# Perfusion!
# Jeopardy or the ultimate advantage?

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# Objective

Objective:  Provide an introduction to perfusion as mode of manufacturing for biotherapeutics

# Outline

- Introduction and background
- Modes of operations:  perfusion vs. fed-batch
- Detailed description of perfusion operations
- Advantages and challenges of perfusion

# Background on CMC Biologics

A contract manufacturing organization that utilizes upon both fed-batch and perfusion

# Manufacturing capabilities

## Seattle

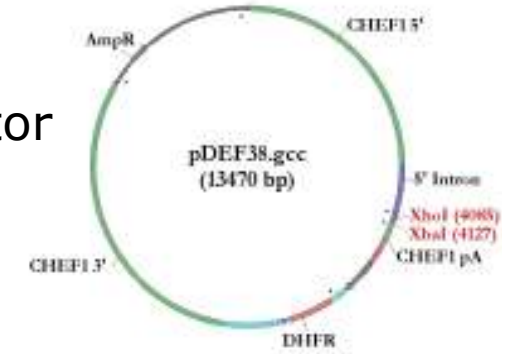
- Clinical and commercial capabilities ranging from 250L to 2500L for fed-batch mammalian processes
- Perfusion capability up to 2000L/day
- Pilot plant capability up to 100L mammalian and microbial processes

## Copenhagen

- Clinical and commercial capabilities ranging from 100L to 750L for fed-batch mammalian processes
- Clinical and commercial capabilities ranging from 400L to 1500L for microbial processes
- Perfusion capability up to 1000L/day
- Pilot plant capability up to 100L mammalian and microbial processes

# CMC's Capabilities

- Cell Line Development
  - Cell line creation using the CHEF1® expression vector
  - Final clone in <14 weeks
  - Stable cell lines without using amplification
  - Titers > 1 g/L




- Process Development
  - Bench scale fed-batch and perfusion capabilities applied to mAbs and complex glycoproteins
  - Experienced staff performing fed-batch and perfusion since 1992

- Analytical
  - Sophisticated analytical tools to examine product quality

# Perfusion –historical perspective

Perfusion– previously a choice of necessity

# History

- Industrial perfusion for the manufacture of proteins matured in the 1980s
  - Poor protein expression resulted in low titers
  - Cell culture media was not sophisticated
- In the 1990s industry moved away from perfusion
  - Media and expression systems improvements led to increase fed-batch productivity
  - Fed-batch was simple; perfusion had high failure rates

# Future

Prediction: Perfusion will make a comeback as the manufacturing method of choice

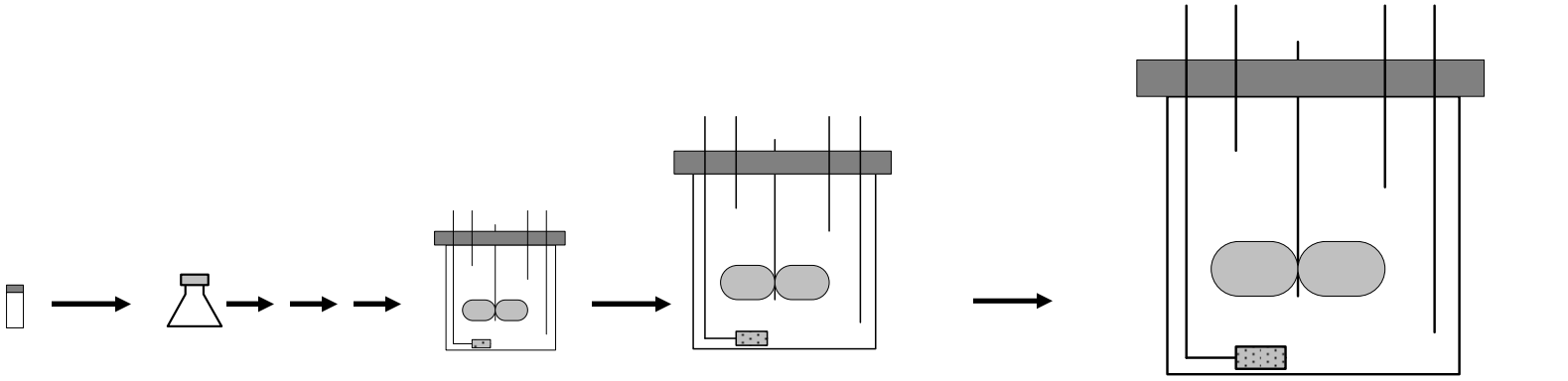
- Cell culture equipment is now more sophisticated
  - Improvements in sterile filtration
  - More sophisticated pumps with feed-back control
  - Improved technology and understanding of perfusion techniques
  - Media improvements
- Driver for perfusion: reducing cost of goods and capital investment
  - Increased competition as products go off patent leading to the rise of biosimilars
  - Pressure to reducing drug costs to patients
  - Continuous manufacturing is more capital equipment efficient

# Overview of the Primary Modes for Industrial Manufacturing of a Recombinant Protein

Fed-batch and perfusion bioreactors

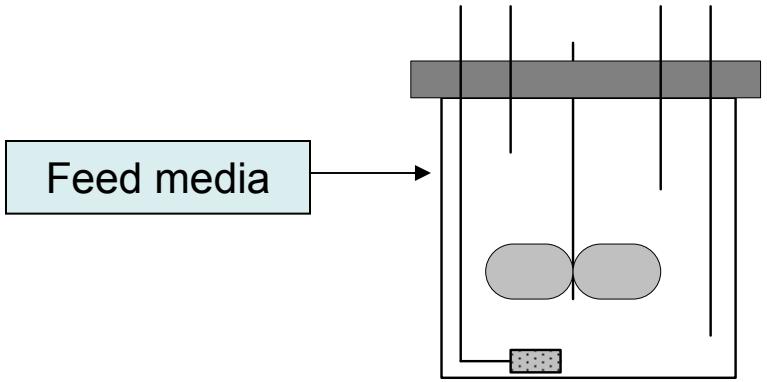
# Bioreactor Operating Modes

- Two basic modes of operation for an industrial cell culture manufacturing process
  - Fed-batch
  - Perfusion
- Both modes begin with thawing a vial and expanding cells in the seed train process
- The seed train is used to inoculate the final bioreactor



# Bioreactor Operating Modes – Fed Batch

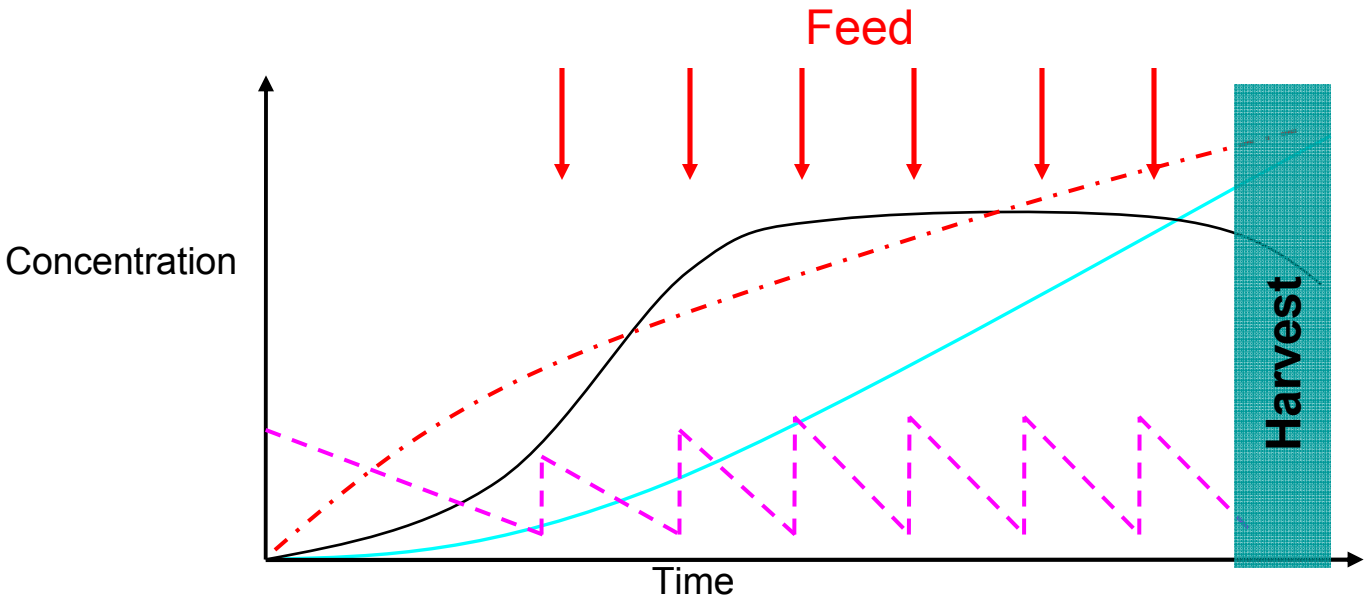
- Inoculated at ~75-90% of the final working volume
- Fed up to the final working volume during the course of the process
- Bioreactor is terminated and the culture harvested based upon a pre-determined criteria (e.g.  % of viable cells)
  - Duration may range from approximately 8 to 21 days
  - Typically harvest by centrifugation and/or depth filtration




production bioreactor

# Fed-batch profiles

## Fed-batch process profile




Cell density

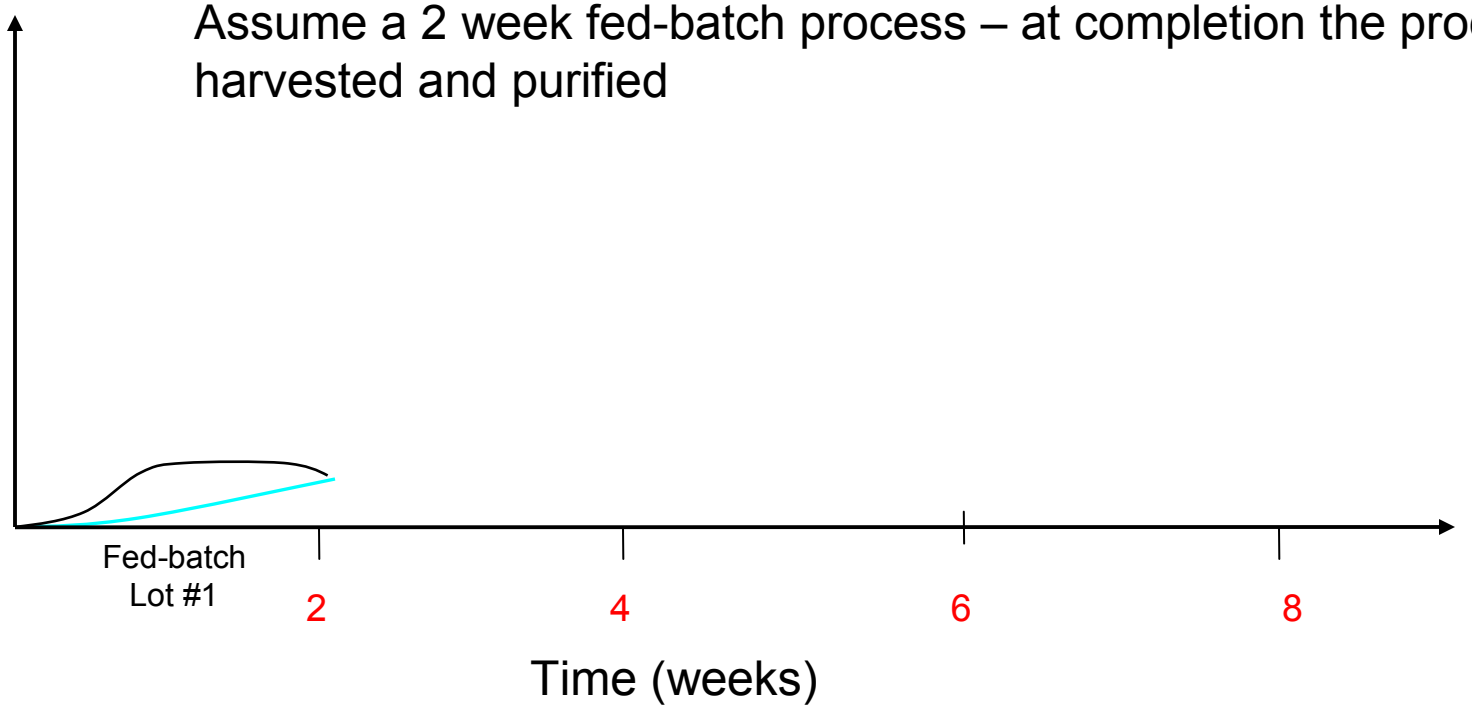
Product of interest

Waste products

Nutrients

# A manufacturing campaign

Assume a 2 week fed-batch process – at completion the product is harvested and purified

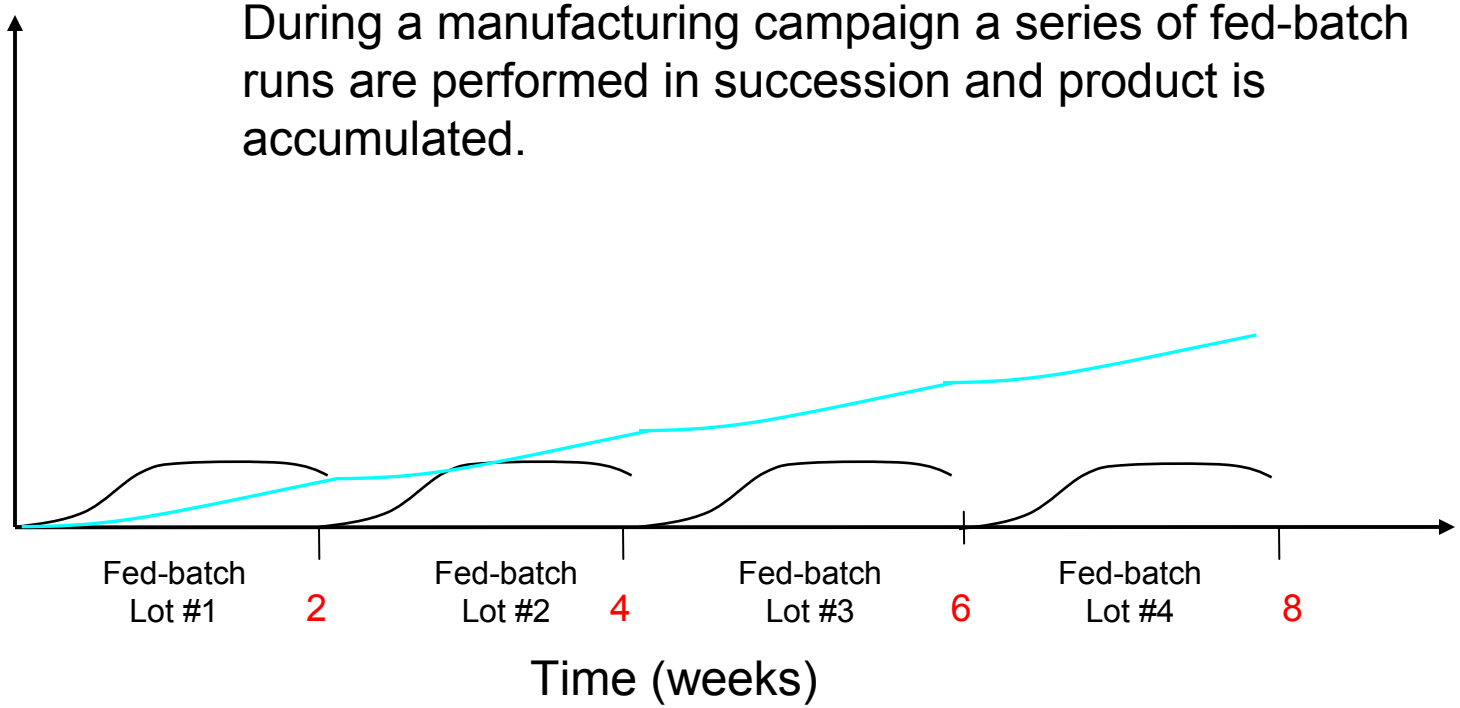



Cell density

Acumulated product of interest (fed-batch)

# A manufacturing campaign

During a manufacturing campaign a series of fed-batch runs are performed in succession and product is accumulated.

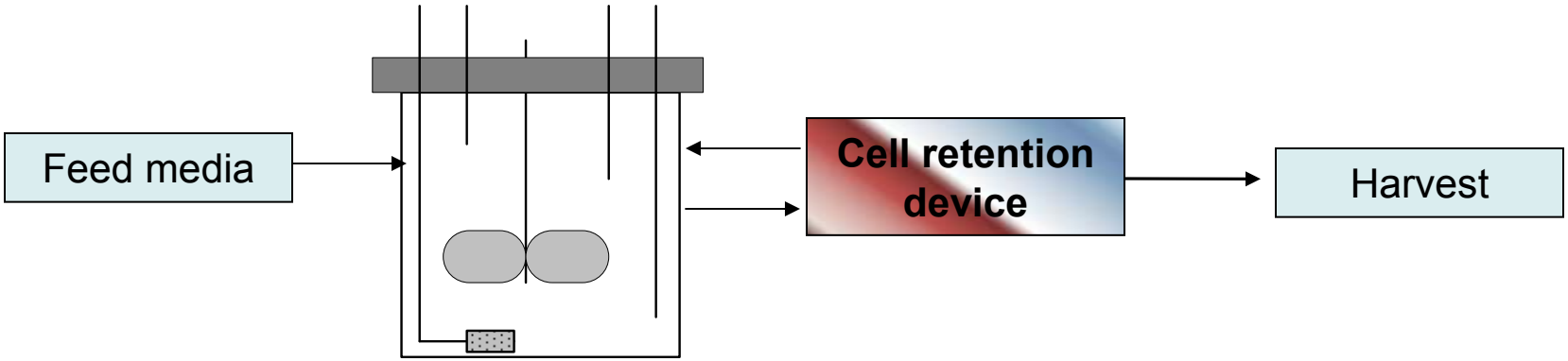



Cell density

Acumulated product of interest (fed-batch)

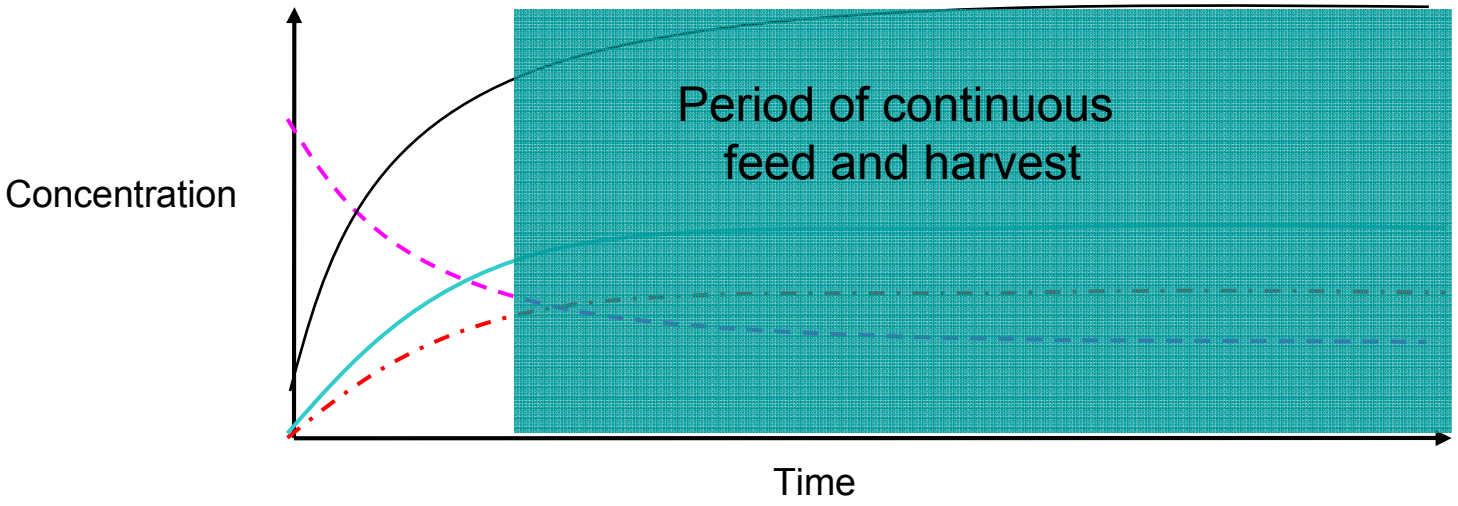
# Bioreactor Operating Modes – Perfusion

- After the reactor is inoculated perfusion is initiated
  - Fresh media is continuously fed into the bioreactor
  - Spent media (perfusate) containing the product of interest is continuously harvested through a cell retention device
  - The cell retention device retains cells within the bioreactor.
  - The operating volume is constant by feeding and harvesting media at the same rate
- The bioreactor run duration typically will range from 21 to 60+ days

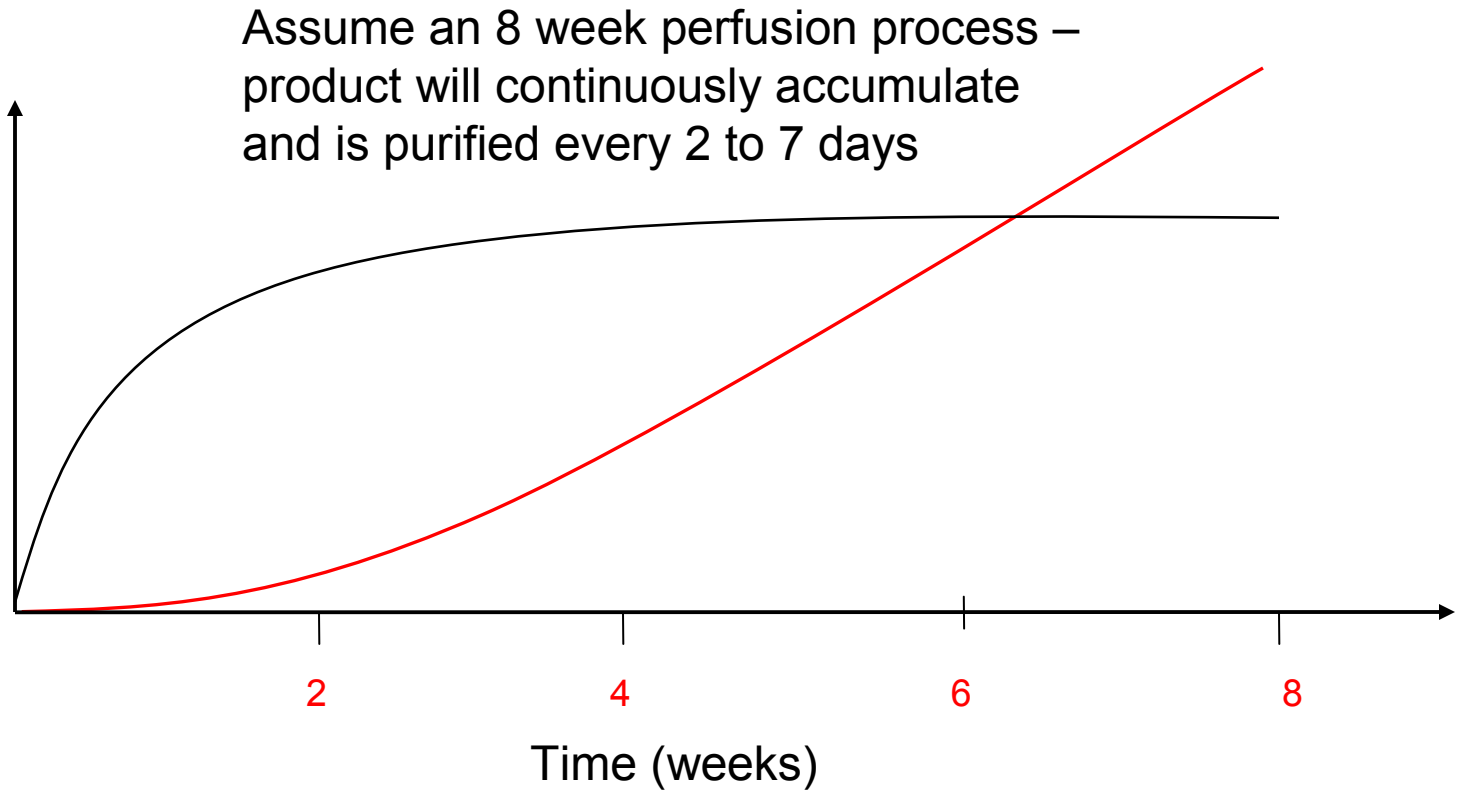


# Perfusion profiles

## Perfusion process parameter profile

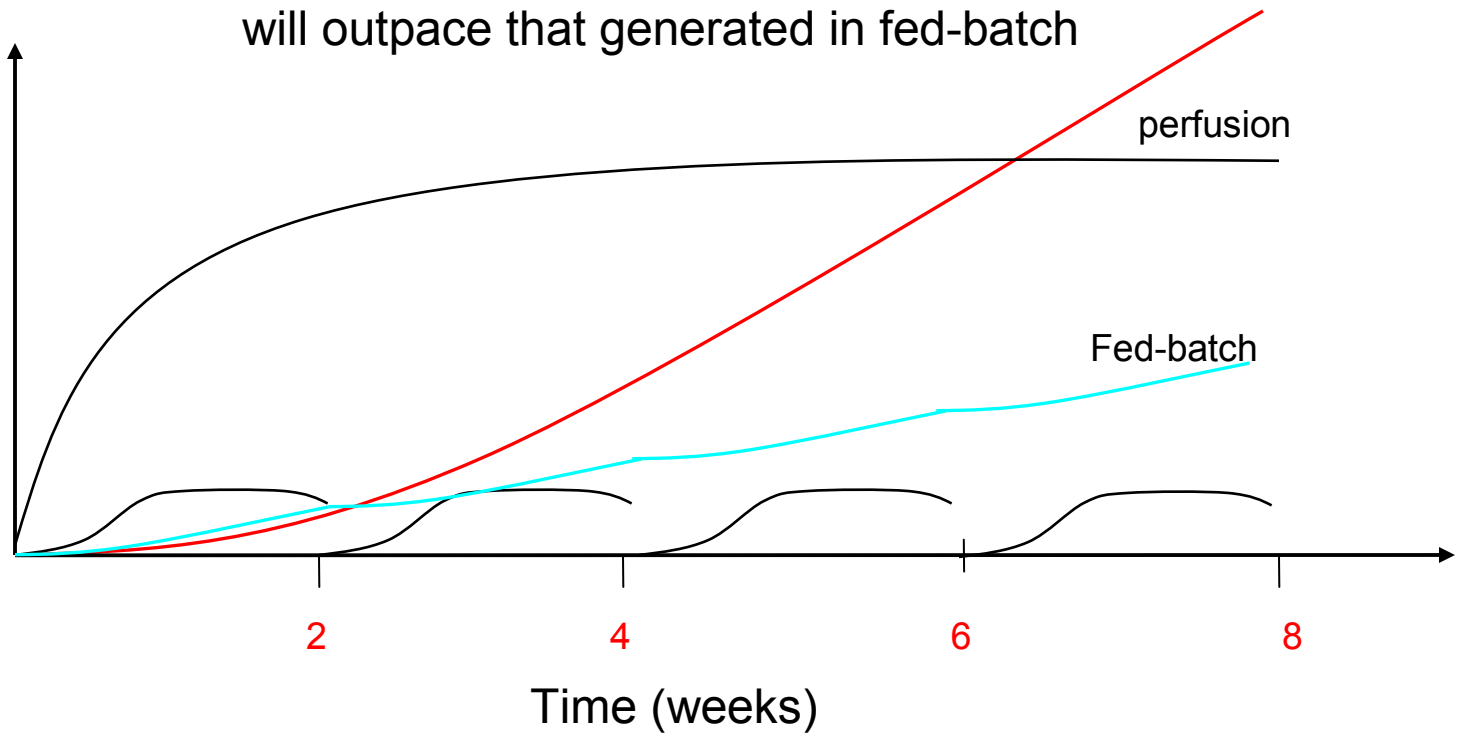


# A manufacturing campaign



# A manufacturing campaign

With time, the cumulative product generated in perfusion will outpace that generated in fed-batch




Cell density

Accumulated product of interest (fed-batch)

Accumulated product of interest (perfusion)

# Perfusion – Advantages

Perfusion – now a choice of efficiency and not just necessity

# Comparison of operating modes

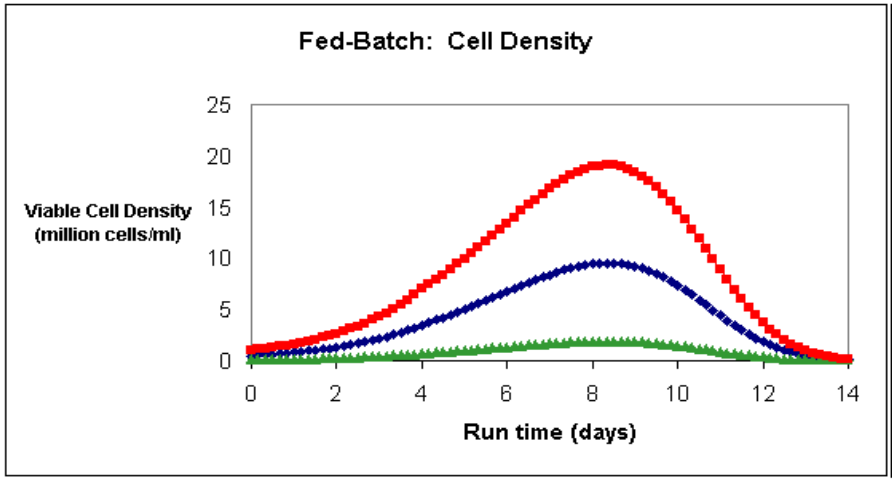
| | Fed batch | Perfusion |
|---|---|---|
| Scale (L) up to wv | 20,000 | 1000L (or larger) |
| Culture time (days) | 10-21 | 21-60+ |
| Cell density ($10^6$/mL) | 5-25 | 30-100 |
| Accumulation of waste-products | Yes | No |
| Product concentration | 0.5 - 5 g/L | ~20% of fed-batch |
| Product residence time | Up to entire culture duration | <36 hours |
| Product stability issues | High | Low |

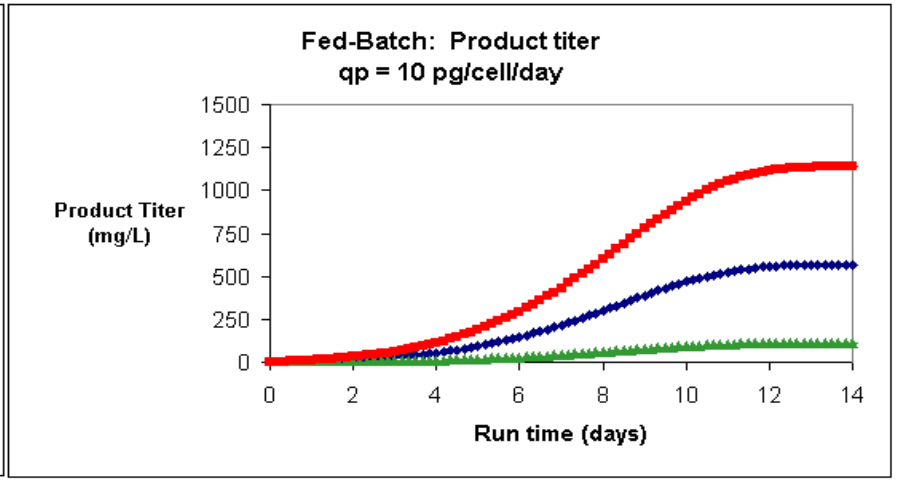
# Advantages

- Ideal for products where there are product quality concerns.
  - The bioreactor is continuous harvested; labile products can be processed quickly
  - The operating conditions of the bioreactor are constant and can be precisely controlled—this may be critical for some products
- High volumetric productivity from the bioreactor as compared to fed-batch
  - Much higher peak cell densities can be achieved than in fed-batch
  - The bioreactor can operate continuously for extended periods of time at high cell density

# Mathematical Modeling

The productivity advantage can be demonstrated through a mathematical model. First, consider a 14 day fed-batch bioreactor with a constant specific productivity.

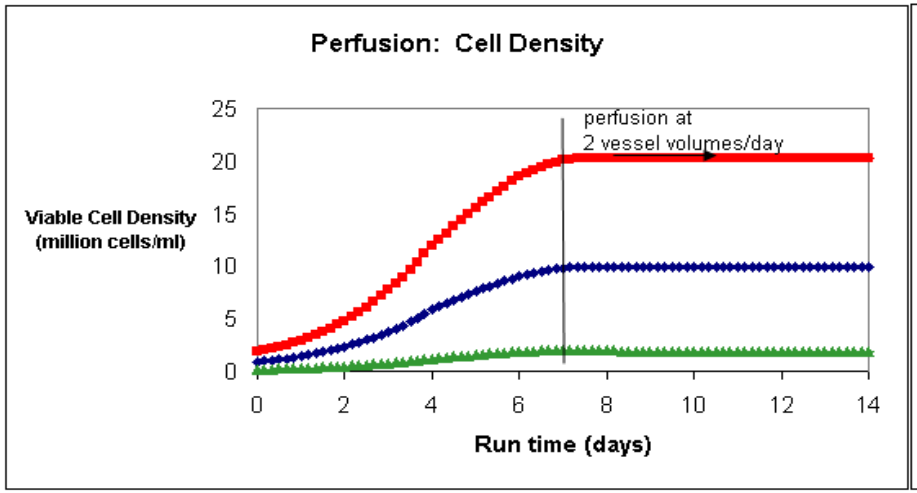


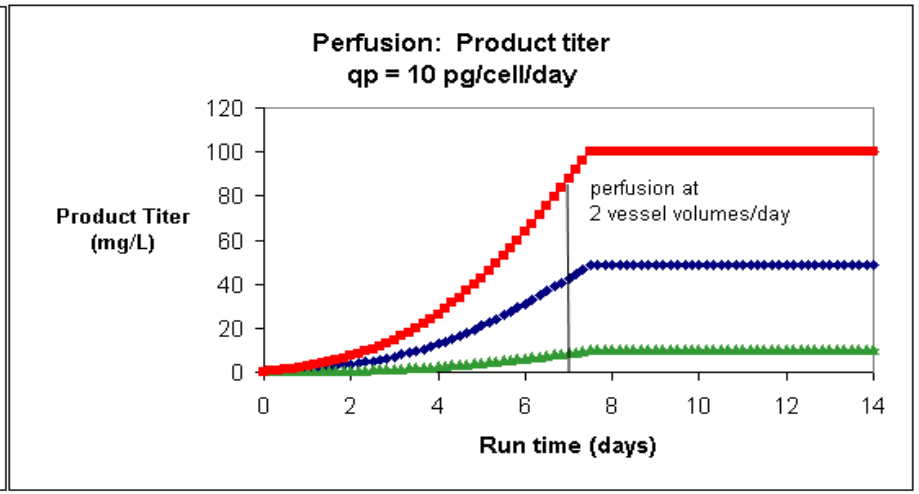




| Peak day 8 cell density (million cells/ml) | volumetric productivity (mg/day/bioreactor liter) during a 14 day period | |
|---|---|---|
| | Fed-batch | Perfusion |
| 20 | 81 | |
| 10 | 41 | |
| 2 | 8 | |

# Mathematical Modeling

Mathematical modeling of a 14 day perfusion bioreactor – harvest 2 vessel volumes/day beginning on day 7



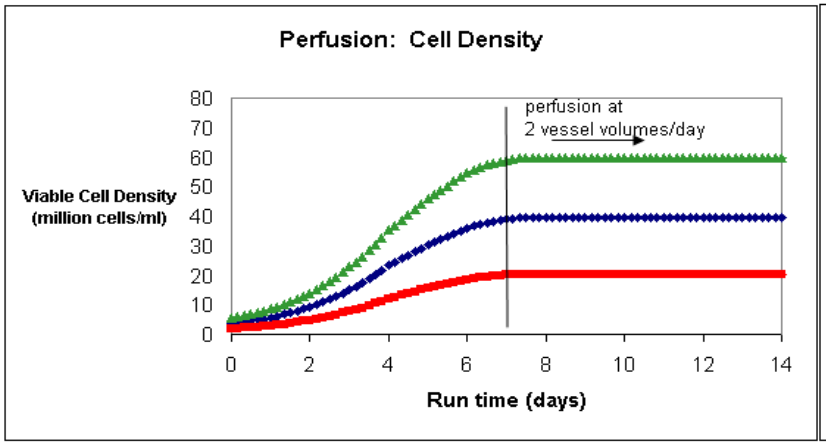



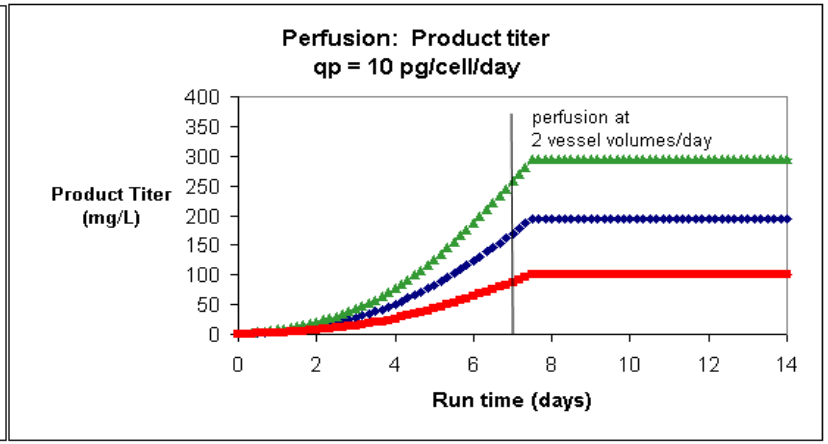

| Peak day 8 cell density (million cells/ml) | volumetric productivity (mg/day/bioreactor liter) during a 14 day period | |
|---|---|---|
| | Fed-batch | Perfusion |
| 20 | 81 | 200 |
| 10 | 41 | 100 |
| 2 | 8 | 20 |

**~150% increase with perfusion**

# Mathematical Modeling

One of the major advantages from perfusion is the ability to achieve increased peak cell densities. Densities as high as 100 million cells/ml are possible.




| Steady state cell density (million cells/ml) | Steady state conditions | | | Product from a 14 day fed-batch 100L bioreactor* (grams) |
|---|---|---|---|---|
| | Product titer (mg/L) | Volumetric productivity at 2 VVD (mg/day/bioreactor liter) | Daily product accumulation from a 100L bioreactor (grams) | |
| 20 | 100 | 200 | 20 | 114 (every 14 days) |
| 40 | 200 | 400 | 40 | |
| 60 | 300 | 600 | 60 | |

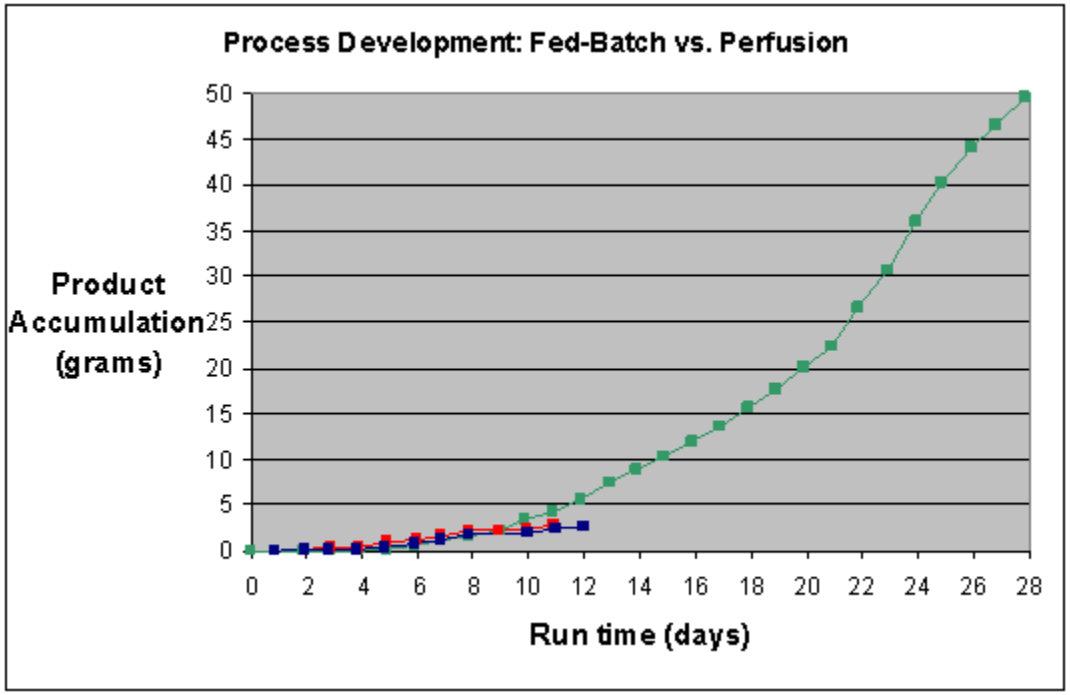
*peak density = 20 million cells/ml

# Perfusion at CMC Biologics

## Example

**Example from CMC Biologics**

- Fed-batch Culture: ~55mg/L/day (12 days), 2.8g total product
- Perfusion: ~ 425mg/L/day (28 days), 50g total product



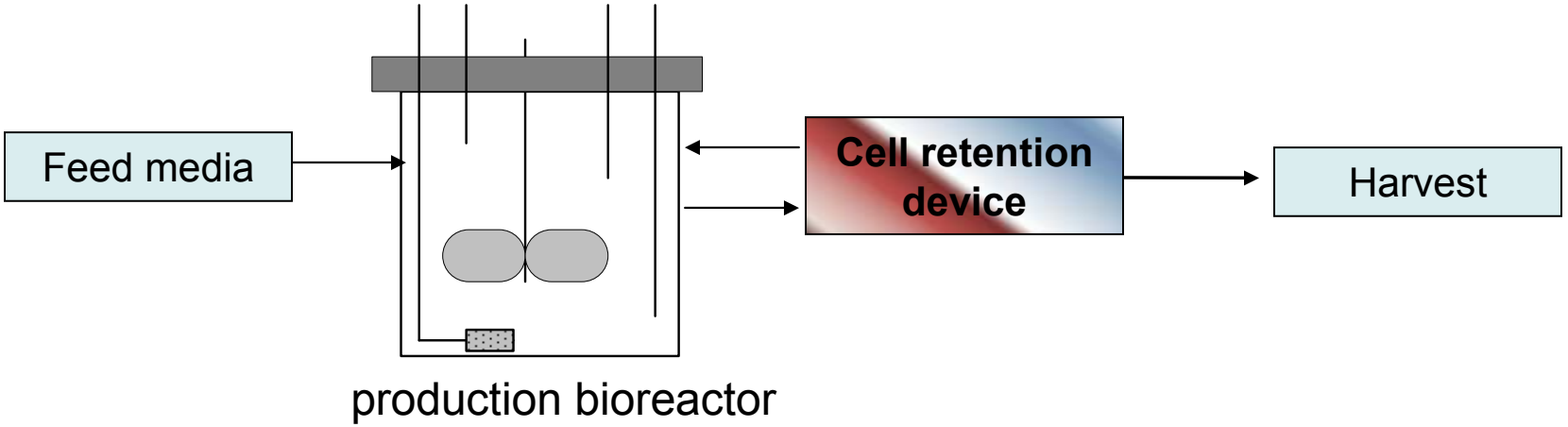

12 day Fed-batch Culture (blue & red)  28 day Perfusion (Green)

# Perfusion – operational details

The elegance of continuous manufacturing

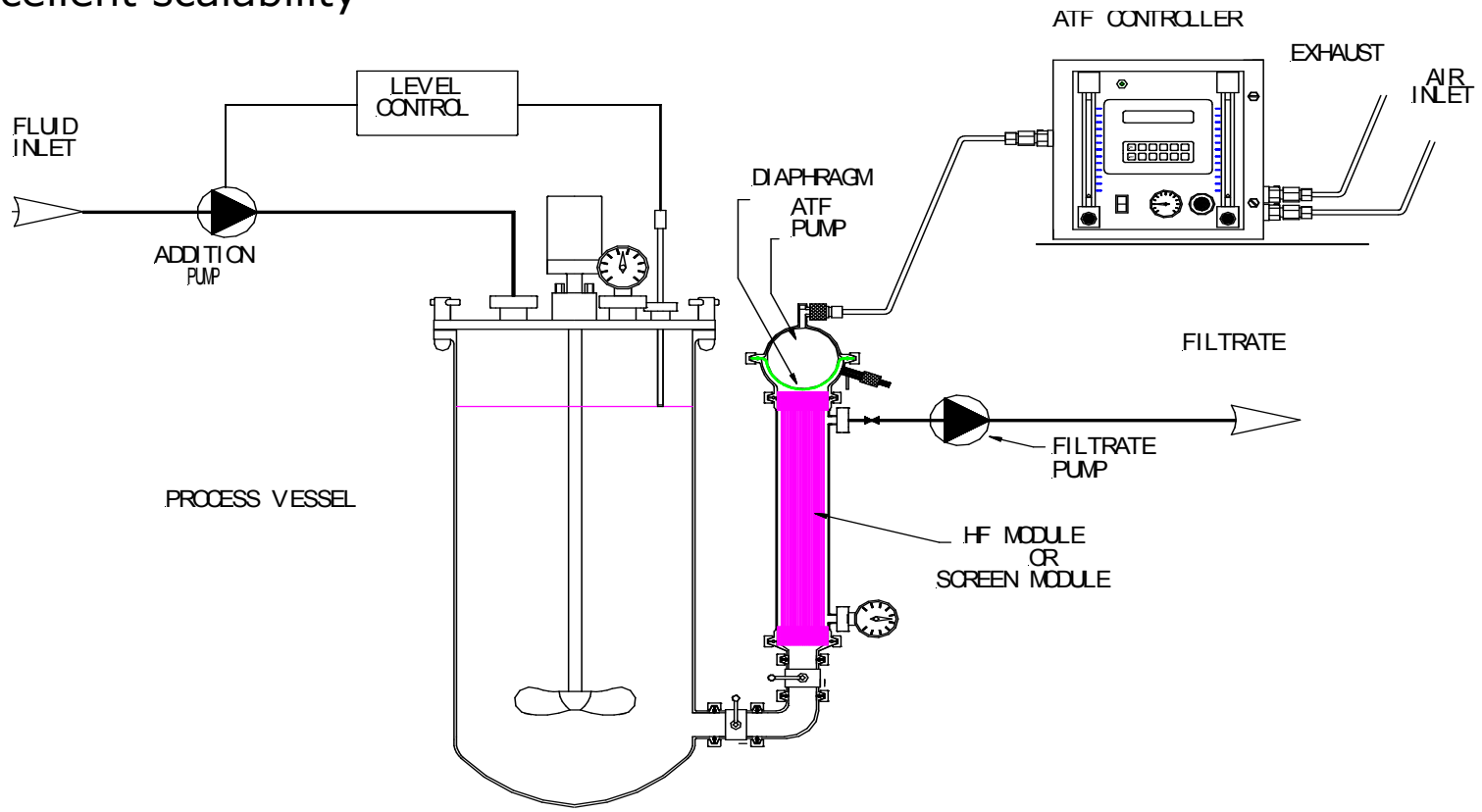
# Cell retention devices

- Types of cell retention devices that can be used
  - Conical and inclined settlers
  - Centrifuges
  - Cell immobilization
  - Spin filters, internal or external
  - Acoustic resonance
  - Microfiltration



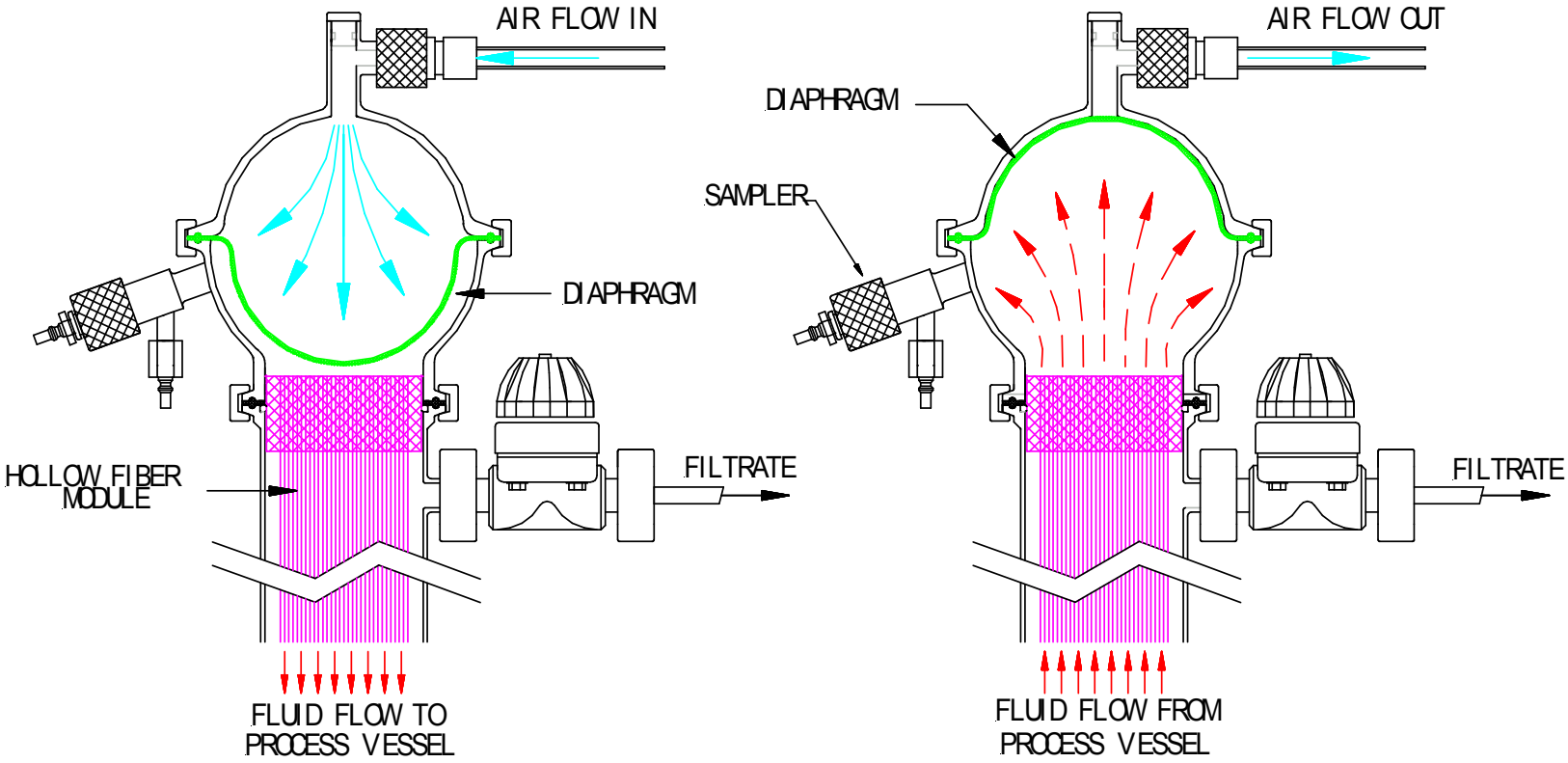
# Alternating Tangential Flow

- CMC has evaluated technologies and chosen the ATF system*
  - Ease and reliability of operation
  - Excellent scalability

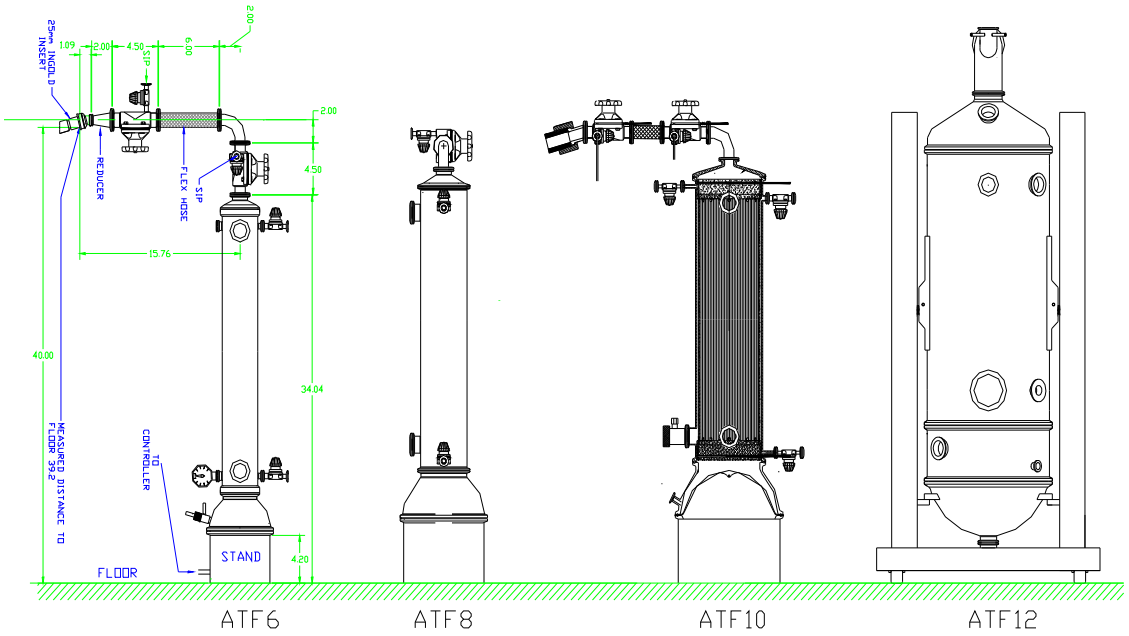



* Refine Technology East Hanover, New Jersey, USA

# ATF Details


AIR FLOW IN
DIAPHRAGM
HOLLOW FIBER MODULE
FILTRATE
FLUID FLOW TO PROCESS VESSEL
AIR FLOW OUT
DIAPHRAGM
SAMPLER
FILTRATE
FLUID FLOW FROM PROCESS VESSEL

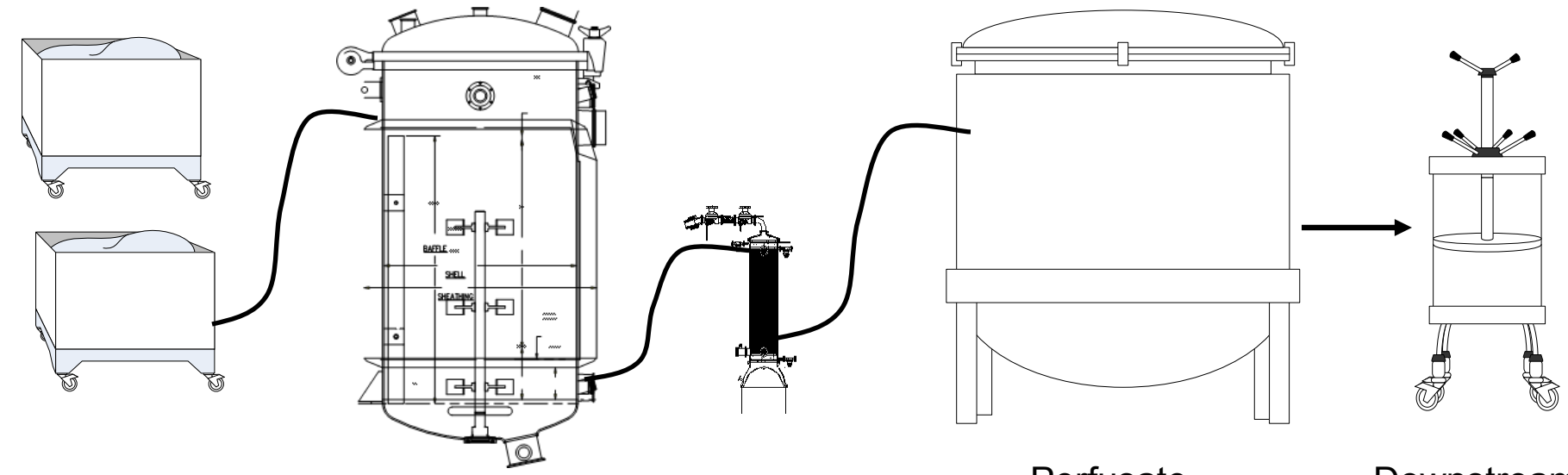
# ATF Scale-up Options




| System | Bioreactor size (L) | Approx. Capacity (L/day) * |
|---|---|---|
| **ATF 2** | 1-3 | 2-10 |
| **ATF 4** | 4-20 | 10-40 |
| **ATF 6** | 20-100 | 40-200 |
| **ATF 8** | 100-180 | 200-600 |
| **ATF 10** | 200-800 | 600-1200 |
| **ATF 12** | 400-1000 | 1200+ |

* these numbers are just a guideline, usually, a flow of 2-3 times the vessel volume per day is possible

# Manufacturing Logistics




Feed media

Production Bioreactor

ATF Perfusion

Perfusate Collection Vessel (2-8°C)

Downstream Processing

Continuous feed and perfusate collection from the production bioreactor

Every 2 – 7 days the perfusate will be forward processed in purification

# Perfusion – Challenges

Process complexity managed through engineering and experience

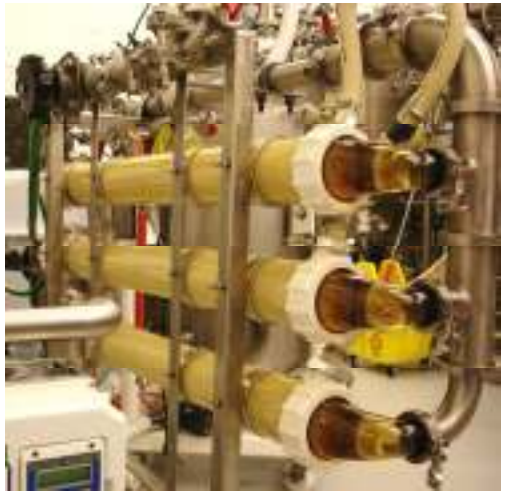
# Comparison of operating modes

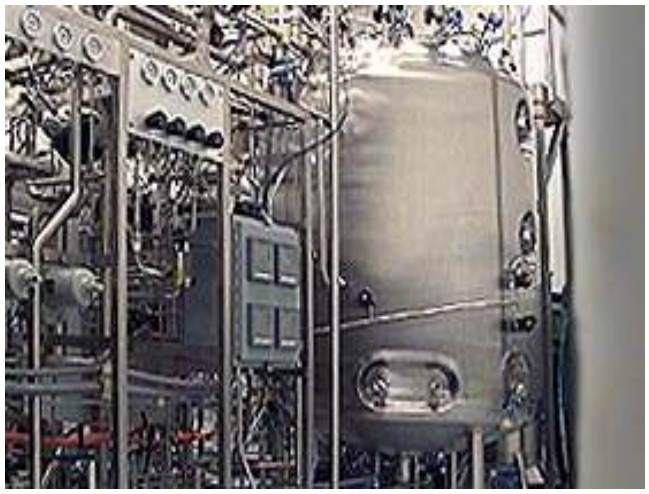
| | Fed batch | Perfusion |
|---|---|---|
| Process Complexity | Moderate | High |
| Process Control Needed | Moderate | High |
| Contamination risk | Moderate | Moderate |
| Operational costs | Moderate | Moderate |
| Cell line stability issues | Moderate | High |

# Challenges – Process Complexity and Control

Perfusion is a complex process. However, the challenges can be managed.

- Reliable scale-down perfusion models
  - Need to model hold times and fluid handling challenges at bench scale
- Experienced staff with diverse technical background
  - Scheduling and raw material logistics is critical
- Robust manufacturing systems with appropriate hardware and control capabilities

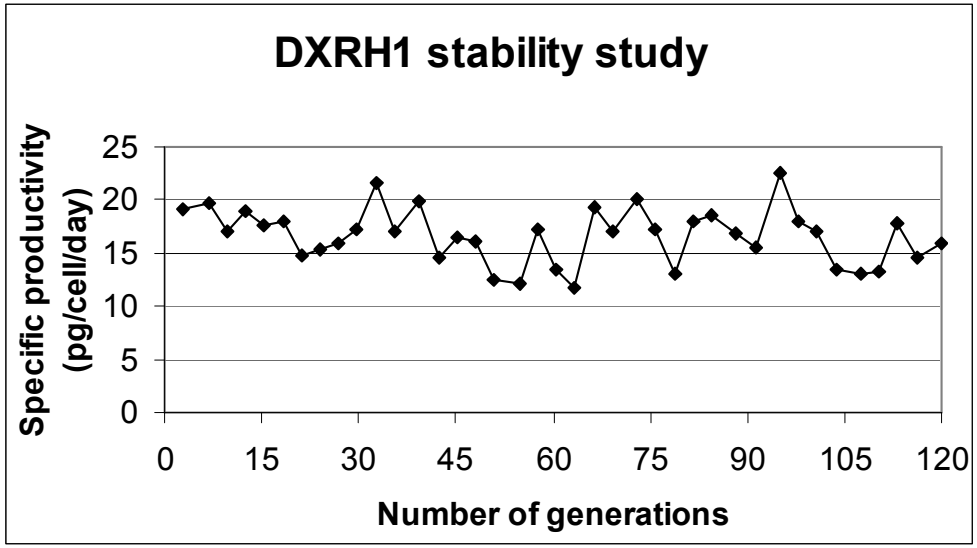




# Challenges – Cell Line Stability

The cell line must stably express the product of interest for the duration of the perfusion process

- An expression system and cell line development platform will permit the isolation of a productive and stable cell line
- Example:  CHEF1 cell line shown to have a stable expression out to 120 generations (~ 120 days)



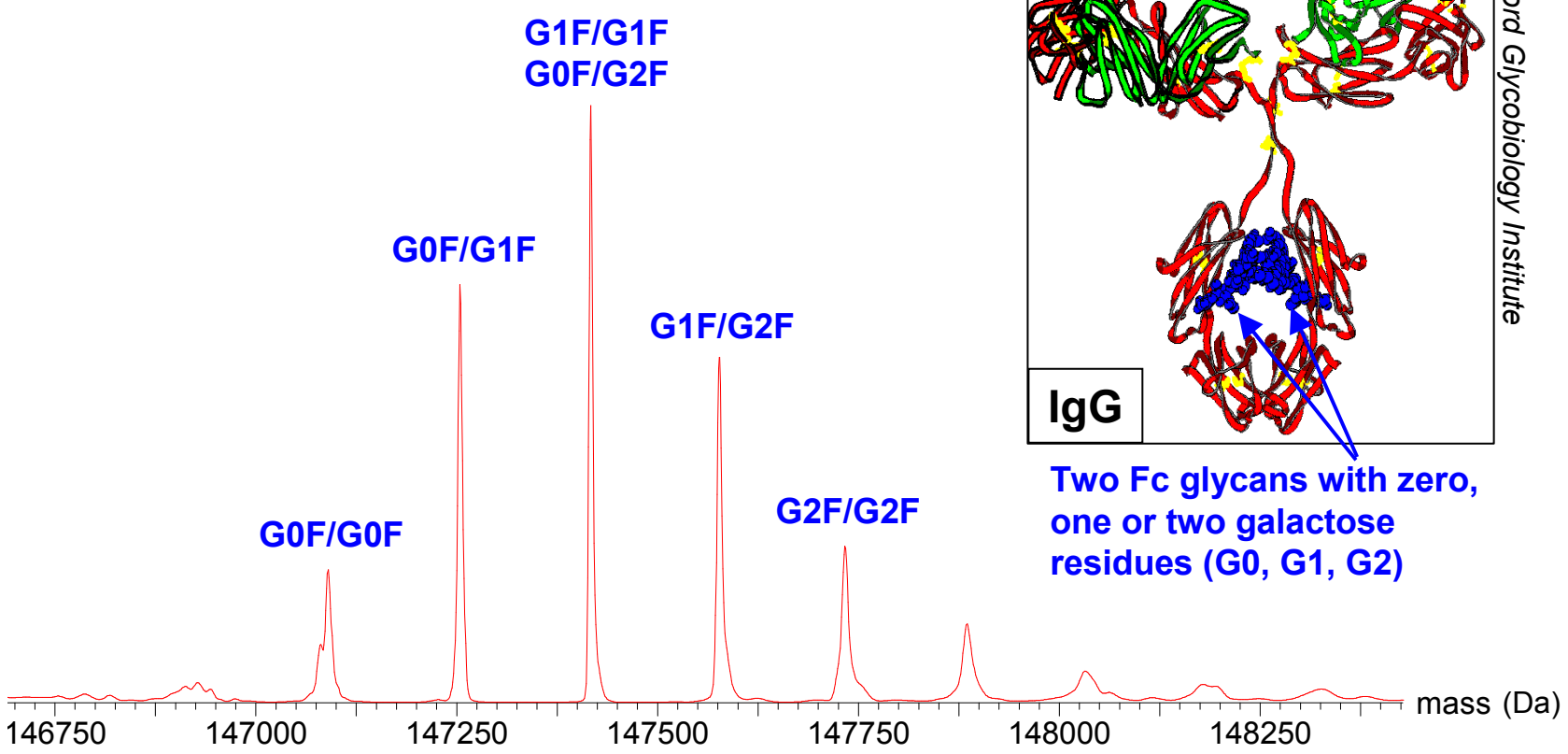
# Challenges – Validation

The manufacturing process must be validated to show consistency for the entire process run. Sophisticated analytical techniques can be applied to monitor the process and product quality

- **Structural Analysis**
  - SDS-PAGE and IEF
  - HPLC: SE, RP and IE
  - Capillary electrophoresis
  - N-terminal sequencing
  - HPLC Peptide mapping
  - Mass spectrometry
    - Instruments: MALDI-TOF, MSD single quad, LTQ ion trap, API4000 triple quadrupole, Synapt HDMS
    - Applications: whole proteins, carbohydrates, peptide maps and process residuals
  - Glycan profiling & sialic acid quantitation
  - DLS and SEC-MALLS Light scattering, CD, fluorescence, FTIR
- **Functional analysis**
  - ELISA, BIAcore and ITC
  - Cell-based assays

# Example: Intact mAb Mass on Synapt HDMS

- High resolution mass spectrometer similar to QTOF plus ion mobility function.
- Single run of intact mAb gives accurate glycoprotein mass for all glycoforms.



# Summary

- Perfusion has a higher volumetric productivity as compared to fed-batch
- Perfusion is more complex from a development, manufacturing, and regulatory perspective.
- However, these challenges can be overcome with good science and experience.
  - Manufacturing organization must be comfortable running complex processes
  - Process development must have sufficient equipment and analytical tools to control the process and monitor the product