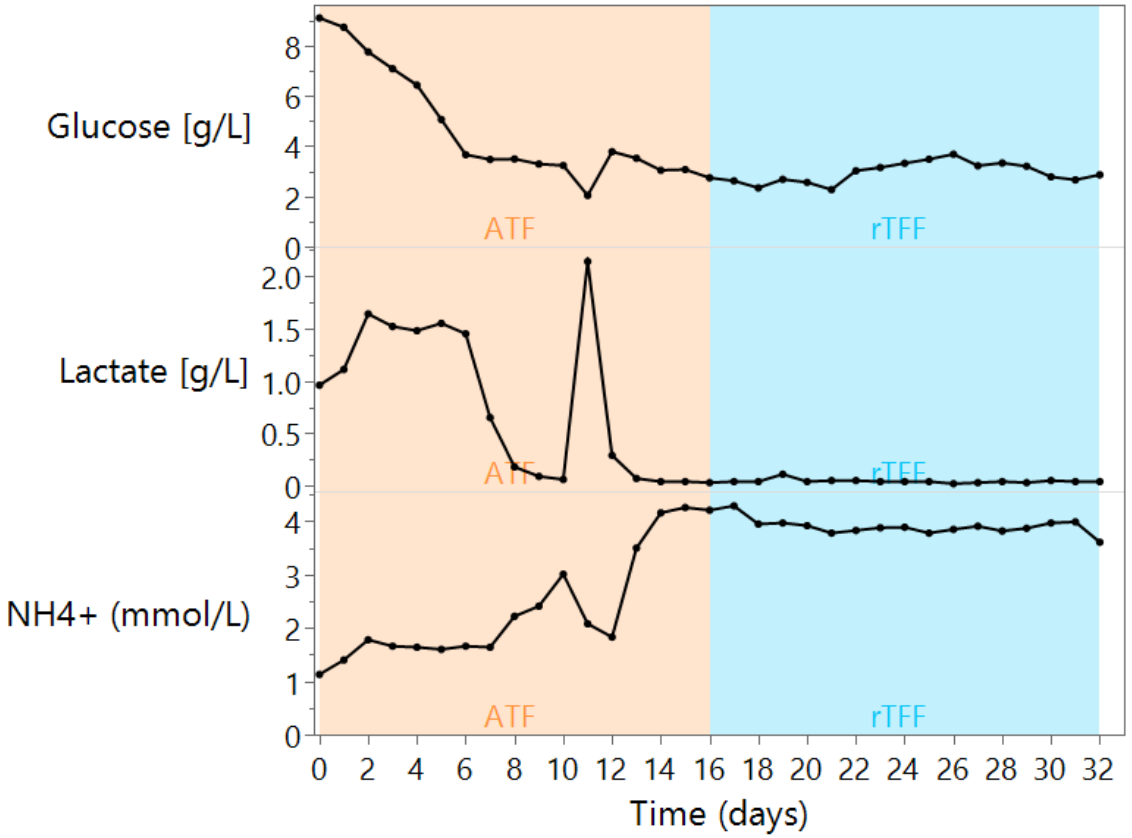




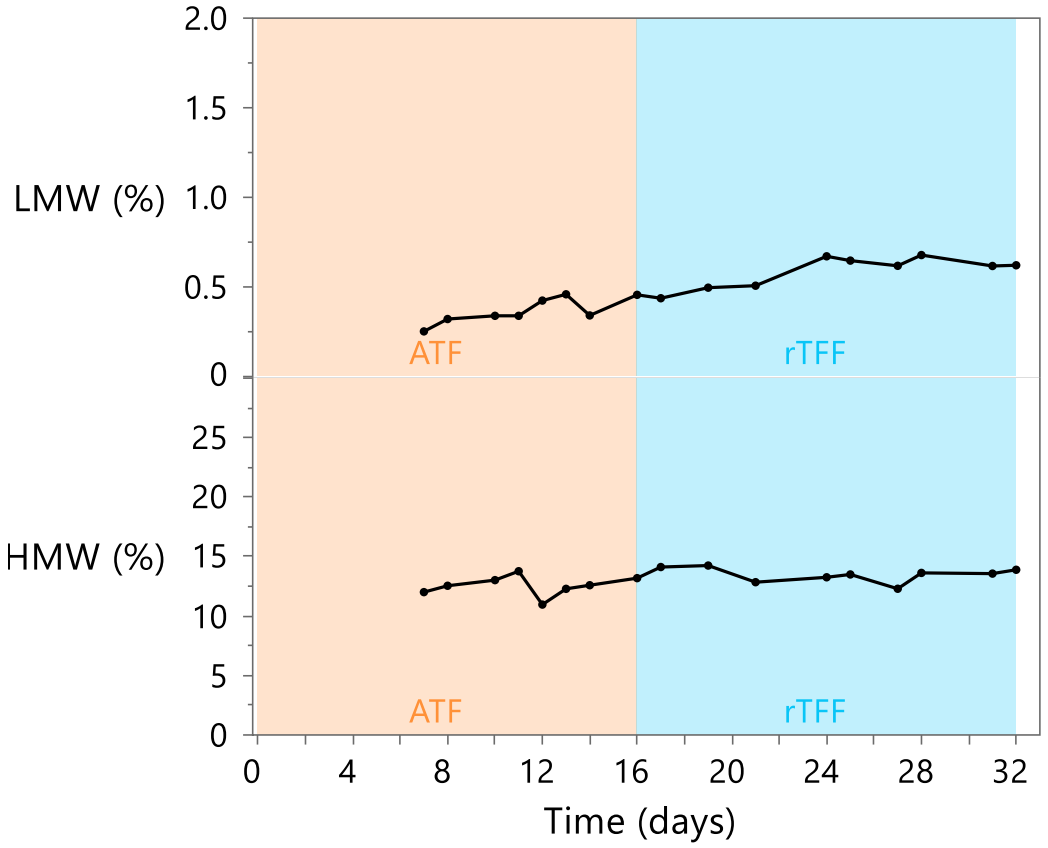
200 L rTFF
Prototype running

200 L rTFF
Similar growth and metabolism between ATF and rTFF



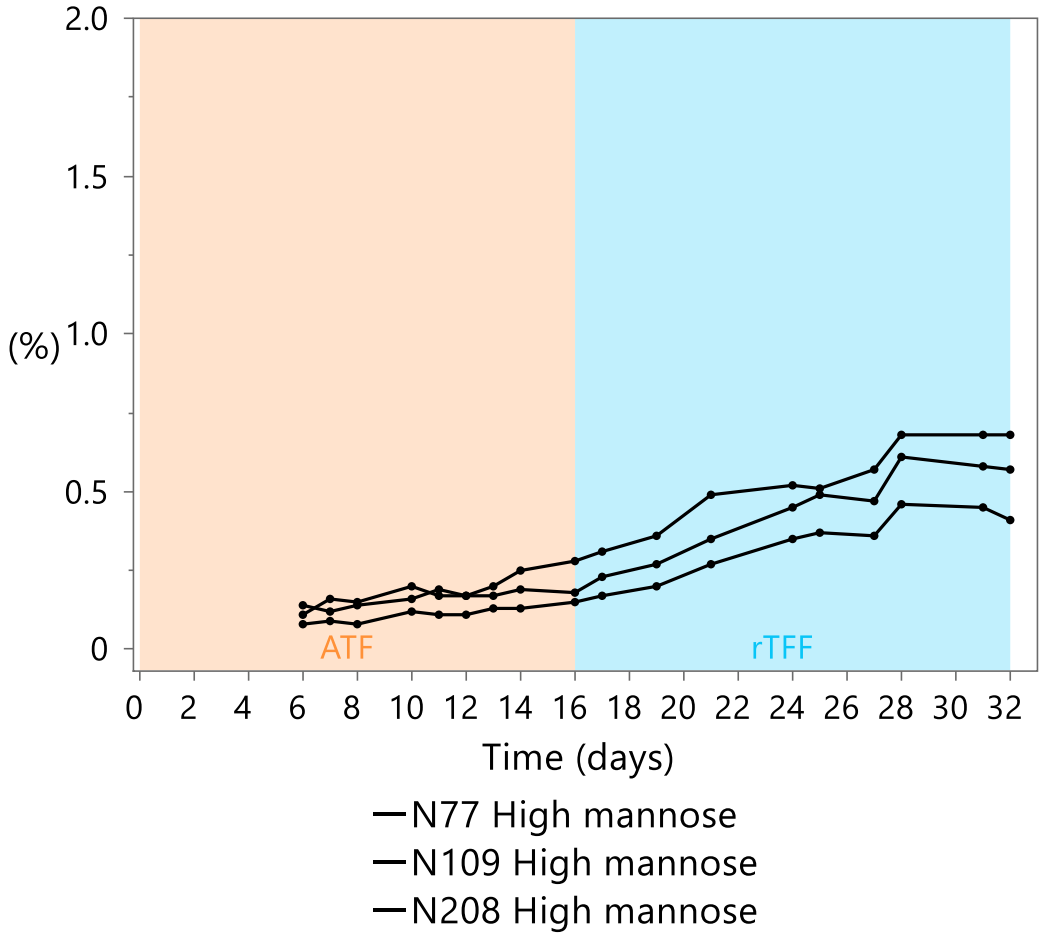


200 L rTFF
No impact on product quality

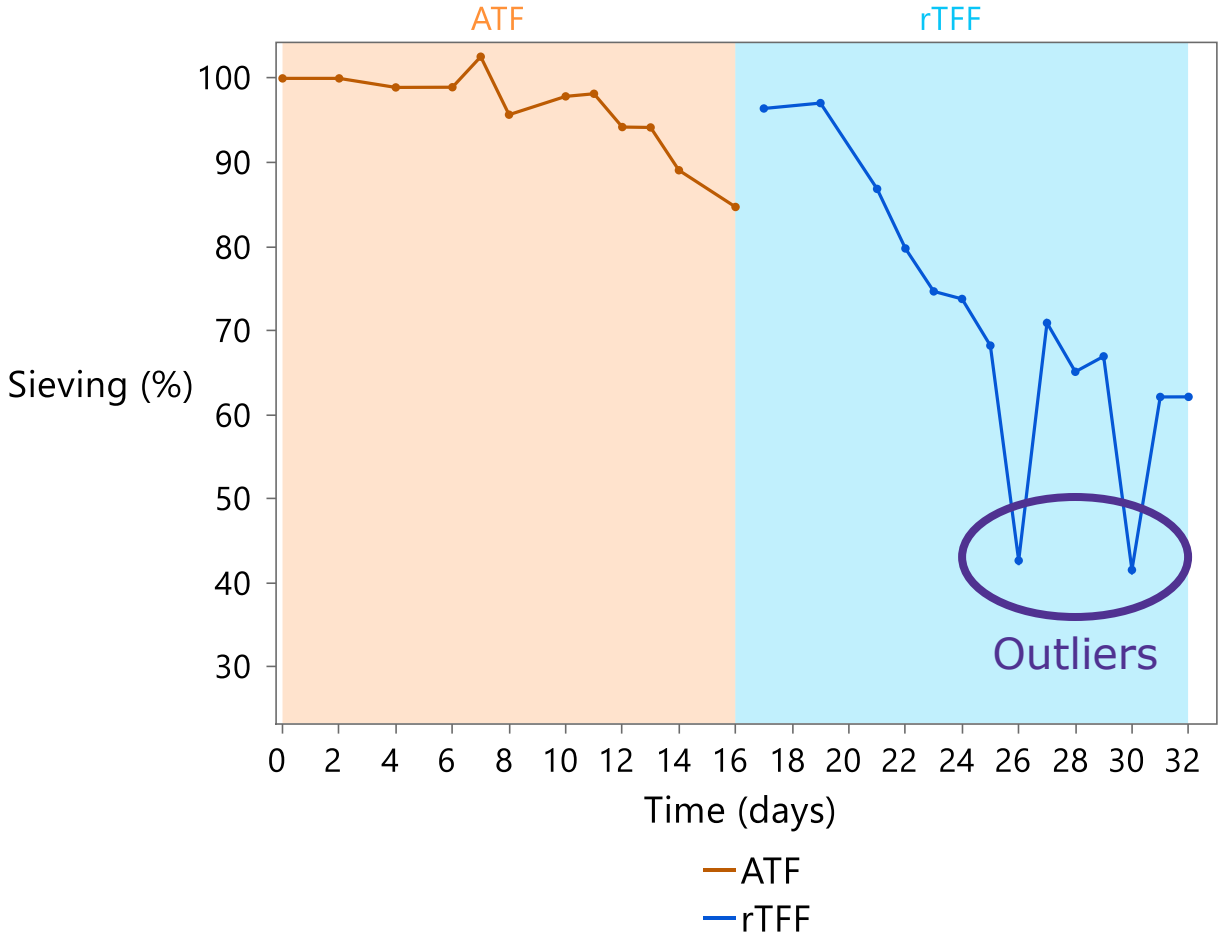


LMW: Low Molecular Weight

HMW: High Molecular Weight



200 L rTFF
Similar sieving profile between ATF and rTFF



Conclusion
rTFF as a cell retention device

Good performance at 20 and 50 L scale

Sieving is equivalent or better

40% cost decrease estimated for cell retention

Easier and more flexible set-up

Conclusion
rTFF as a cell retention device

- Good performance at 20 and 50 L scale
- Sieving is equivalent or better
- 40% cost decrease estimated for cell retention
- Easier and more flexible set-up

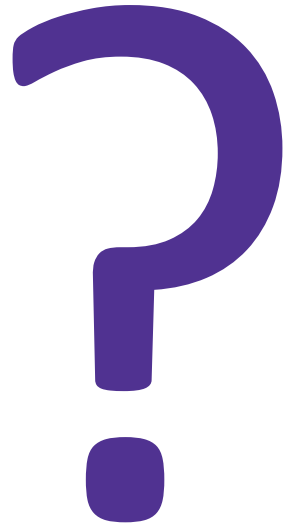
Next Steps

- Improve set-up and assembly at 200 L
- Test different membranes at 3.5 L
- Continue testing on 200 L pilot scale

Conclusion
Open questions

- How to implement rTFF in GMP production?
- How to make a smart design at 2 kL?
- What are the risks and challenges associated with this technology?
- How to wet efficiently the filters?
- Should we adapt conditions (LMH, crossflow, etc) for each process?
- Could we build model to predict performances of filters swap?
- Could we clean filters to optimized duration?

Thanks !

Any questions 

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