Ultra Scale-down Tools to Accelerate Process Development

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Outline

- Significance of ultra scale-down (USD) technology
- Ultra Scale-down device for crossflow filtration process
- Modelling system resistance
- Predict large scale flux and TMP relationship
- Conclusions

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The Challenge for Pharmaceuticals

- Medicines are becoming more complex
- Healthcare markets are becoming more segmented
- Governments are working to contain healthcare costs
- Greater emphasis on speed to market and improved bioprocesses

What Is Ultra Scale-down (USD)?

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- The ability to research at a small scale and provide new insights into how a bioprocessing material is impacted by the process engineering environment
- The ability to inform on how to scale up and provide material representative of full scale

Why Do It?

- Test cell and product candidates for "manufacturability", early and at low cost
- Address novel bioprocessing solutions, early and at low cost
- Resolve large-scale manufacturing challenges, early and at low cost
- Help meet regulatory challenges
- Move with speed to

New Engineering Tools to Accelerate Bioprocess Development

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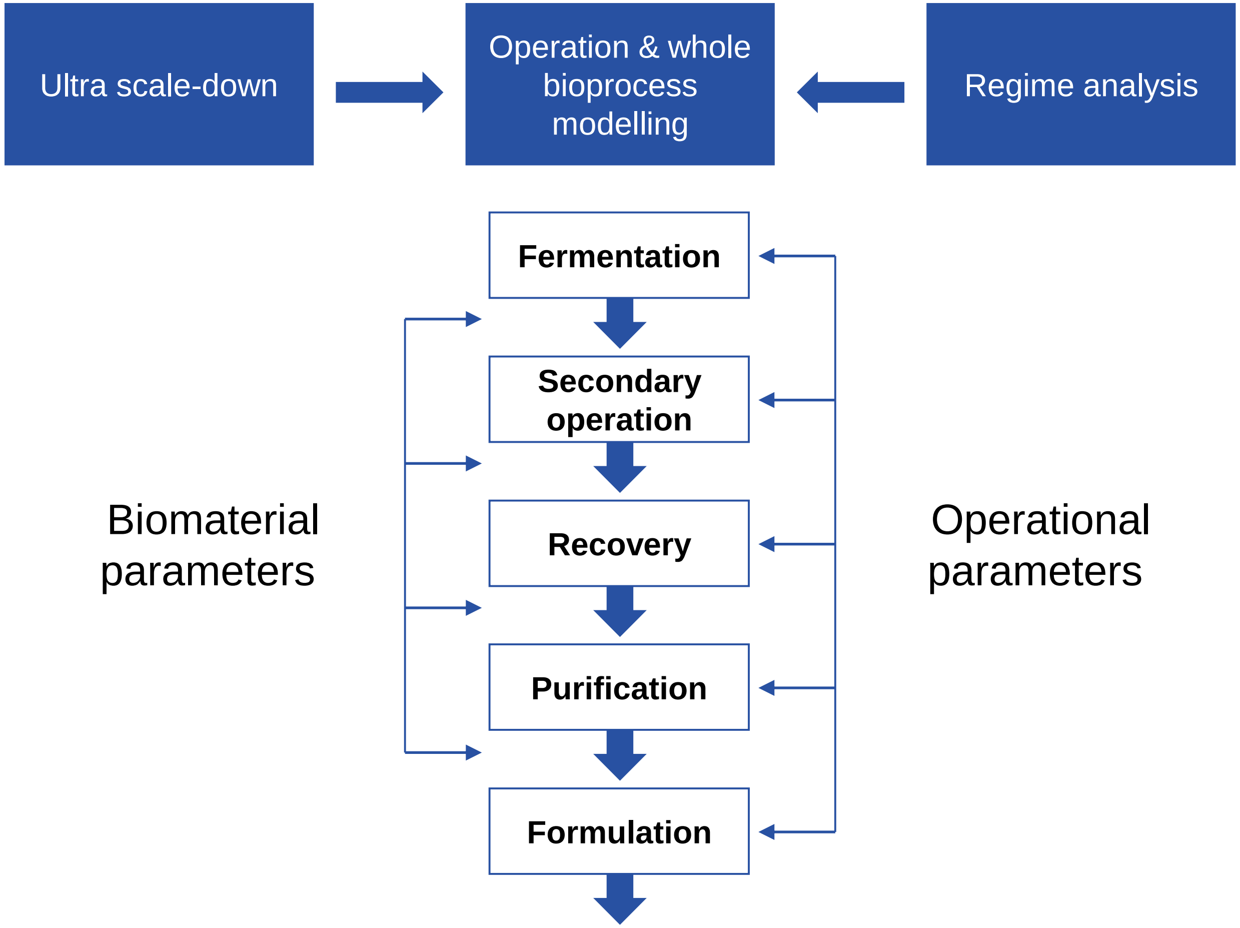

1. Specify operations and whole bioprocess
2. Identify regimes where changes in process stream properties may occur
3. Use ultra scale-down to mimic such regimes and test impact on process stream

Achievements in Ultra-scale Down Technology

1. Fermentation
2. Centrifugation
3. Dead-end filtration
4. Depth filtration
5. Chromatography
6. Cross-flow filtration

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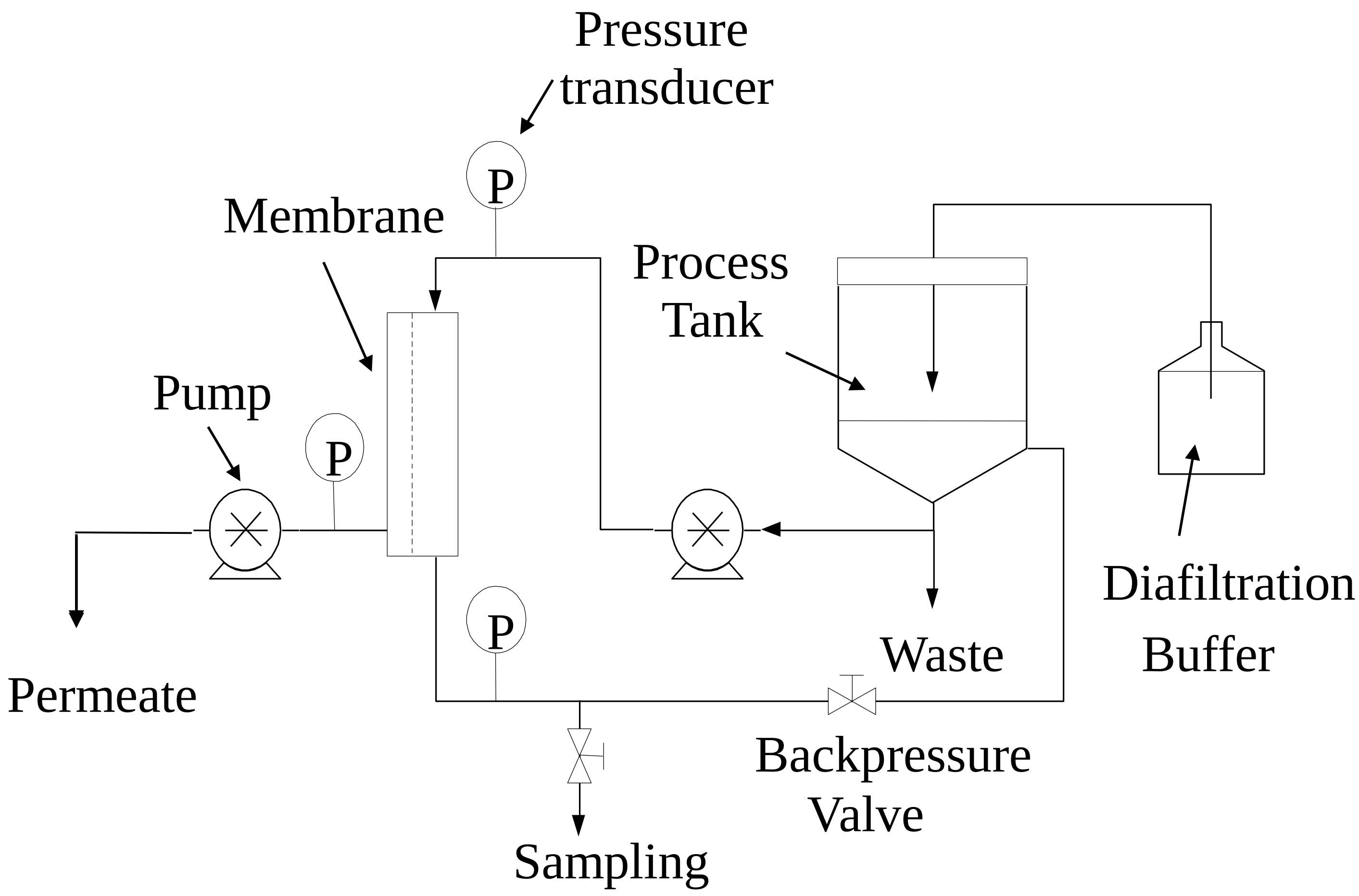
Cross-flow Filtration



USD Device

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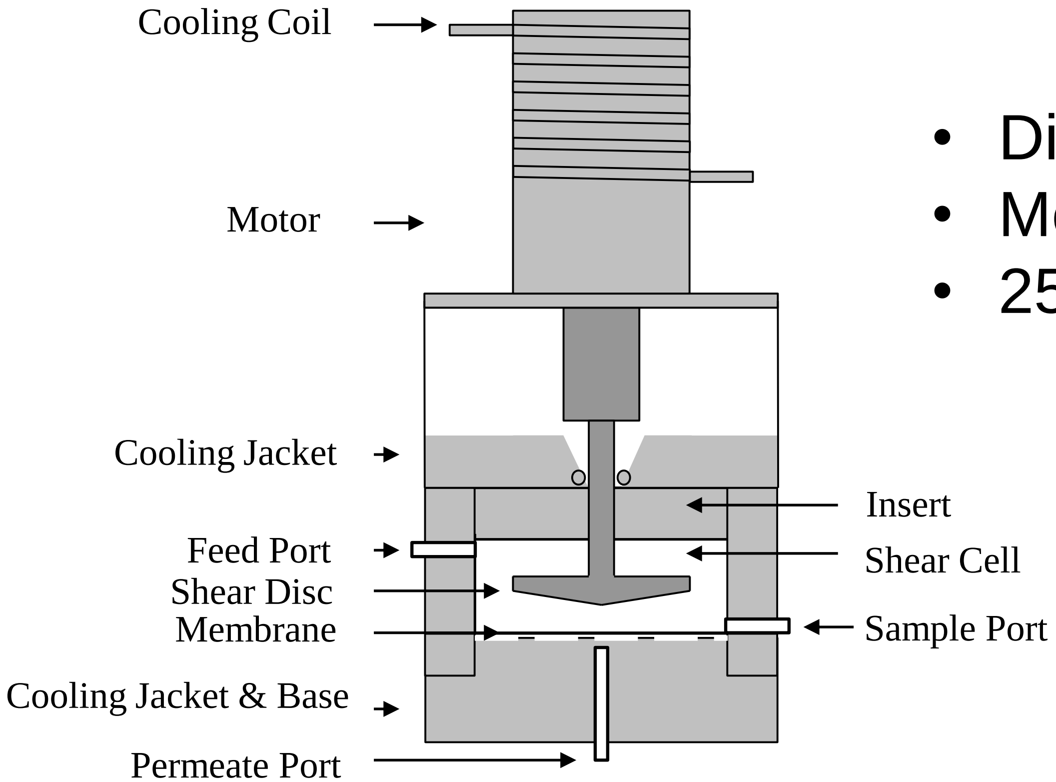
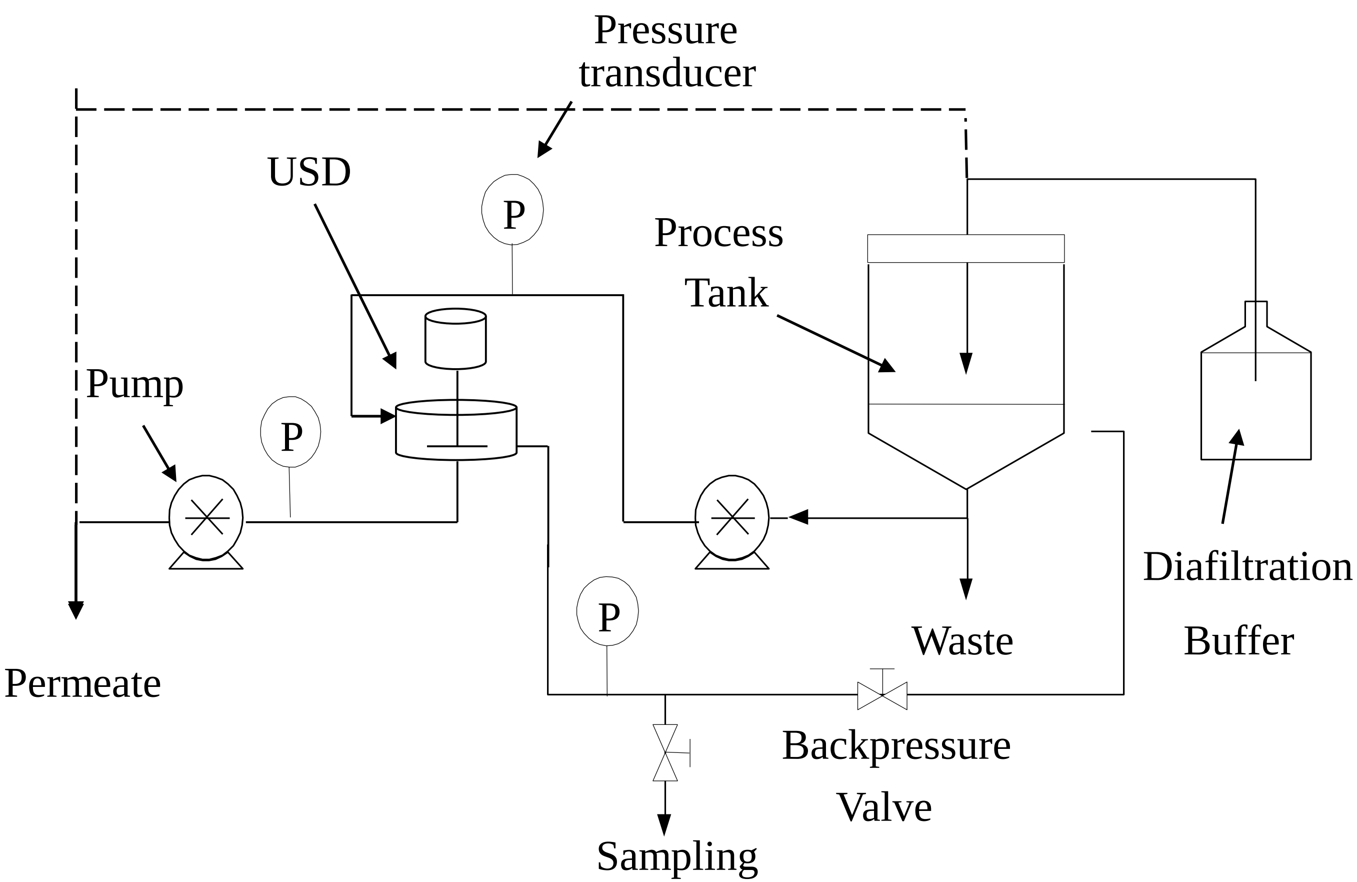
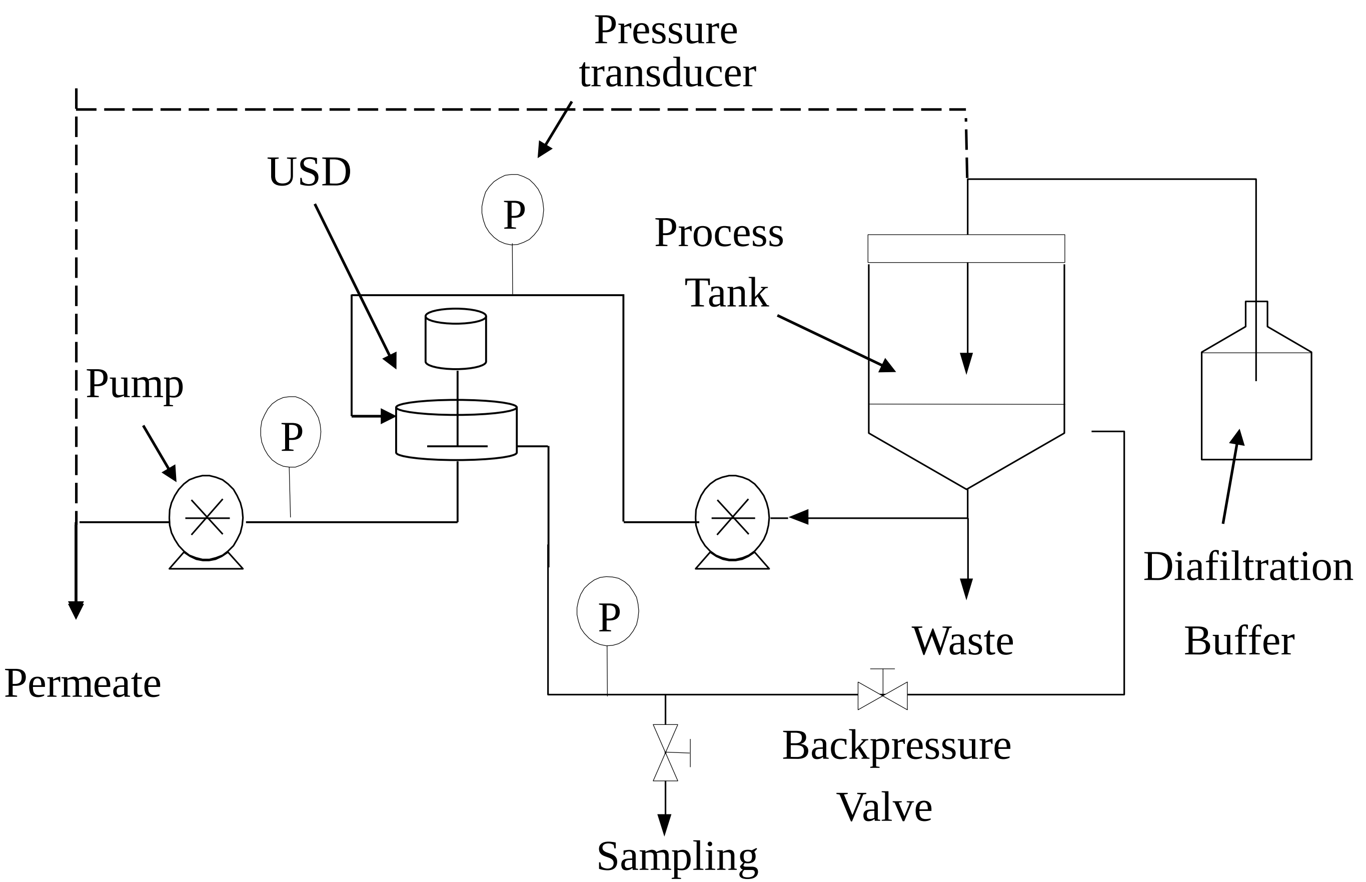


- Diameter = 21 mm
- Membrane area =3.64 cm^2
- 25 ml holding volume

Cross-flow filtration with USD

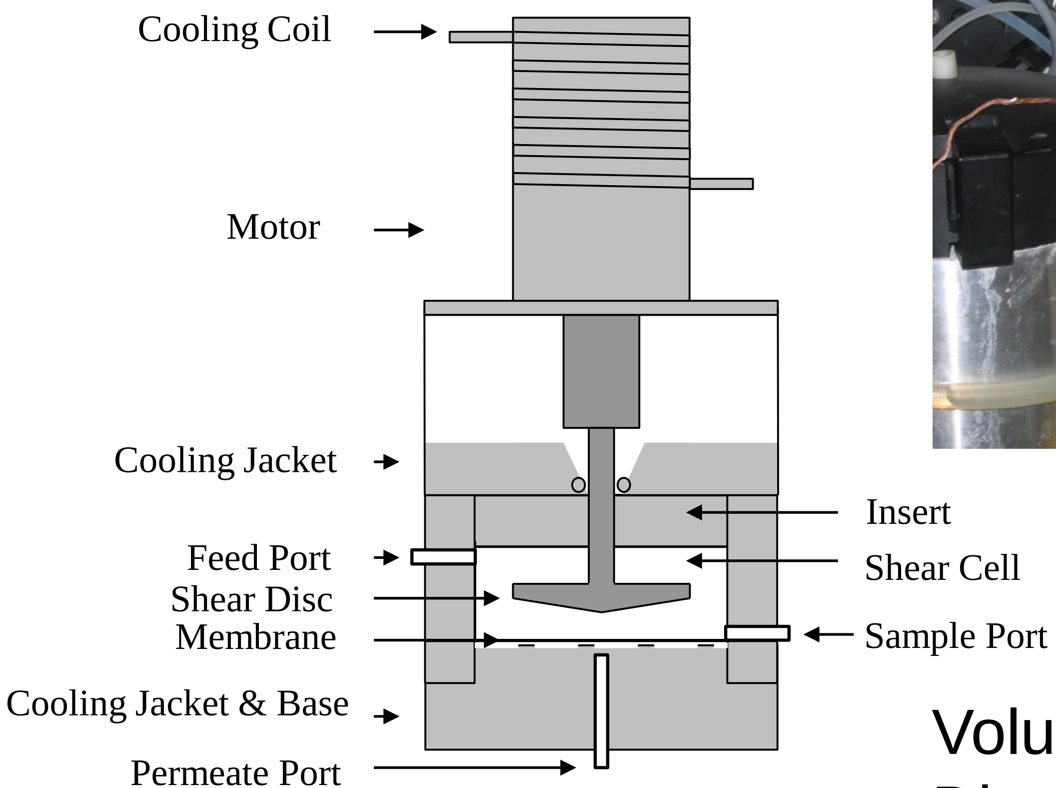


Microfiltration with USD



- Noise on pressure
- Volume and membrane area ratio is too high
- Unable to mimic transmission

USD Device

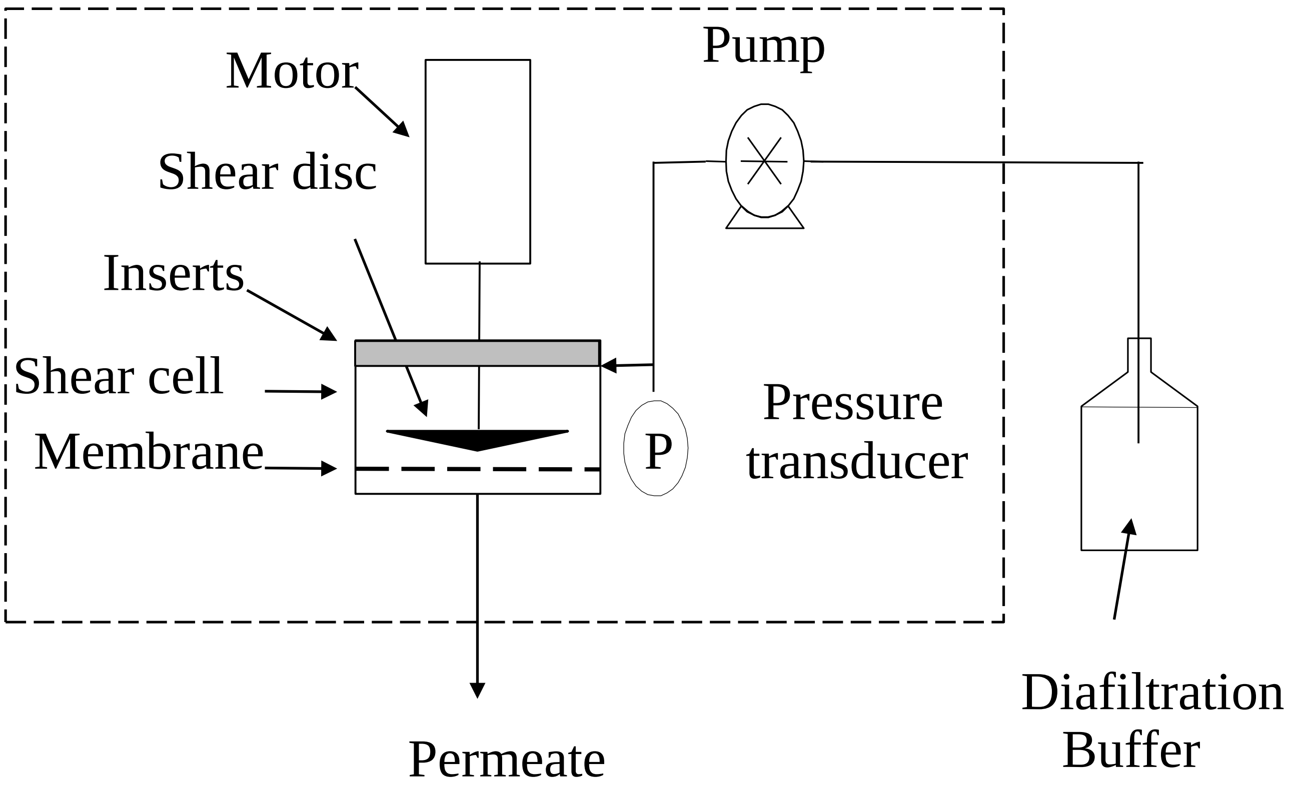


Volume = 1.5-3 ml

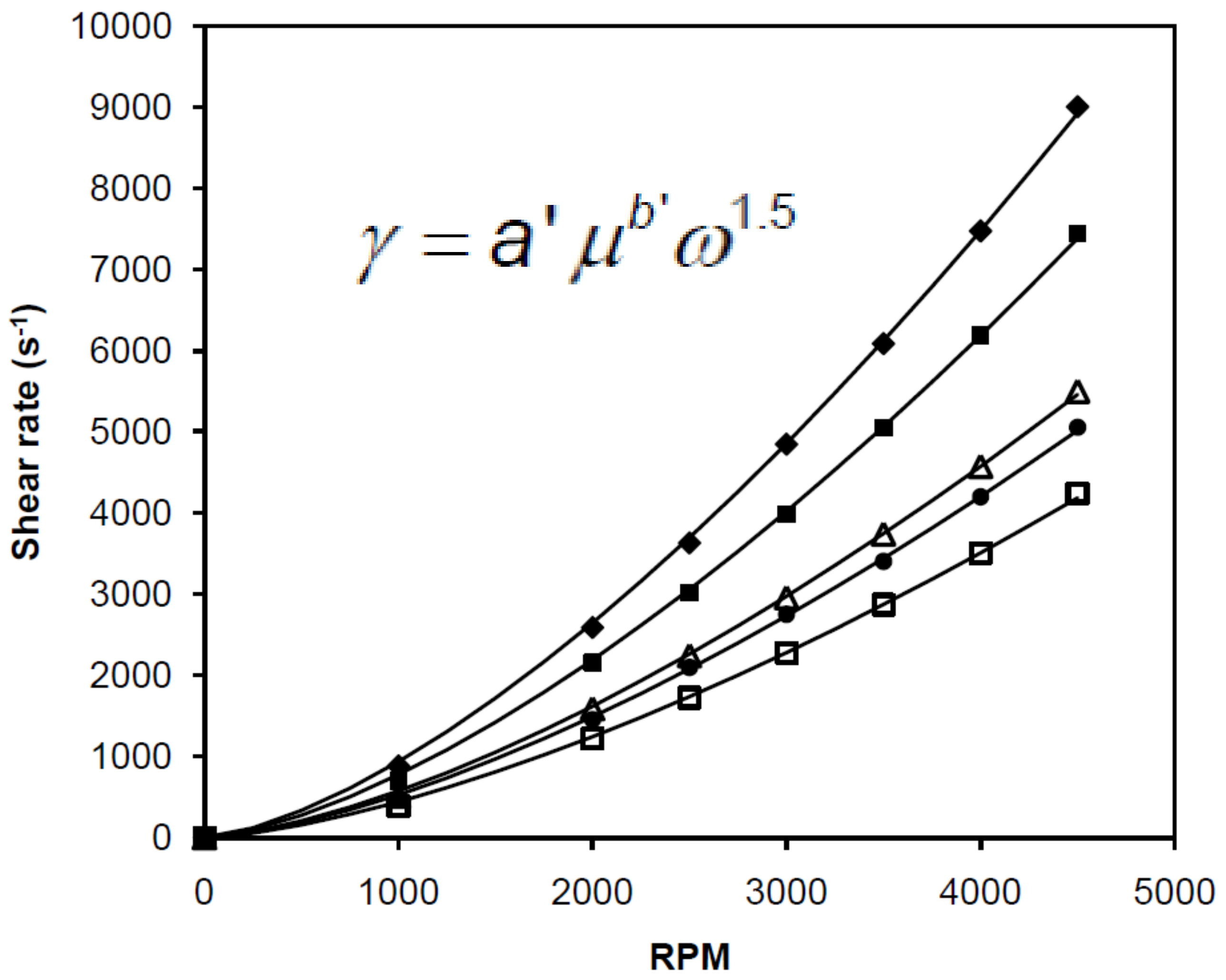
Diameter = 21 mm

Membrane area =3.64 cm^2

Dead-end Method



Shear Rate



$\gamma = a'\mu^{b'}\omega^{1.5}$
Shear rate (s^{-1})
RPM
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
0
1000
2000
3000
4000
5000

Material & Method

Feed material

- Fab expressed E coli; 450 L fermentation, harvested by a CSA-1 disc-stack centrifuge
- Periplasmic extraction buffer of 100 mM tris-base and 10 mM disodium EDTA dissolved in deionized water, pH of 7.4, T of 40 ^{o}C used to lyse the cells, then heat at 60 ^{o}C for 3 hours
- Cell concentration is 47 g DCW/L

Diafiltration buffer

- 90 mM sodium chloride

Material & Method

Small pilot plant experiment

- a Proflux® M12 rig
- a 1,000 kDa polyethersulfone membrane, 0.1 m^2
- BIOMAX cassette, turbulence screens (V-screen)

USD experiment

- USD device
- a 1,000 kDa polyethersulfone membrane, 3.46 cm^2

Material & Method

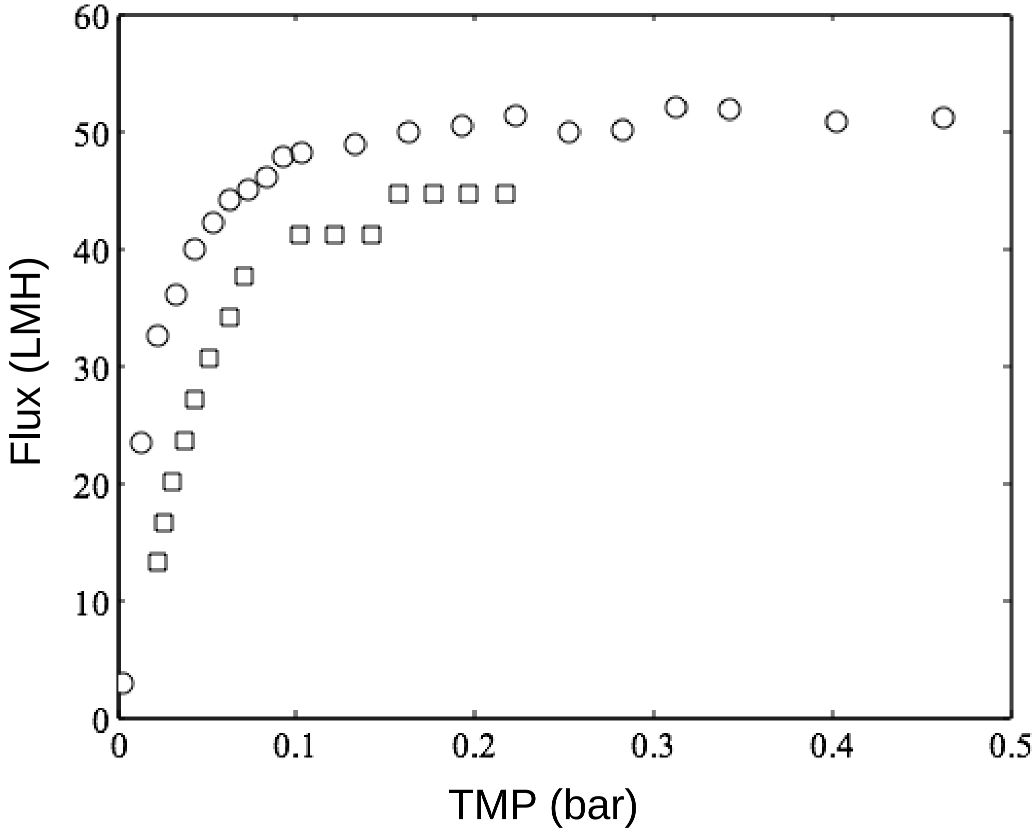
Experimental method

•Constant flux operation for both USD and small pilot scale experiments

Process aim

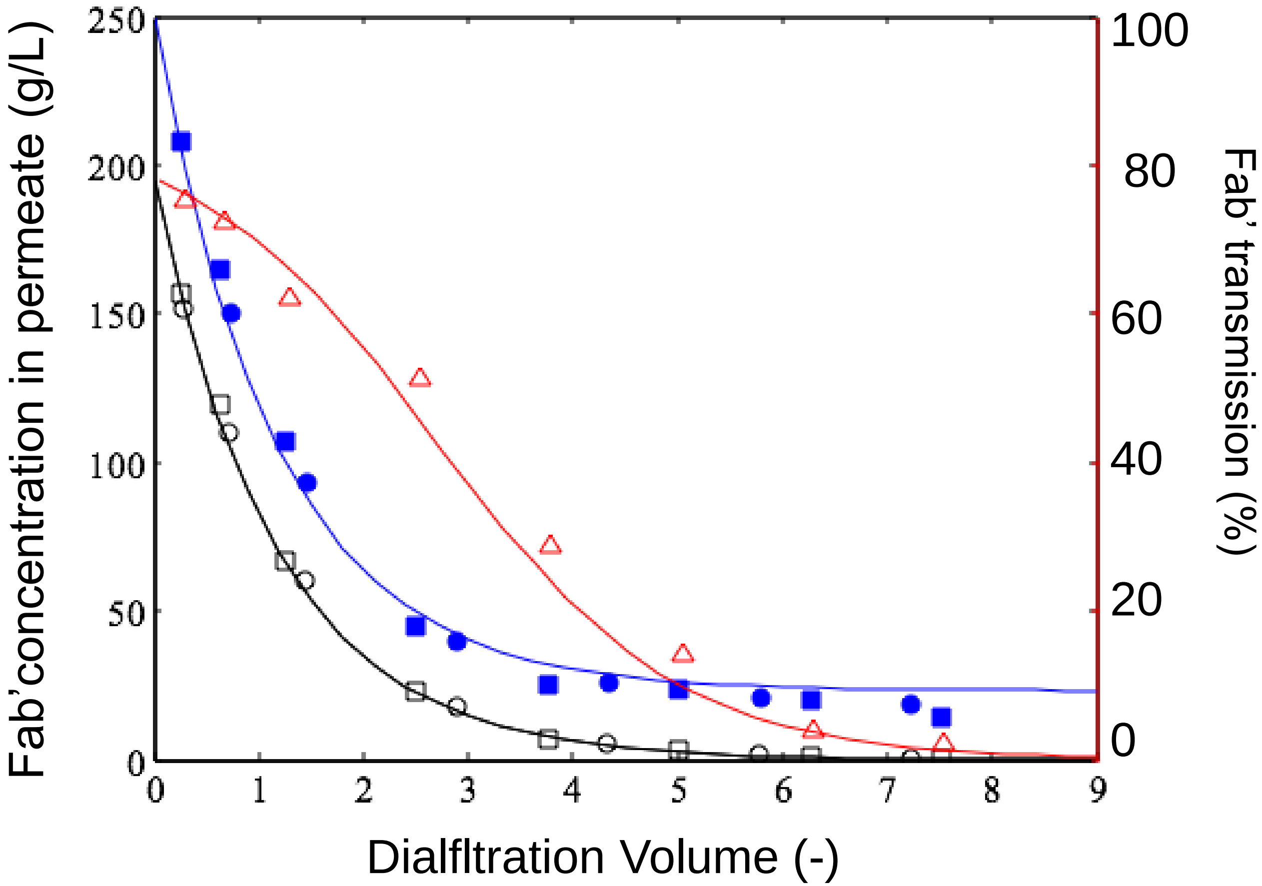
•Recover Fab from lysate
•Efficient flux and TMP

Large Scale Verification



(□) Large scale experiment and (○) USD experiment

Large Scale Verification



Fab’concentration in permeate □ labscale; ○ USD.
Fab’ concentration in retentate (■) labscale; (●) USD.
Fab’ transmission (red line) and (Δ) experimental results.

Observation

- Different system configuration of USD and large scale such as screened flow paths in large scale equipment
- System resistance - includes the membrane resistance, the cassette resistance and the resistance of the tubing in the 3 measurement points of pressures
- $J = \frac{TMP}{\mu R}$, TMP=0 when J=0. However TMP is not equal to zero at large scale.

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Modelling TMP

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$$\mathrm{TMP} = \frac{P_i + P_o}{2} - P_p$$

$$\mathrm{TMP} = \Delta P_A - \frac{\Delta P_C}{2}$$

$\Delta P_A = P_i - P_p$, Applied pressure drop

$\Delta P_C = P_i - P_o$, Channel pressure drop

Modelling TMP When Flux is Zero

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Water

$$\text{TMP} = GQ^m$$

$$G = C_{Aw} - \frac{C_w}{2}$$

Lysate

$$\text{TMP} = G'Q^m$$

$$G' = C_{Aw}\frac{\mu_m}{\mu_w} - \frac{C_w}{2}\left(\frac{\mu_m}{\mu_w}\right)^n$$

Modelling System Resistance

$$R_S = \frac{\text{TMP}}{\mu J}$$

When G = 0

$$R_S = R_A - \frac{R_C}{2}$$

When G $\neq$ *0*

Water test at large scale

$$\Delta P_A = C_A(Q - JA)^m + \mu R_A J$$

$$\Delta P_C = C(Q - JA)^m + \mu R_C J$$

$$\text{TMP} = \Delta P_A - \frac{\Delta P_C}{2} = G(Q - JA)^m + \mu R_S J$$

Modelling System Resistance

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Water

Lysate

$$R_S = R_A - \frac{R_C}{2}$$

$$R_A = R_P + R_M$$

R_F: membrane resistance

R_P: permeate system resistance

$$\Delta P_A = C_A(Q - JA)^m + \mu R_A J \longrightarrow \Delta P_{Am} = C_A \frac{\mu_m}{\mu_w}(Q - JA)^m + (\mu_m R_F + \mu_P R_P)J$$

$$\Delta P_C = C(Q - JA)^m + \mu R_C J \longrightarrow \Delta P_{Cm} = C_w \left(\frac{\mu_m}{\mu_w}\right)^n (Q - JA)^m + \mu_P R_C J$$

$$MP = \Delta P_A - \frac{\Delta P_C}{2} = G(Q - JA)^m + \mu R_S J \longrightarrow TMP = G'(Q - JA)^m + \left(\mu_m R_F + \mu_P (R_S - R_M)\right)J$$

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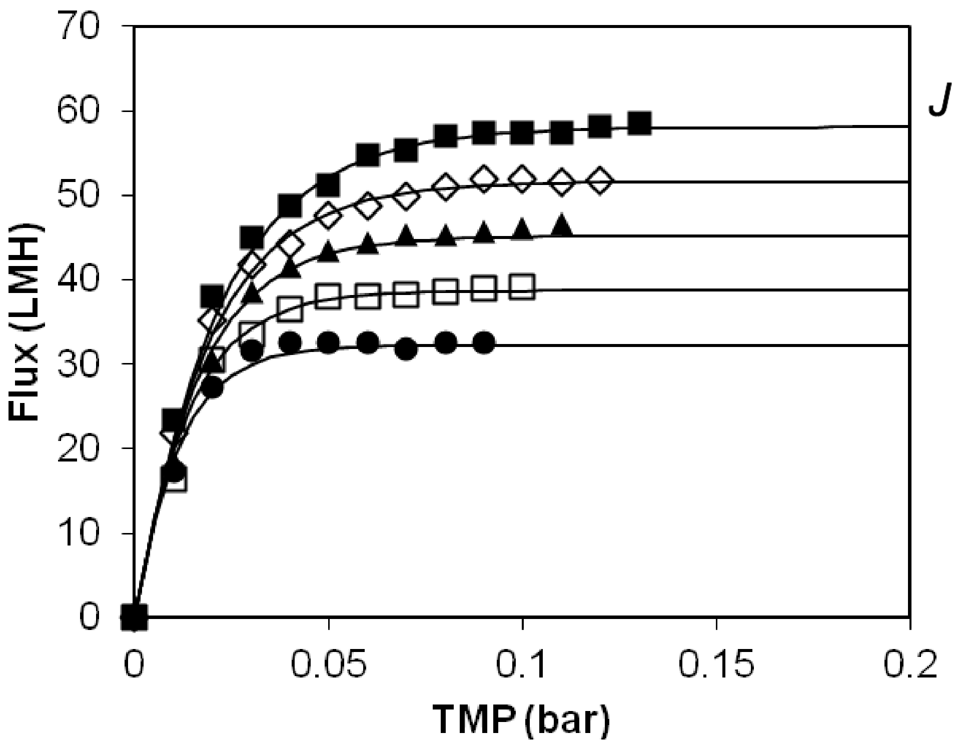
Predict Large Scale Flux and TMP Relationship

- USD modelling
- Water test at large scale when flux is zero to estimate flow parameters
- Water test at large scale to estimate system resistance
- Establish the new model between TMP and flux
- Use the model to predict flux and TMP relationship

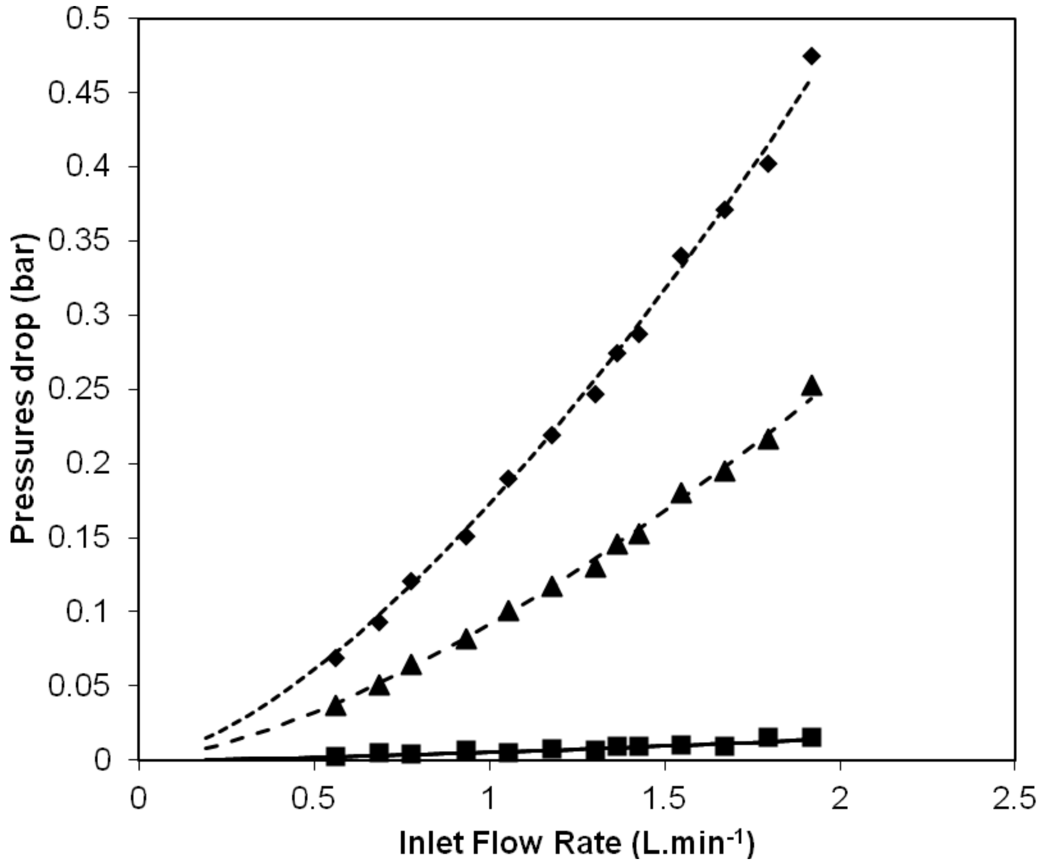
USD Modelling



$$J = (0.01\gamma + 15.4)(1 - \exp(2.256\times 10^{4}\gamma^{-0.74}\mathrm{TMP}))$$

Flux and TMP relationship determined with USD by using 90 mM NaCl solution at shear rates of 1,760 s-1 (●), 2,320 s-1 (□), 2,920 s-1 (▲), 3,570 s-1 (◊), and 4,260 s-1 (■). Solid line is the flux prediction by equation.

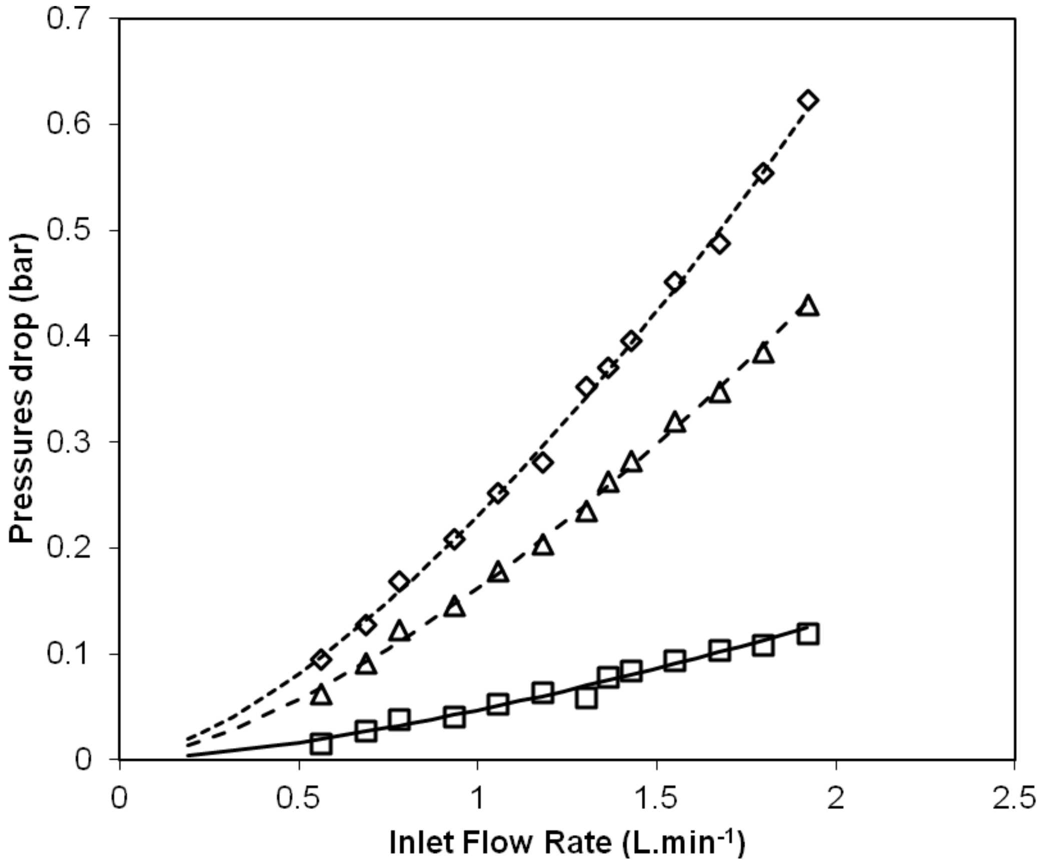
Water Test at Large Scale When Flux is Zero



$$TMP = GQ^m = 0.006Q^{1.5}$$

The effect of inlet flow rate during water test on channel pressure drop (♦), applied pressure drop (▲), and TMP (■).

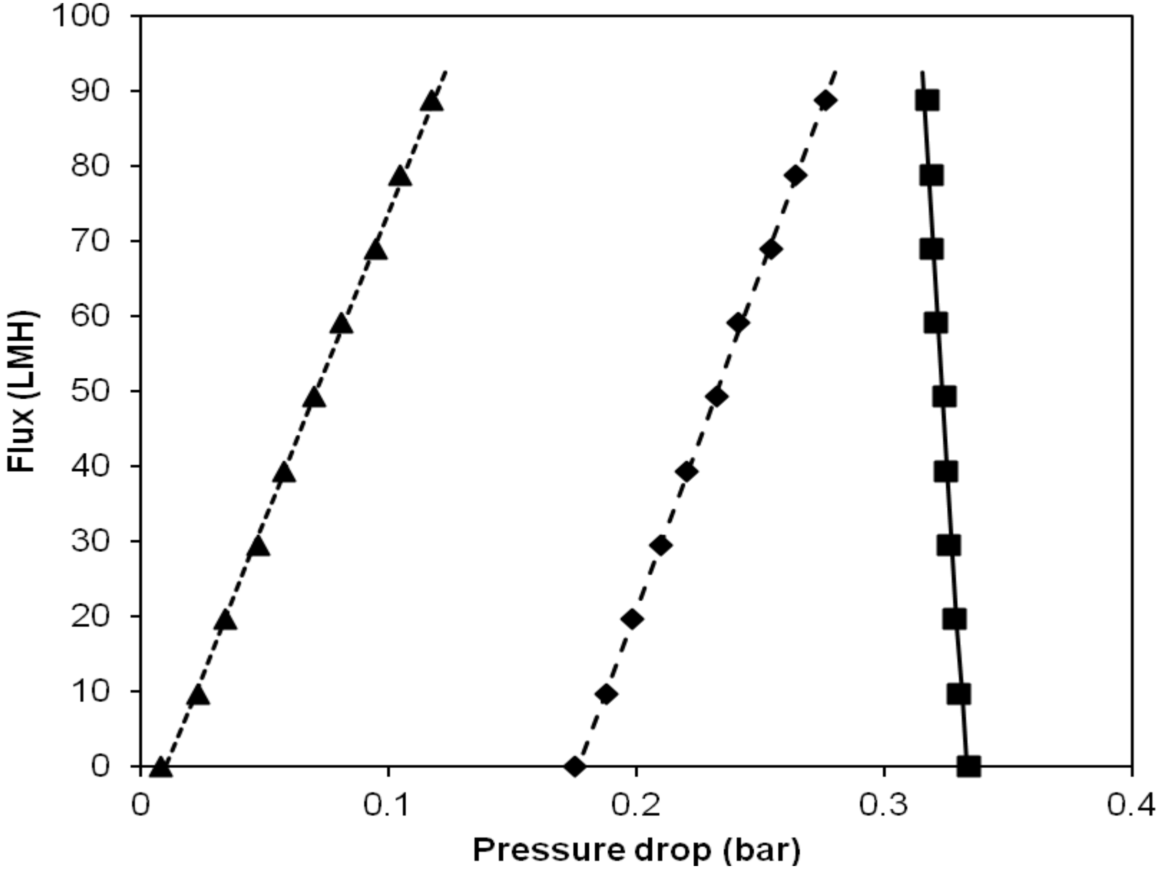
Lysate Test at Large Scale When Flux is Zero



$$TMP = G'Q^m = 0.049Q^{1.5}$$

The effect of inlet flow rate on pressure drops for *E. coli* lysate test. The lysate test results were channel pressure drop (◊), applied pressure drop (Δ), and TMP (□). The viscosity of lysate was 1.78 × 10^{-3} Pa.s.

Water Test at Large Scale Flux and TMP Relationship



R_C =1.20×10^{11} m^{-1}.

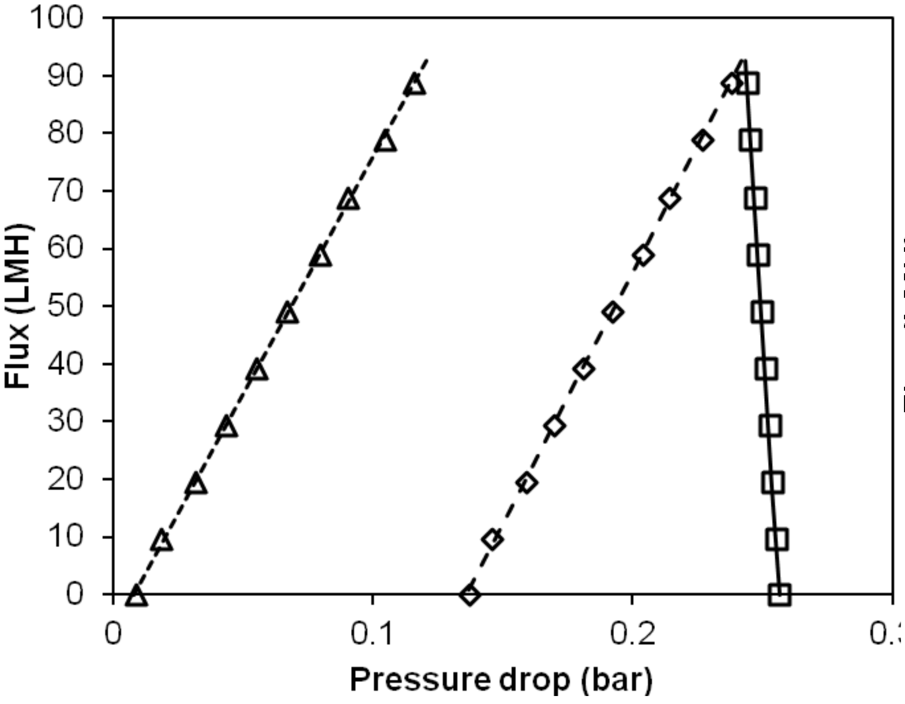
R_A = 5.03×10^{11} m^{-1}.

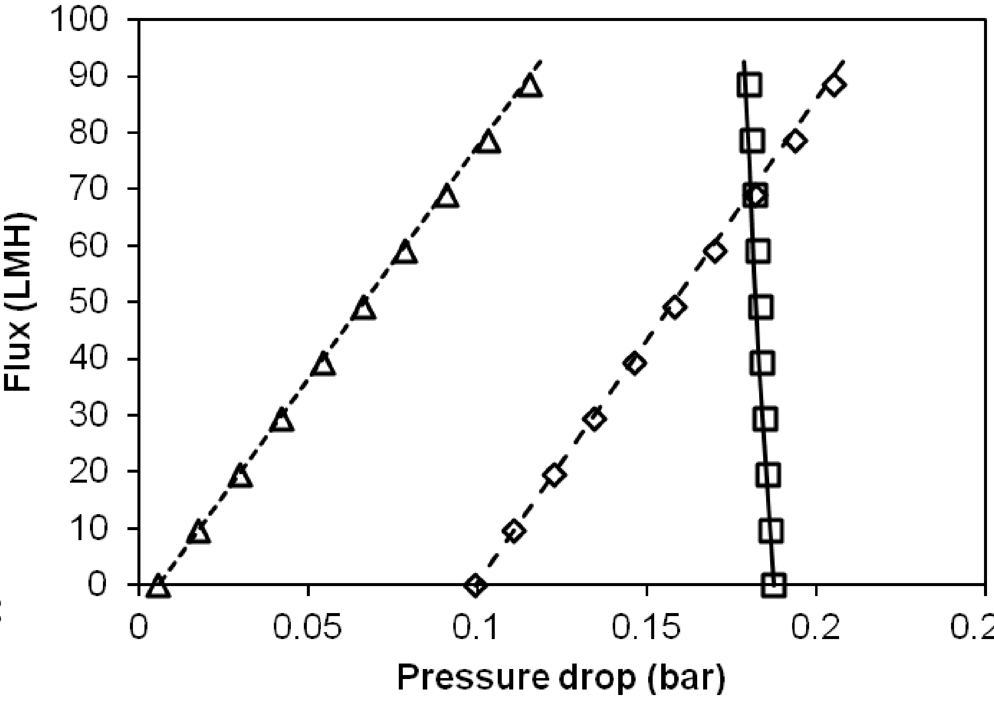
RS= 4.43×10^{11} m^{-1}.

The relationships between flux and pressure drops for water flux test at an inlet flow rate of 1.55 L.min^{-1}: channel pressure drop (■), applied pressure drop (♦), and TMP (▲).

Validation of Water Flux and TMP Relationship

$$TMP = \Delta P_A - \frac{\Delta P_C}{2} = G(Q - JA)^m + \mu R_S J$$

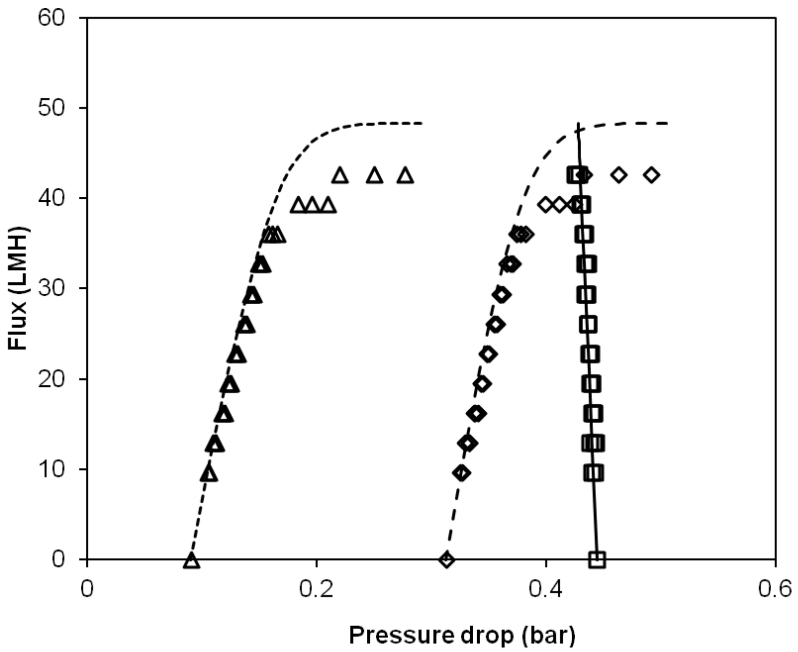




The relationships between flux and pressure drops for water flux test at an inlet flow rate of 1.30 $L.min^{-1}$ (A) and at an inlet flow rate of 1.06 $L.min^{-1}$ (B).

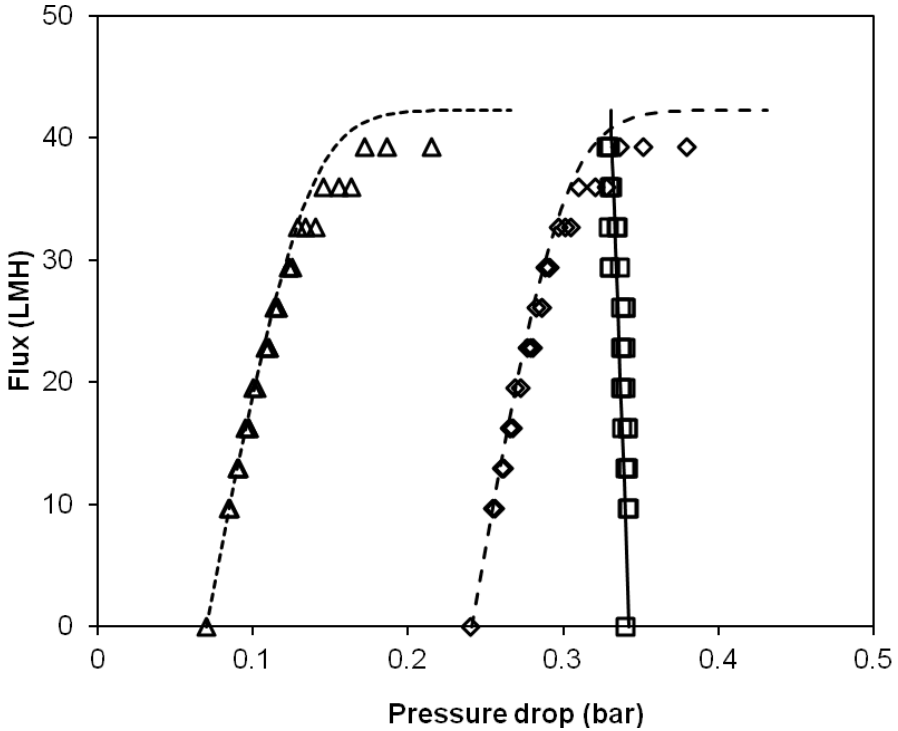
Predict large Scale Flux and TMP Relationship for Lysate

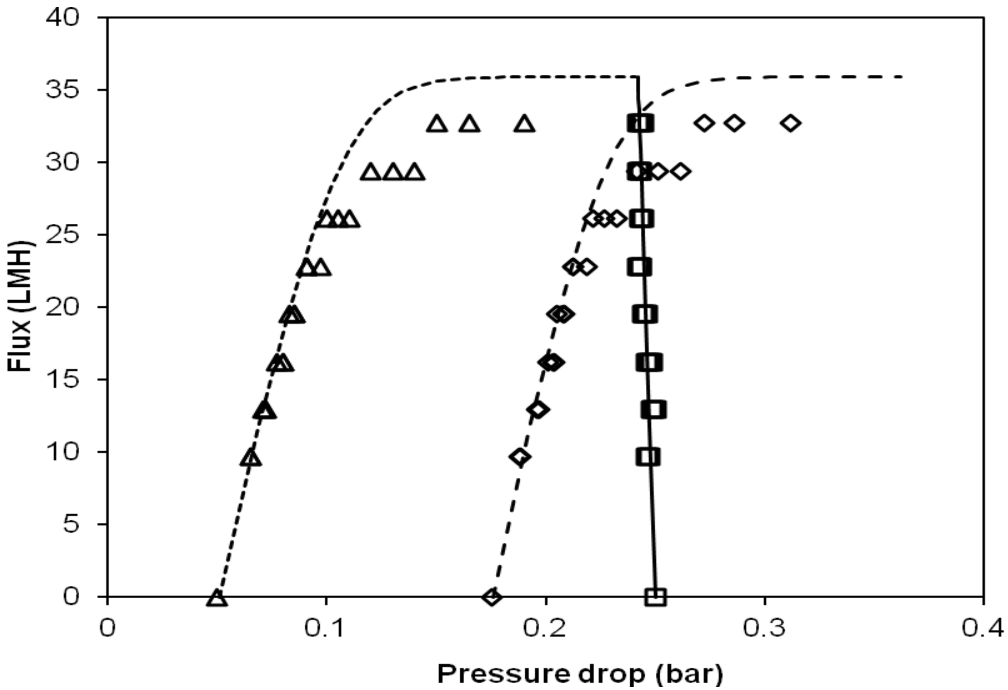
$$TMP = 0.049(Q - 1.67 \times 10^{-3}J)^{1.5} + \frac{Ln\left(1 - \frac{J}{\alpha}\right)}{-\beta J} + 1.23 \times 10^{-3}J$$



The relationships between flux and pressure drops of critical flux determination for *E. coli* lysate at inlet flow rates of 1.55 L.min^{-1} : channel pressure drop (□), applied pressure drop (◊), and TMP (Δ). The viscosity of lysate was 1.78 × 10^{-3} Pa.s and the viscosity of permeate was 1.09×10^{-3} Pa.s.

Predict large Scale Flux and TMP Relationship for Lysate





Inlet flow rates of 1.30 $L.min^{-1}$

Inlet flow rates of 1.06 $L.min^{-1}$

Conclusions

- USD device operated via dead-end mode allows to predict the membrane resistance impacted by fouling
- Water test can be used to calibrate the initial TMP and system resistance
- The combination of USD experiments and water tests establishes the scaling rule
- The benefit of USD membrane device can be realised fully
- USD tool enables the acceleration of bioprocess development, early and at low cost