Fouling Phenomena in Alternating Tangential Flow Filtration

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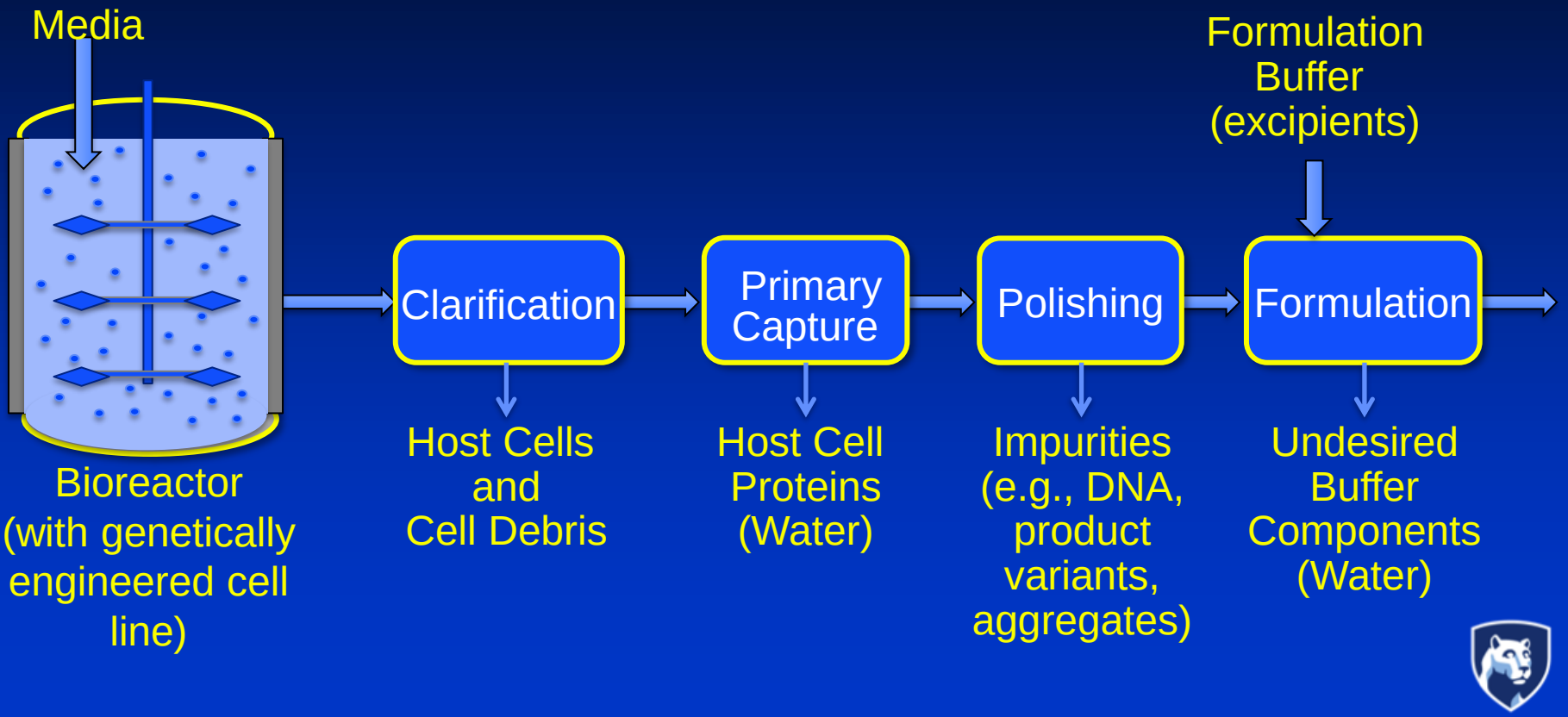
Antibody (mAb) Production



Antibody (mAb) Production

- Batch operations – each step performed sequentially
- Large stainless steel tanks (20,000 L bioreactors)
- Requires ≈100 days per batch (start-to-finish)

Robert Bradway – CEO Amgen

"Today's systems for producing drugs in bacterial or animal cells and then isolating them are hugely expensive and can take months. With more efficient processes in place, companies could swiftly increase production of drugs in high demand, and they could produce medicines for rare diseases more cost-effectively as well."

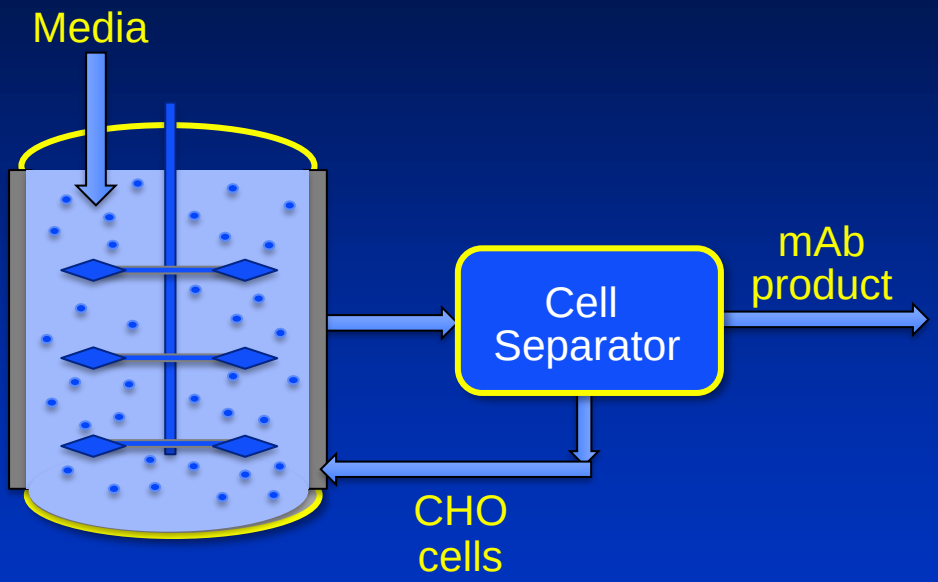
MIT Technology Review (2013)

Antibody (mAb) Production

- Batch operations – each step performed sequentially
- Large stainless-steel tanks (20,000 L bioreactors)
- Requires ≈100 days per batch (start-to-finish)

- Continuous and / or intensified processing could significantly improve productivity while dramatically reducing capital costs and increasing manufacturing flexibility

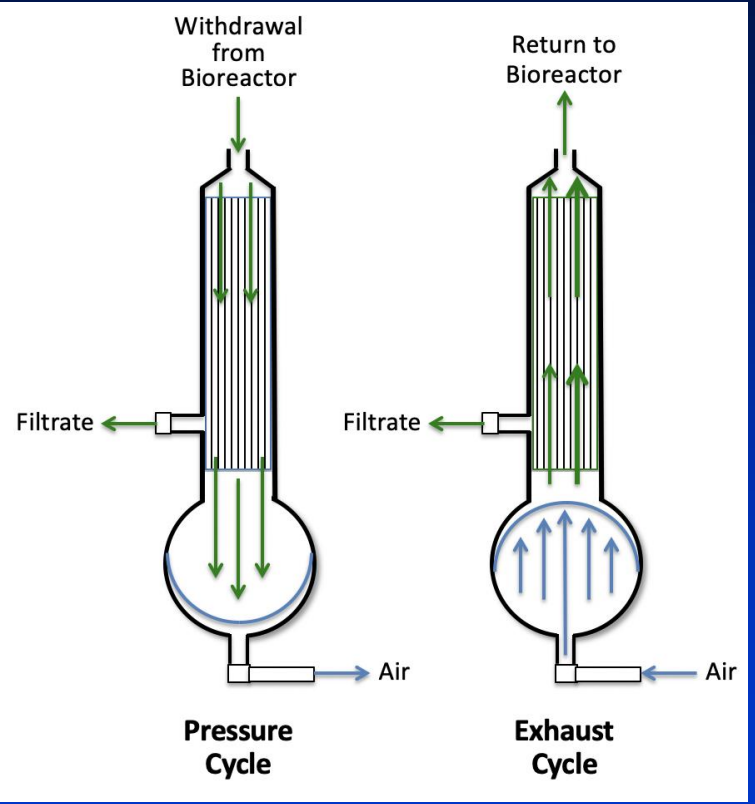
Perfusion Bioreactor



- Cells recycled to bioreactor with continuous product recovery
  - Dramatically reduces residence time in bioreactor → improved product quality
- Cell separator:
  - **Microfiltration membranes**
  - Spin filter (within bioreactor)
  - Continuous centrifuge
  - Acoustic wave system
  - Spiral flow fluidic separator
  - Inclined settler

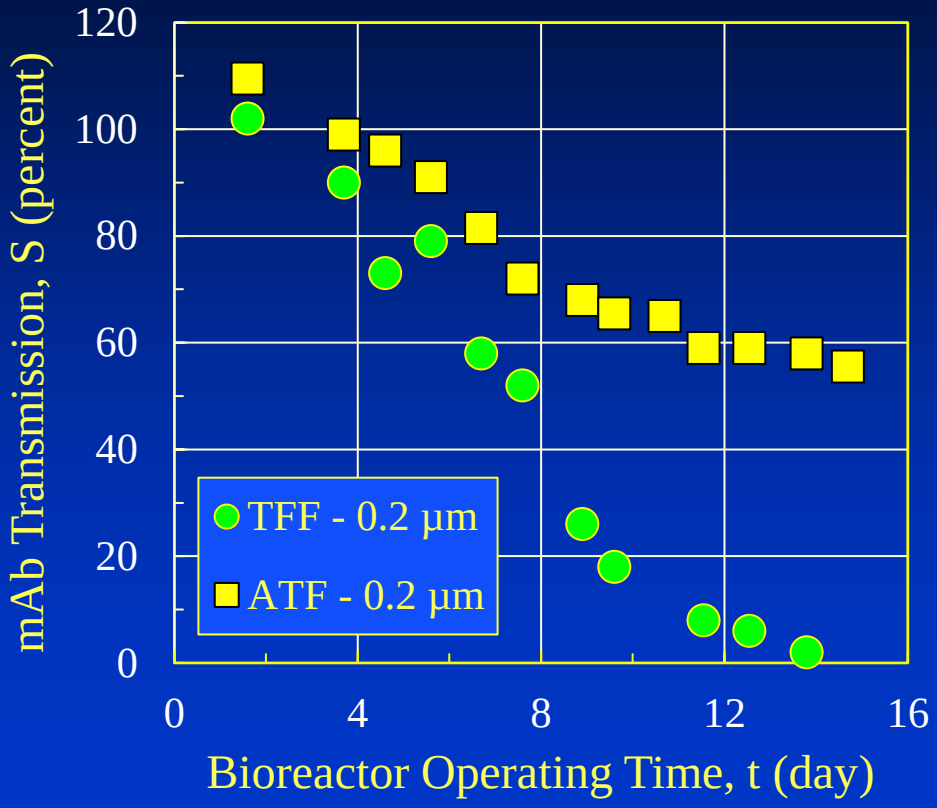
Alternating Tangential Filtration - ATF

- Hollow fiber membrane module operated with alternating feed flow direction
- Alternating flow direction helps unclog fibers
- Low-shear diaphragm pump minimizes cell lysis
- Operation at low flux to reduce membrane fouling
- Flow withdrawn and returned through only single port in bioreactor



ATF vs TFF - Fouling

- Data for mAb transmission during operation of a CHO cell perfusion bioreactor
  - Decline in mAb transmission due to fouling limits operating time
  - ATF outperforms conventional TFF using exact same hollow fiber membrane, but fouling is still problematic

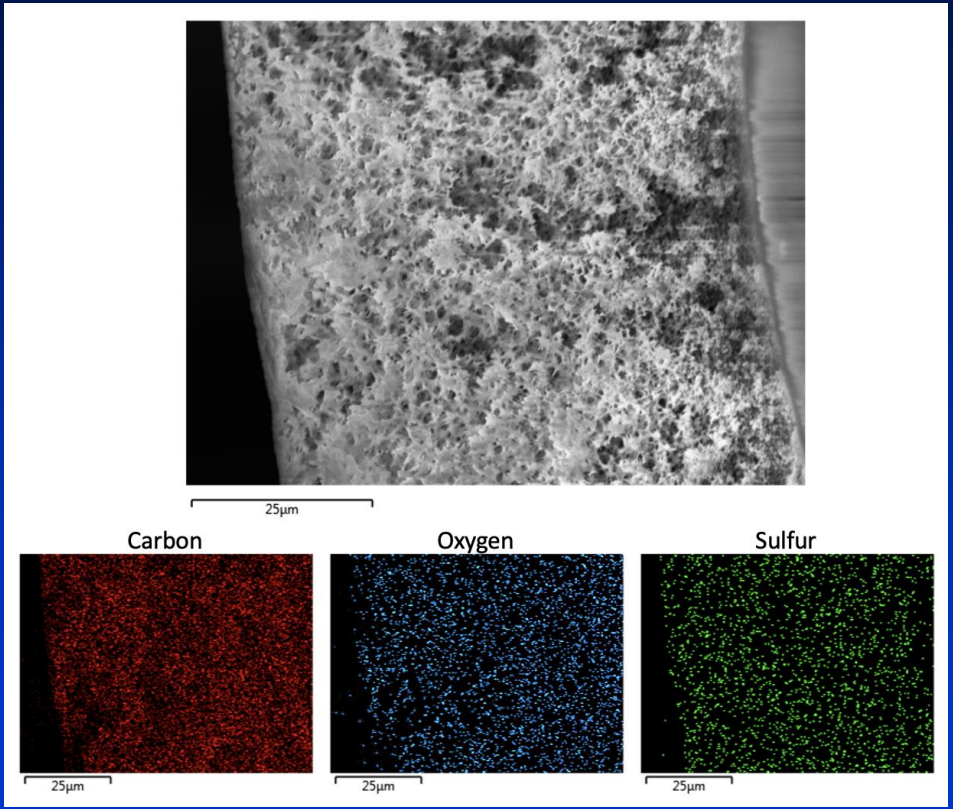


Wang et al. J Biotechnology, 246, 52 (2017)

Key Questions

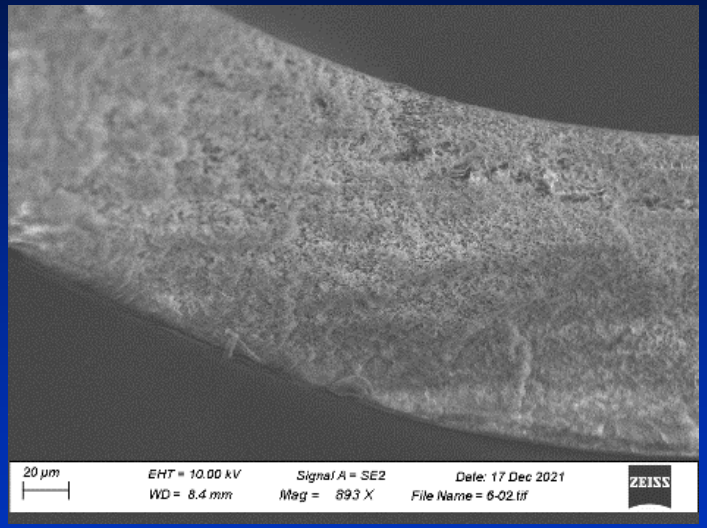
- Where is fouling occurring within the ATF module?
  - Use Scanning Electron Microscopy (SEM) + Energy Dispersive Spectroscopy (EDS) to evaluate location of fouling in autopsied hollow fiber modules after perfusion operation
- What is the nature of the key foulants in perfusion systems?
  - Use SEM + EDS to distinguish foulant classes
  - Use proteomics to evaluate specific proteins eluted from previously fouled membranes

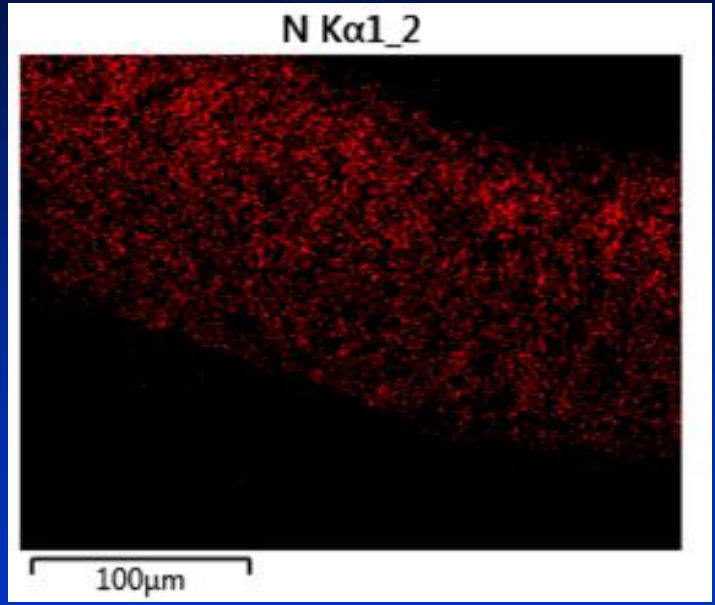
SEM-EDS Analysis of Membranes



- Energy dispersive spectroscopy
  - SEM-EDS identifies atomic composition at micron scale
- Atomic composition
  - Clean PES membrane contains only Carbon, Oxygen, and Sulfur (uniform through fiber)
  - Potential foulants contain Nitrogen (protein + DNA), Phosphate (DNA), Silicon (antifoam)

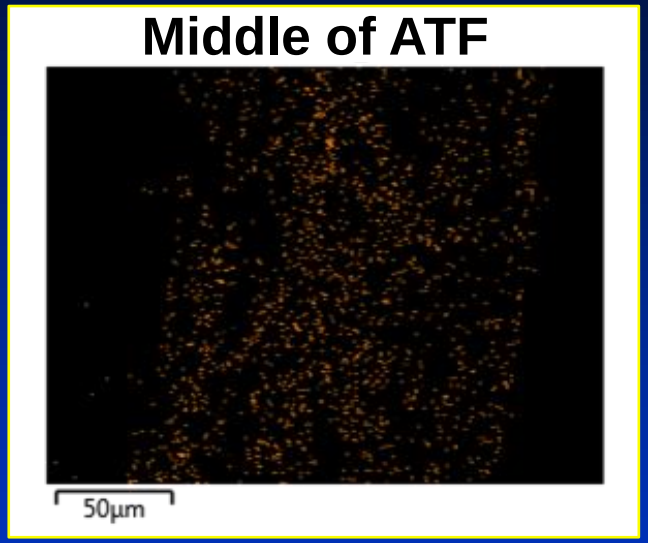
Fouling – Filter Cross-Section

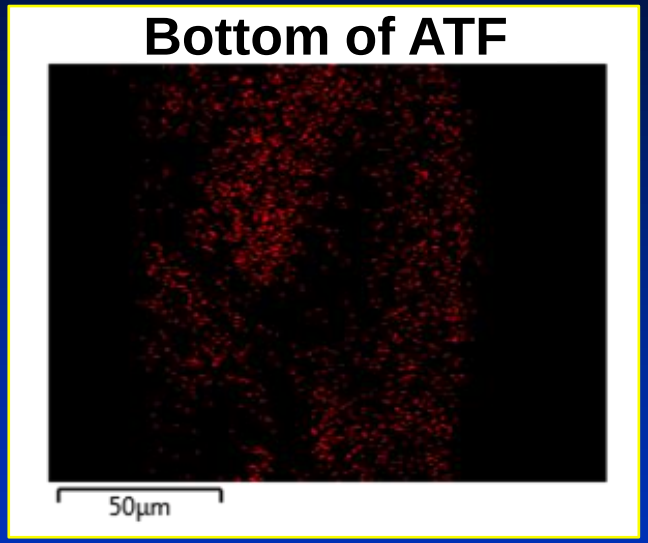




- Nitrogen signal clearly visible (particularly near fiber lumen)
  - Consistent with both protein / DNA fouling
  - No evidence of Phosphate → protein fouling dominant

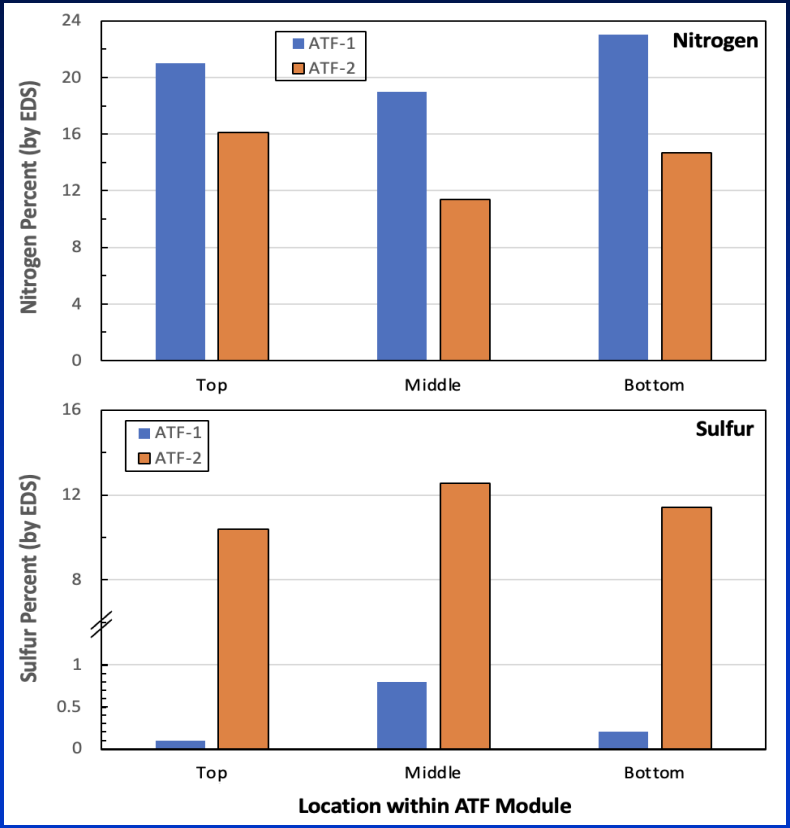
Fouling – Axial Variation





- Nitrogen signal stronger in section from bottom of ATF
  - Suggests axial variation in fouling
  - High nitrogen signal also obtained at top of ATF

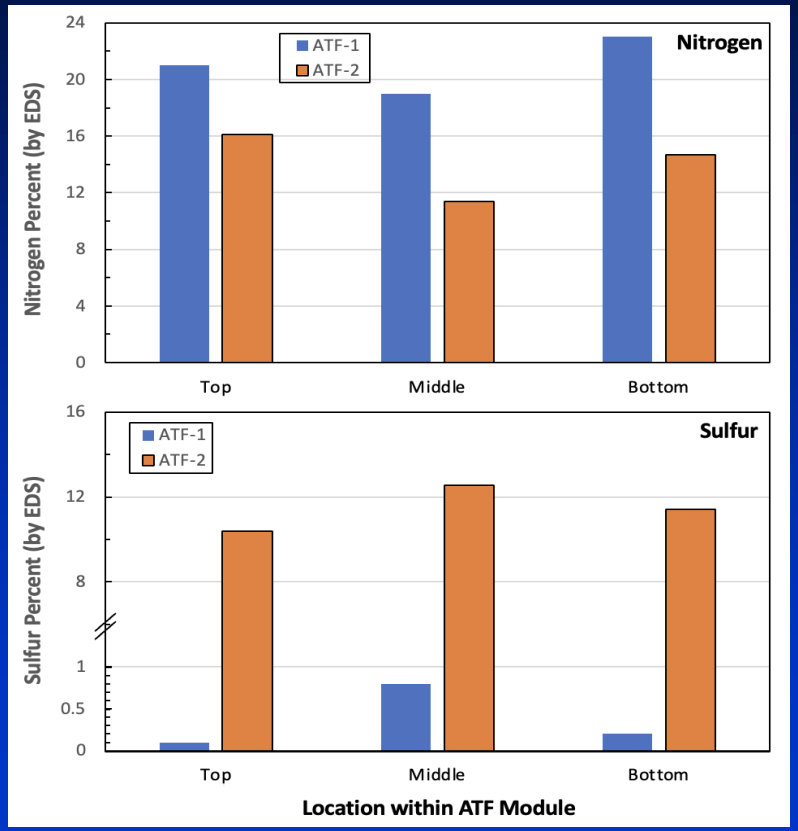
Spatial Variation in Fouling

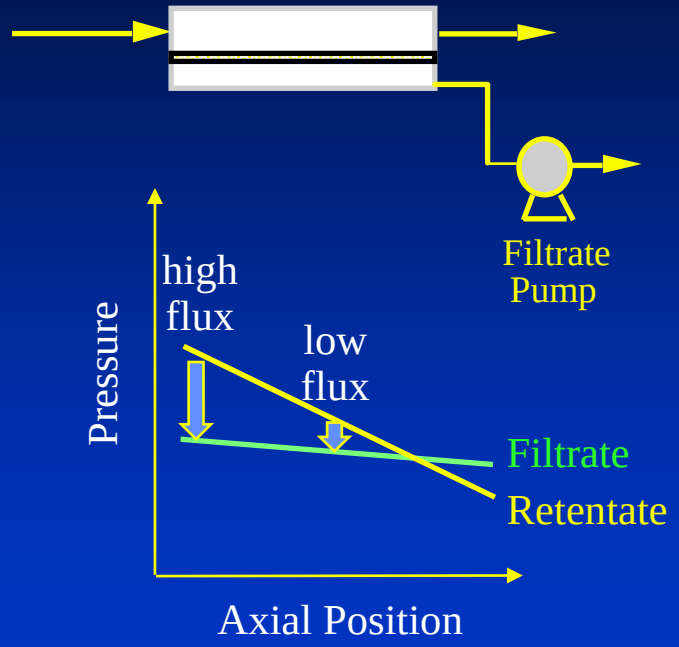


- ATF modules used for CHO cell processing in perfusion bioreactors
  - Two different industrial sites
- Individual fibers analyzed by SEM-EDS
  - Nitrogen peak is greatest at top and bottom of fiber (inlet regions during positive filtration)
  - Sulfur peak from PES greatest at middle of fiber

Sundar et al., Biotech Prog, 39: e3336 (2023)

Axial Variation





High filtrate flux → high degree of fouling

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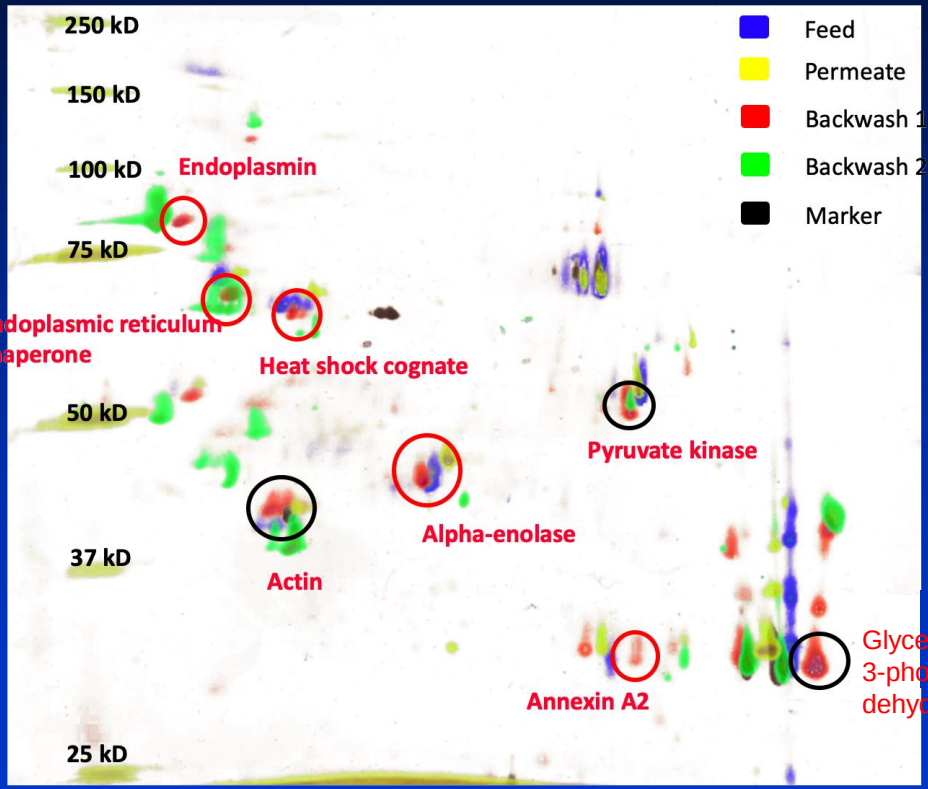
Proteomic Analysis of Fouling in ATF



- HCP extracted from fouled membranes at pH 10, high salt
  - Conditions identified to provide nearly complete extraction of foulants
- Individual HCP identified by 2D-gels
  - Most HCP associated with protein coacervates, e.g., extracellular vesicles, exosomes, or G bodies

Summary

- SEM + EDS can identify nature and location of foulants
  - Fouling dominated by proteins based on N signal
  - Fouling occurs throughout depth of hollow fiber
  - Fouling greatest at top / bottom of ATF → regions of highest flux
- Fouling mechanism
  - Key foulants are proteins associated with extracellular vesicles
  - Results suggest that larger vesicles deposit on / within membrane pores in regions where filtrate flux is highest
  - Potential opportunities to improve performance by controlling spatial variation in filtrate flux and / or vesicle formation

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